Apidologie 30 (1999) 37–41

Original article

Tau-fluvalinate content of Apistan® strips

Mark F. Feldlaufer

USDA, Agricultural Research Service, Bee Research Laboratory, Bldg 476, BARC-East, Beltsville, MD 20705, USA

(Received 1 July 1998; accepted 12 November 1998)

Abstract – The tau-fluvalinate content of 13 lot numbers of Apistan® strips was determined by high performance liquid chromatography (HPLC). The average percent tau-fluvalinate in strips from each lot number ranged from 9.95 to 10.64 %, which falls within the range specified in the Confidential Statement of Formula (CSF) on file with the Environmental Protection Agency. It is not likely that the reduced efficacy in parasitic mite (*Varroa jacobsoni*) control reported in certain areas of the US is due to the tau-fluvalinate content of Apistan® strips. © Inra/DIB/AGIB/Elsevier, Paris

tau-fluvalinate content / Apistan® / liquid chromatography / Apistan® resistance

1. INTRODUCTION

Apistan® (Wellmark International, Bensenville, IL) is the trade name for plastic strips impregnated with the synthetic pyrethroid tau-fluvalinate (10 % w/w) that are used to control the honey bee parasitic mite *Varroa jacobsoni* Oudemans. Tau-fluvalinate is considered relatively non-toxic to honey bees, and Apistan® strips are the sole legal treatment available to beekeepers in the US for the control of *V. jacobsoni*. During the summer of 1997, anecdotal reports surfaced that beekeepers in certain geographic areas of the US were experiencing reduced mite control when using Apistan® [8]. While the possibility of mite resistance to tau-fluvalinate was being investigated, concerns were raised about the possibility of formulation and/or release rate problems associated with Apistan® strips. In this study, we determined the tau-fluvalinate content of Apistan® strips from a variety of years and lot numbers in an effort to explain the reduced efficacy of Apistan® strips in certain situations.

E-mail: mfeldlau@asrr.arsusda.gov
Disclaimer: Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

2. MATERIALS AND METHODS

2.1. Strips

Apistan® strips for this study were obtained from several sources: i) existing Bee Research Laboratory stocks, which were used both experimentally and for routine (twice-yearly) control of *V. jacobsoni*; ii) from unopened boxes supplied by the manufacturer (Wellmark Intl., Bensenville, IL); and iii) from several beekeepers experiencing control problems. In addition, we obtained and analyzed a number of 'Section 18 emergency strips' that were used when *V. jacobsoni* was first encountered in the US. These particular strips were formulated with a phthalate plasticizer not currently used. All strips examined in this study were unused.

2.2. Extraction procedure

Three Apistan® strips from each lot number were taken for analysis. Three disks (~4 mm in diameter) were punched from different areas (top, middle, bottom) of each strip, weighed, and placed in standard scintillation vials to which 10 mL methylene chloride was added. After capping, samples were shaken on an orbital shaker (Bellco; Vineland, NJ) for 15 min and subsequently left to stand at room temperature for a minimum of 72 h. Prior to analyses, all samples were re-shaken as above. Previous tests indicated this protocol was sufficient in extracting all of the tau-fluvalinate in the disks.

2.3. High performance liquid chromatography (HPLC)

A constaMetric® 4100 solvent delivery system (Thermo Separation Products; Piscataway, NJ) was used in conjunction with a Rheodyne 7125 injector (5 µL sample loop) and a Waters Model 481 UV detector. Samples were analyzed by injecting 30 µL (six loop volumes) of the methylene chloride extract onto a C_8 column (150 mm × 4.6 mm; 5 µm particle size; YMC Inc., Wilmington, NC) eluting 90 % acetonitrile-water at 1 mL/min. The eluant was monitored at 254 nm and column temperature was held constant at 33 °C. Under these conditions, tau-fluvalinate eluted at approximately 4.5 min. Tau-fluvalinate in each sample was quantified with a Shimadzu CR3A integrator (Columbia, MD), by comparing the peak area in each sample with the peak area obtained from a tau-fluvalinate stock solution. Percent tau-fluvalinate was calculated by dividing the amount of tau-fluvalinate by the weight of the individual disks. All analyses were performed in triplicate. All solvents for extraction and analysis were from Burdick & Jackson (Baxter Healthcare Corp., McGaw Park, IL). The tau-fluvalinate standard was supplied by Wellmark Intl.

