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First report of *Varroa destructor* resistance to pyrethroids in the UK

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Abstract – *Varroa destructor* resistance to pyrethroids has been reported in mainland Europe since the early 1990’s. *V. destructor* was first detected in the UK in 1992 and since then there has been widespread use of the only two authorised pyrethroid treatments. A routine national screening programme for resistance to pyrethroids was established in 2000 and in August 2001 resistance was detected in southwest England. The resistance outbreak was limited to 25 apiaries, was associated with product misuse, and the resistance factors to fluvalinate and flumethrin were approximately 10 fold when compared to susceptible mites. There was no cross-resistance with amitraz, coumaphos or cymiazole. This level of resistance is far lower than that detected following widespread colony collapse in Italy and highlights the importance of the correct use of varroacides and of early detection of resistance to enable its control.

*Varroa destructor* / resistance / pyrethroid / monitoring

1. INTRODUCTION

*Varroa destructor* Anderson and Trueman (Anderson and Trueman, 2000) a major pest of the European honeybee (*Apis mellifera* L.) was first reported in western Europe in the late 1970’s/early 1980’s. If untreated, infested colonies generally die within 2–3 years. The pyrethroid acaracides (e.g. Apistan (fluvalinate) and Bayvarol (flumethrin)) were introduced in the 1980’s and proved very effective in the mite control, relatively non-toxic to the bees and easy to use. However, *V. destructor* resistance to these pyrethroid acaracides, detected due to high levels of colony losses, was first reported in Europe in 1992 (Watkins, 1997). Trouiller (1998) monitored the spread of resistance to pyrethroids throughout Europe and his data supported the theory that the resistant strain originated in Italy in the early 1990s and spread to Slovenia, Switzerland, France, Belgium and Austria. In 1998 the detection of fluvalinate resistant mites in the United States was reported associated with lack of control of the mites with Apistan (Baxter et al., 1998; Elzen et al., 1998). Many cases of resistance in Europe were associated with the use of agricultural formulations of the pyrethroids (Watkins, 1997) or the high use of varroacide strips which significantly increase the selection pressure for resistant mites (Milani, 1999). Resistance is usually associated with decreased fitness leading to a decrease in the frequency of the resistance allele (reversion). Reversion of fluvalinate resistance of approx. 50% per year has been reported in Italy following withdrawal of the active ingredient (Trouiller, 2001). Resistance is not limited to the pyrethroid varroacides, it has also been reported for coumaphos in Italy (Abed and Ducos...
de Lahitte, 1993; Milani and Della Vedoiva, 1996; Spreafico et al., 2001) and amitraz in the former Yugoslavia after only 4 years of use (Dujin et al., 1991 as cited by Milani, 1999). The basis of *V. destructor* resistance and its management is reviewed by Milani (1999).

*Varroa destructor* was first reported in the UK in 1992 in Devon, since when it has spread throughout England and Wales and into many parts of Scotland. There are only two registered treatments in the UK, both pyrethroids. This paper reports the first case of resistance to pyrethroids to be detected in the UK. This is also the first case to have been detected as part of a routine national screening programme rather than in response to reported lack of mite control by beekeepers (Milani, 1999). Early detection is crucial in reducing economic damage (Milani, 1999). The detection of resistance to Apistan required assessment of cross-resistance with the only other registered treatment (Bayvarol) and with other treatments, Apivar (amitraz), Apitol (cymiazole) and CheckMite (coumaphos) which could be authorised for use under “Special Treatment Authorisation” issued by the UK Veterinary Medicines Directorate to control the outbreak.

Several methods have been used to define pyrethroid resistance in the field and laboratory. We used those developed by Trouiller (1998) and Milani (1995) so as to ensure comparability with data collected elsewhere in Europe.

2. MATERIALS AND METHODS

2.1. Chemicals

Tau-fluvalinate (94%), flumethrin (97.7%) and coumaphos (95%) were obtained from Sigma Chemical Company, Poole, Dorset. Amitraz was obtained as Apivar strips (3.2%) from Laboratoires Biove, France. Cymiazole as Apitol (17.5%) and Apistan package bee strips (2.5% tau-fluvalinate) were a gift from Vita (Europe) Ltd, UK.

2.2. Field trials

From early 2000 country-wide field trials were conducted by CSL Appointed Bee Inspectors with field test kits using Apistan package bee strips. A minimum of two colonies were tested in each apiary.

The kits were based on those developed by Vita (Europe) Ltd (Trouiller, pers. comm.) in which bees from test colonies were exposed to the Apistan package bee strips for 4 hours to determine the number of susceptible mites dropping onto a sticky surface on the floor of the cage. The number of mites remaining on bees was determined by washing in detergent. The efficacy was calculated by expressing the number knocked off bees by exposure to the Apistan package bee strips as a percentage of the total. When efficacy was detected at 60% or less mites were submitted to the laboratory for confirmatory tests. In addition, data were submitted by beekeepers using the field kit or the equivalent Beltsville test method (Pettis et al., 1998).

2.3. Laboratory studies

2.3.1. Sources of mites

Mites for dose-mortality assessments were sampled from two susceptible colonies (95–100% efficacy using field test kits) from a single apiary site at CSL, York, and from three resistant colonies from a single apiary identified by the field test in Devon (showing 2–5% efficacy using field test kits). Mites from each colony were kept separate and included as a replicate in each assay thus allowing any differences in sensitivity between colonies to be identified. Mites from the country-wide survey were sent to the laboratory for confirmation of resistance whenever field test kits showed less than 60% efficacy.

Infested combs were brought in to the laboratory from the CSL apiary, or sent by courier overnight from the apiary. Combs were sampled as soon as possible after arrival and never more than 3 days after collection (combs were stored at 30–32 °C and 60–80% relative humidity in the dark). Mites were extracted from combs and any which were contaminated with larval fluid, damaged or not fully pigmented were rejected. Mites were put into petri dishes with damp filter paper and honeybee larvae from just capped cells within 24 hours prior to assay. The assays were carried out between 28 August and 22 September 2001.

2.3.2. Bioassays

Laboratory studies were undertaken to confirm resistance in colonies identified in field tests and to determine the susceptibility of mites to a number of varroacides. Full dose-response curves were developed for five different varroacides. These dose-response curves were also used to identify a suitable
The dose of fluvalinate for use in laboratory confirmation studies of resistance.

The response of *V. destructor* mites to fluvalinate, flumethrin and coumaphos was determined using the methods developed by Milani (1995) and Milani and Della Vedova (1996). The active ingredients were dissolved in hexane (fluvalinate and flumethrin) or acetone (coumaphos) and then incorporated into paraffin wax (melting point 46–48 °C) and the solvent allowed to evaporate. The concentration levels in wax for fluvalinate were 23, 46, 90, 185, 500 and 2000 mg/kg, for flumethrin were 0.2, 0.5, 2.0, 5.0, 20 and 50 mg/kg and for coumaphos were 5.0, 10, 25 and 75 mg/kg. Control paraffin was prepared with the same volume of solvent as the treated paraffin. Glass discs and steel rings (supplied by Vita (Europe) Ltd) were coated with the treated paraffin, the remaining solvent allowed to evaporate off overnight and the discs placed together to form capsule and stored at 30–32 °C and 60–80% relative humidity in the dark before use.

Mites were placed in the treated capsule for 6 hours for fluvalinate and flumethrin and 4 hours for coumaphos before transfer onto untreated larvae in glass petri dishes. Three replicates of ten mites were used per treatment. The mortality was observed at transfer, 24 and 48 hours. The assay was repeated if control mortality was greater than 15%.

The efficacy of amitraz was assayed using the method of Faucon et al. (1996). A piece of Apivar strip (0.4–0.5 g) was placed in a petri dish cage with five bees in the absence of *V. destructor* mites for 2 hours (30–32 °C, 60–80% relative humidity) before the introduction of five mites. Five replicates were set up for each assay, i.e. 25 mites per assay. The time for mites to drop off the bees was determined and compared to that of control bees in which no Apivar strip was present (there was no control mortality).

The efficacy of cymiazole was determined by direct application of Apitol dissolved in water (0.2 µl) to the ventral side of *V. destructor* mites placed on their dorsal side on sticky tape. Doses of 0.5, 0.9, 1.8 and 3.7 µg cymiazole per mite were applied to 30 mites per dose and mortality observed at 24 hours. Control mites were dosed with water containing the same level of sucrose as the highest Apitol dose.

The CSL Probit 1 programme was used to calculate an LC50 for fluvalinate, flumethrin, coumaphos and cymiazole and an LT50 (time to 50% mortality) for amitraz. This program takes into account control mortality (there was no control mortality in the amitraz assay) and calculates 95% confidence limits and slope (Finney, 1971).