3. RESULTS

Apistan® strips manufactured in years 1994 through 1997 were included in this study, as well as 'Section 18 emergency' strips (approximately 10 years old). The percent tau-fluvalinate in each strip analyzed is given in *table I*. The average percent tau-fluvalinate in each lot number ranged from 9.95 % (± 0.06 %) in strips obtained from a Pennsylvania beekeeper to 10.64 % (± 0.09 %) in strips obtained from the manufacturer. All individual strips analyzed contained the amount of tau-fluvalinate specified in the Confidential Statement of Formula (CSF) on file with the Environmental Protection Agency.

4. DISCUSSION

Analysis for tau-fluvalinate residues in honey and other bee products has been previously described [1, 6, 9 10]. Most analyses utilized gas chromatography (GC) with an electron capture detector (ECD) owing to its greater sensitivity and selectivity [7]. While GC-ECD has also been used to determine the tau-fluvalinate content of Apistan® strips [3], we chose high performance liquid chromatography (HPLC) for our analyses since accuracy, not sensitivity, was our primary concern. Using a reversed-phase HPLC protocol modified from [1], we were able to sample directly from the extraction vial, thereby eliminating the need for multiple dilutions and transfers, potential sources of error.

Table I. Percent fluvalinate in Apistan® strips from different lot numbers determined by high performance liquid chromatography (HPLC).

Lot number[a]	Source[b]	% Fluvalinate			Ave. % ± SEM
		Strip 1	Strip 2	Strip 3	
94020627	BRL	10.27[c]	10.51	10.43	10.40 ± 0.07
94072948	W	10.08	10.23	10.30	10.20 ± 0.06
94124727	BRL	10.49	10.55	10.71	10.58 ± 0.07
950918641R	W	10.42	10.42	10.15	10.33 ± 0.09
960729308	PA	10.12	9.96	9.85	9.98 ± 0.08
960730319	W	10.13	10.19	11.58	10.63 ± 0.47
960731333	PA	10.01	9.95	9.89	9.95 ± 0.06
960911486	FL	10.53	10.37	10.46	10.45 ± 0.04
961119807	W	10.56	10.34	10.36	10.42 ± 0.07
970304596	W	10.52	10.81	10.59	10.64 ± 0.09
970602183	FL	10.04	10.06	10.21	10.10 ± 0.08
970602184	FL	10.38	10.31	10.08	10.26 ± 0.09
970602186	FL	10.33	10.21	10.16	10.23 ± 0.05
Sec. 18	FL	10.18	9.85	9.87	9.97 ± 0.10

[a] The first two digits represent the year of manufacture; 'Sec. 18' are strips that were produced about 10 years ago and formulated with a phthalate plasticizer.
[b] BRL = Bee Research Laboratory; FL = Florida; PA = Pennsylvania; W = Wellmark.
[c] Represents the average of nine analyses per strip (3 disks/strip × 3 injections/disk). Fluvalinate content was calculated by comparing the peak area of each injection with the peak area obtained from the injection of a fluvalinate standard. Percent fluvalinate was obtained by dividing the amount of fluvalinate by the weight of the disk.

Since all of the Apistan® strips analyzed nominally contained the advertised amount of tau-fluvalinate (10 %; w/w), it is unlikely that reduced efficacy with regard to *V. jacobsoni* control can be attributed to the tau-fluvalinate content of these strips. Rather, a recent report [2] suggests that certain populations of *V. jacobsoni* in the US are exhibiting resistance to tau-fluvalinate, a condition that has been previously documented in Europe [4, 5, 11].

ACKNOWLEDGEMENTS

We thank L. Cutts (Gainesville, FL), C. Randall (Umatilla, FL), G. & C. Tschida (Lake Wales, FL), D. VanGundy (Dallas, TX) and K. Young (Cogan Station, PA), for supplying Apistan® strips. The technical expertise of K. Wilzer, Jr is greatly appreciated. We thank D. Liewehr and J.S. Pettis for helpful discussion.