2.3.3. Laboratory confirmation of field test resistance

When field test kits showed 60% efficacy or less comb samples were sent to the laboratory for confirmation. Mites were sampled from the submitted combs as above and confirmation of resistance was undertaken using the bioassay with a single concentration of 200 mg/kg fluvalinate (3 replicates of 10 mites) and control capsules (3 replicates of 5 mites). The mortality was determined after 48 h and resistance confirmed if the efficacy was 70% or less and control mortality was 15% or less. The data were also compared with those of the field test kits to determine the comparability of field and laboratory data.

3. RESULTS

3.1. Field trials

In 2000 and 2001 a total of over 450 colonies were tested in nearly 300 apiaries in England and Wales. Resistance was detected for the first time in Devon and Cornwall in August 2001. Further targeted testing in the area showed 38 colonies with resistance in 25 apiaries but no resistance was detected outside this area (see Fig. 1). Of the 415 reports received...
from resistance tests 47% had a total of less than 5 mites (56% of these had no mites detected), suggesting that resistance is not a problem in these colonies due to the low total numbers of mites present. Of the colonies showing resistance the mean number of mites knocked down was 4.7 (SD 7.8) and the mean number washed off was 18 (SD 19) giving a mean efficacy of 19% (SD 17%). Fifteen colonies (39%) gave less than 10% efficacy in the field test.

3.2. Laboratory studies

The dose-response curves for the mites from CSL, York and the resistant apiary in Devon are shown in Figures 2–6.

3.2.1. Fluvalinate

The susceptibility of mites from colonies at York (LC50 42.7 mg/kg, 95% confidence limits (CL) 25.4–59.6 mg/kg, slope 1.9) and those from the resistant apiary identified in Devon (LC50 477 mg/kg, 95% CL 300–906 mg/kg, slope 1.4) showed an 11 fold difference in the LC50 for fluvalinate. The LC95 for the York colonies (326 mg/kg) was 20 fold lower than that estimated for the Devon colonies (6957 mg/kg) showing the curves deviated from parallel at levels which would affect efficacy of Apistan in the Devon colonies. The 200 mg/kg discriminatory concentration used in the laboratory confirmation studies following field detection of resistance according to the reference curves resulted in a mean of 85% mortality of susceptible mites and 30% mortality of resistant mites.

3.2.2. Flumethrin

Figure 3 shows that mites from both sources were far more sensitive to flumethrin than to fluvalinate but showed a similar order of difference in susceptibility. The mites from the York colonies (LC50 0.47 mg/kg, 95% CL 0.25–0.73 mg/kg, slope 1.4, LC95 6.4 mg/kg) showed a 13 fold lower LC50 and LC95 than the mites from the Devon colonies (LC50 6.3 mg/kg, 95% CL 3.9–10 mg/kg, slope 1.5, LC95 81 mg/kg).

3.2.3. Coumaphos

The sensitivity of the mites from York and those from Devon to coumaphos is shown in Figure 4. The curves are very similar and there is no difference in the LC50 between the mites from the two sources (York LC50 14 mg/kg, 360 H.M. Thompson et al.

Figure 2. Proportion of dead V. destructor mites in fluvalinate assays (± SD) carried out with mites from colonies at CSL, York (solid line) and in Devon (dotted line) (30 mites per point).
Figure 3. Proportion of dead *V. destructor* mites in flumethrin assays (± SD) carried out with mites from colonies at CSL, York (solid line) and in Devon (dotted line) (30 mites per point).

Figure 4. Proportion of dead *V. destructor* mites in coumaphos assays (± SD) carried out with mites from colonies at CSL, York (solid line) and in Devon (dotted line) (30 mites per point).
Figure 5. Proportion of dead *V. destructor* mites in cymiazole assays (± SD) carried out with mites from colonies at CSL, York (solid line) and in Devon (dotted line) (30 mites per point).

Figure 6. Time to knockdown for *V. destructor* mites in amitraz assays (± SD) carried out with mites from colonies at CSL, York (solid line) and in Devon (dotted line) (25 mites).
95% CL 11–17 mg/kg, slope 3.3; Devon LC\textsubscript{50} 11 mg/kg, 95% CL 7.7–14 mg/kg, slope 2.7).