Résumé – Teneur en tau-fluvalinate des lanières d'Apistan®. On a déterminé la teneur en tau-fluvalinate des lanières d'Apistan® afin d'essayer d'expliquer la baisse d'efficacité du produit contre *Varroa jacobsoni* dans certaines régions des États-Unis. Les lanières d'Apistan® utilisées provenaient de diverses sources, années et lots : i) stocks présents au Bee Research Laboratory, qui étaient utilisés à des fins expérimentales et en routine deux fois par an contre *V. jacobsoni* ; ii) boîtes non ouvertes fournies par le fabricant (Wellmark Intl., Dallas, TX) ; et iii) lanières de divers apiculteurs ayant rencontré des problèmes de traitement. Toutes les lanières étaient neuves. Trois disques de 4 mm de diamètre environ ont été découpés dans le haut, le milieu et le bas des lanières, pesés et mis dans des flacons à scintillation normalisés, dans lesquels on a

ajouté 10 mL de chlorure de méthylène. Après obturation des flacons, les échantillons ont été placés sur un agitateur orbital pendant 15 min puis laissés à température ambiante durant 72 h au minimum. Tous les échantillons ont été de nouveau agités comme indiqué ci-dessus, puis analysés en chromatographie liquide à haute performance (HPLC) par injection de 30 µL (six fois le volume de la boucle) de l'extrait de chlorure de méthylène sur une colonne C_8 (150 mm × 4,6 mm; taille des particules 5 µm) puis élué avec un mélange acétonitrile-eau à 90–10 (ou bien : acétonitrile à 90 % (v/v) dans l'eau) à la vitesse de 1 mL/min. L'élution a été suivie à 254 nm et la température de la colonne maintenue constante à 33 °C. Dans ces conditions, le tau-fluvalinate est élué en 4,5 min environ. La quantité de tau-fluvalinate dans chaque échantillon a été mesurée avec un intégrateur Shimadzu CR3A, par comparaison de la surface du pic de chaque échantillon avec la surface du pic obtenu à partir d'une solution de tau-fluvalinate standard. Le pourcentage de tau-fluvalinate a été calculé en divisant la quantité de tau-fluvalinate par la masse de chaque disque. Toutes les analyses ont été répétées trois fois.

Le taux moyen de tau-fluvalinate a varié de 9,95 à 10,64 %, ce qui entre dans la gamme des valeurs mentionnées dans le *Confidential Statement of Formula* (CSF) de l'Agence de Protection de l'Environnement des États-Unis. L'efficacité réduite de l'Apistan® rencontrée dans certaines régions des États-Unis dans la lutte contre *V. jacobsoni* n'est vraisemblablement pas due à la teneur en tau-fluvalinate des lanières d'Apistan®.

***Varroa jacobsoni* / résistance / Apistan® / tau-fluvalinate / HPLC**

Zusammenfassung – Gehalt von Tau-Fluvalinat in Apistan® Streifen. Für eine Mögliche Erklärung der reduzierten Wirksamkeit von Apistan® Streifen bei der *Varroa jacobsoni* Kontrolle in bestimmten geographischen Bezirken der Vereinigten Staaten wurde der Gehalt von Tau-Fluvalinat in Apistan® Streifen bestimmt. Die Apistan® Streifen stammten aus verschiedenen Jahren und aus einer großen Zahl verschiedener Quellen: i) aus den vorhandenen Lagern der Versuchslaboratorien für Bienen, die sowohl für Versuche als auch für Routinekontrollen von *V. jacobsoni* genutzt wurden, ii) aus ungeöffneten Paketen direkt aus der Fabrik (Wellmark Intl., Dallas, TX); und iii) von verschiedenen Imkern, die Schwierigkeiten mit der Varroabehandlung hatten. Alle Streifen waren noch unbenutzt. Drei Scheiben (d ≈ 4 mm) wurden aus verschiedenen Stellen (oben, Mitte, unten) der einzelnen Apistan® Streifen ausgestanzt , gewogen und in Standard Scintillations Gefässe überführt, in die 10 mL Methylenchlorid zugefügt wurden. Nach dem Verschließen wurden die Proben für 15 min in einem Orbitalschüttler geschüttelt und anschließend für mindestens 72 h bei Raumtemperatur stehen gelassen. Vor der Analyse wurden alle Proben noch einmal wie oben geschüttelt. Die Proben wurden in einem Hochdruck-Flüssigchromatographen (HPLC) analysiert. Dazu wurden 30 mL (das Volumen von 6 Schleifen) des Methylenchloridextraktes auf eine C_8 Säule (150 mm × 4,6 mm; 5 µm Korngröße) injiziert und mit 90 % Acetonitrilwasser mit 1 mL/min eluiert. Das Eluat wurden bei 254 nm kontrolliert, die Säulentemperatur wurde konstant bei 33 °C gehalten. Unter diesen Bedingungen wurde jede Probe mit einem Shimadzu CR3A Integrator quantifiziert, indem man die Peakfläche jeder Probe mit der Peakfläche einer Tau-Fluvalinat Stammlösung verglich. Der Anteil Tau-Fluvalinat wurde berechnet durch die Division der Tau-Fluvalinat Menge durch das Gewicht der einzelnen Scheiben. Alle Analysen wurden 3 mal wiederholt.