### 3.2.4. Cymiazole

The sensitivity of the mites from York and those from Devon to cymiazole is shown in Figure 5. Again the curves are very similar and there is no difference in the LC\textsubscript{50} between the mites from the two sources (York LD\textsubscript{50} 0.9 µg/mite, 95% CL 0.73–1.1 µg/mite, slope 3.3; Devon LD\textsubscript{50} 0.8 µg/mite, 95% CL 0.62–0.91 µg/mite, slope 5.4).

### 3.2.5. Amitraz

The sensitivity of the mites from York and those from Devon to amitraz is shown in Figure 6. The time to knockdown is very similar and there is no difference in the LT\textsubscript{50} between the mites from the two sources (York LT\textsubscript{50} 35 min, 95% CL 31–39 min, slope 5.9; Devon LT\textsubscript{50} 39 min, 95% CL 38–41 min, slope 9.2).

### 3.3. Laboratory confirmation of field test resistance

A comparison of the results from laboratory and field resistance test results for the same colonies is shown in Figure 7. Although the data is limited, due the small number of colonies in which resistance has been detected, there was a correlation between the laboratory and field data ($r = 0.89$). This shows that the field kit can reliably detect resistance when a threshold of 60% efficacy is used, although false positives are shown by the field kit near the threshold. For the 7 samples from colonies confirmed as resistant in the laboratory the field efficacy using the 2.5% Apistan package bee strip was 8% (SD 5%) and the laboratory efficacy using fluvalinate at 200 mg/kg was 37% (SD 13%).

### 4. DISCUSSION

This is the first reported incidence of \textit{V. destructor} resistance to pyrethroids in the UK and the first in Europe detected prior to reports of colony losses by beekeepers. Devon was also site of the first reported case of \textit{V. destructor} in the UK in 1992 and therefore use of pyrethroid treatments may have been occurring for up to ten years. In this case, the beekeeper was well known by the local regional bee inspector to misuse Apistan strips, through long-term continual use of strips throughout the year and overdosing, despite repeated advice to the contrary. Many other cases in Europe have been the
result of migratory beekeeping and importation of resistant mites rather than new outbreaks (Watkins, 1997; Trouiller, 1998). However, the localised nature of this outbreak, with no importation of bees by the beekeeper or by those affected in the locality, makes the development of resistance within the area more probable than the importation of resistant mites.

The toxicity of fluvalinate to the susceptible mites from CSL, York was slightly higher than that reported by Milani (1995) (LC$_{50}$ 13–19 mg/kg) suggesting generally lower susceptibility than mites in Italy but similar to that reported by Mozes-Koch et al. (2000) in Israel (41.5 mg/kg) and Trouiller (1998) (25 mg/kg). The LC$_{50}$ in resistant mites was similar to that reported in Tirano, Italy (385 mg/kg) but lower than that reported in Como (857 mg/kg) or reported by Trouiller (1998) in Italy (9 234 mg/kg). When comparing the mites tested under the same conditions the 11 fold resistance factor in LC$_{50}$ shown by the mites from the Devon apiary is far lower than the 440 fold resistance in Italy reported by Milani (1995) or 369 reported in Italy by Trouiller (1998) but higher than the 2 fold resistance factor reported for Israel by Mozes-Koch et al. (2000). The 200 mg/kg discriminatory concentration for detecting resistant mites is the same as that identified by Trouiller (1998).

The toxicity of flumethrin is similar to that reported by Milani (1995) (0.28–0.36 mg/kg) in susceptible mites and but slightly lower than the 11.4–20.4 mg/kg in resistant mites in Italy. These data clearly demonstrate the cross-resistance to fluvalinate and flumethrin.

The toxicity of coumaphos was similar to that reported by Milani and Della Vedova (1996) and Spreafico et al. (2001) for susceptible mites in Italy (9.8–12.2 mg/kg) and well below the LC$_{50}$ reported for resistant mites (21.5–554 mg/kg) (Spreafico et al., 2001). This confirmed that there was no cross-resistance or multi-resistance to coumaphos in the mites from the fluvalinate resistant colonies. The toxicity of amitraz as Apivar strips was determined as a LT$_{50}$ due to the instability of amitraz and showed similar results in the CSL, York and Devon colonies to those reported by Faucon et al. (1995) (13–47 minutes) who also showed that there was no cross-resistance between fluvalinate and amitraz. The toxicity of cymiazole to mites from the CSL, York and Devon was also similar to that recorded for susceptible mite populations (Watkins, pers. comm.).