Im Durchschnitt lag der Prozentsatz von Tau-Fluvalinat zwischen 9,95 % und 10,64 % (*Tabelle I*), der innerhalb des Bereiches liegt, der in der Confidential Statement of Formula (CSF) in den Akten im US Umwelt-

schutzamt dokumentiert ist. Es ist unwahrscheinlich, daß die geringere Wirksamkeit bei der parasitischen Milbe (*V. jacobsoni*) in verschiedenen Bezirken der USA vom Gehalt des Tau-Fluvalinat in den Apistan® Streifen abhängig ist.

Tau-Fluvalinat Gehalt / Apistan® / HPLC / Apistan® Resistenz / *Varroa jacobsoni* / USA

REFERENCES

[1] Atienza J., Jimenez J.J., Bernal J.L., Martin M.T., Supercritical fluid extraction of fluvalinate residues in honey. Determination by high performance liquid chromatography, J. Chromatogr. A 665 (1993) 95–99.

[2] Baxter J., Eischen F., Pettis J., Wilson W.T., Shimanuki H., Detection of fluvalinate-resistant varroa mites in U.S. honey bees, Am. Bee J. 138 (1998) 291.

[3] Cabras P., Floris I., Garau V.L., Melis M., Prota R., Fluvalinate content of Apistan® strips during treatment and efficacy in colonies containing sealed worker brood, Apidologie 28 (1997) 91–96.

[4] Colin M.E., Vandame R., Jourdan P., Di Pasquale S., Fluvalinate resistance of *Varroa jacobsoni* Oudemans (Acari: Varroidae) in Mediterranean apiaries of France, Apidologie 28 (1997) 375–384.

[5] Faucon J.P., Drajnudel C., Fléché C., Decrease in Apistan® efficacy used against *Varroa* disease in the honeybee (*Apis mellifera*), [Mise en évidence d'une dimunition de l'efficacité de l'Apistan® utilisé contre la varroose de l'abeille (*Apis mellifera* L.), Apidologie 26 (1995) 291–296.

[6] Fernandez-Muino M.A., Sancho M.T., Muniategui S., Huidobro J.F., Simal-Lozano J., Acaracide residues in honey: Analytical methods and levels found, J. Food Protection 58 (1995) 449–454.

[7] Fitch W.L., Helisten C.C., Visser I.M., Miller W.W., Fluvalinate, Analytical Methods for Pesticides and Plant Growth Regulators XIII, 1984, pp. 79–102.

[8] Fore J., T. H., Florida beekeepers question Apistan efficacy, The Speedy Bee 26 (1997) 3.

[9] Kubik M., Nowacki J., Marcinkowski J., Michalczuk L., Pidek A., Penetration of fluvalinate into bee-products, J. Fruit and Ornamental Plant Res. 3 (1995) 13–22.

[10] Kubik M., Nowacki J., Marcinkowski J., Pidek A., Michalczuk L., Penetration of fluvalinate into bee products. II. The role of fluvalinate vapour, J. Fruit and Ornamental Plant Res. 4 (1996) 55–68.

[11] Lodesani M., Colombo M., Spreafico M., Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy), Apidologie 26 (1995) 67–72.