Comparable data for a range of varroacides in mites from the same colonies are not possible due to resistance being detected by poor V. destructor control and collapsing colonies (Milani, 1995). As the resistance was detected in the early stages this is the first time that truly comparable data have been generated for five different varroacides in mites from the same colonies. These laboratory data have allowed cymiazole, amitraz and coumaphos to be identified as control measures for the fluvalinate resistant mite.

The use of the field kits as a strategy to detect resistance at an early stage has allowed the situation to be managed before it became widespread or resulted in colony collapse. The laboratory confirmation tests have shown that the field kits can reliably detect resistance when a threshold of 60% is used, although false positives may occur around this value underlining the need for laboratory confirmation of apparent new outbreaks. The mean efficacy detected in the outbreak in the southwest of England (18% ± 16%) was slightly greater than that detected in Florida, USA, using the same package bee strips (7.1 ± 7.9%) (Elzen et al., 1998), and similar to that reported in colonies elsewhere in Europe since 1995, e.g. Switzerland 0–26% efficacy (Trouiller, 1998). The kits and laboratory confirmation will be continued to be used in monitoring the situation in England and Wales and in encouraging beekeepers to monitor their own colonies to ensure outbreaks are detected at an early stage and information can be provided on appropriate management strategies.

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Résumé – Résistance de Varroa destructor aux py-rétrinoïdes en Grande-Bretagne : premier cas signalé. La résistance de l’acarien Varroa destructor aux pyréthrinoïdes est signalée en Europe continentale depuis le début des années 90. L’acarien a été détecté pour la première fois au Royaume-Uni en 1992 et depuis cette date l’usage des deux seuls traitements autorisés aux pyréthrinoïdes s’est largement répandu. Un programme national de dépistage appliqué en routine a démarré en 2000 et, en août 2001, la résistance a été détectée dans le sud-ouest de l’Angleterre. Le foyer de résistance était limité à 25 ruchers et associé à une mauvaise utilisation du produit. La toxicité de cinq varroacides (fluvalinate, fluméthrine, coumaphos, amitraze et cymiazole) a été comparée sur des acariens provenant de deux sources : (i) des colonies apparemment sensibles situées au CSL, York et (ii) des colonies du sud-ouest de l’Angleterre identifiées comme étant résistantes. La toxicité du fluvalinate pour les acariens sensibles du CSL, York était légèrement supérieure à celle mentionnée par Milani (1995) en Italie (9,8 à 12,2 mg/kg) et bien inférieure à la CL50 mentionnée par Trouiller (1998) en Italie (385 mg/kg), mais inférieure à celle signalée à Come (857 mg/kg) ou à celle mentionnée par Mozes-Koch et al. (2000) en Israël (41,5 mg/kg) et par Trouiller (1998) (25 mg/kg). La CL50 pour les acariens résistants était semblable à celle signalée à Tiran, Italie (385 mg/kg), mais inférieure à celle signalée à Come (857 mg/kg) ou à celle mentionnée par Trouiller (1998) en Italie (9234 mg/kg). Lorsque l’on compare les acariens testés dans les mêmes conditions, la résistance pour la CL50 présentée par les acariens du rucher du Devon (facteur de 11) est beaucoup plus basse que la résistance en Italie mentionnée par Milani (1995, facteur de 440) ou celle mentionnée en Italie par Trouiller (1998, facteur de 369), mais plus forte que la résistance mentionnée en Israël par Mozes-Koch et al. (2000, facteur de 2). La dose discriminante de 200 mg/kg pour détecter les acariens résistants était la même que celle identifiée par Trouiller (1998). La toxicité de la fluméthrine était semblable à celle mentionnée par Milani (1995) en Italie pour les acariens sensibles (0,28–0,36 mg/kg) mais légèrement inférieure à la toxicité pour les acariens résistants (11,4–20,4). Ces données prouvent clairement la résistance croisée au fluméthrine et à la fluméthrine. La toxicité du coumaphos était semblable à celle mentionnée par Milani et Della Vedova (1996) et par Sparaefico et al. (2001) pour les acariens sensibles en Italie (9,8 à 12,2 mg/kg) et bien inférieure à la CL50 mentionnée pour les acariens résistants (21,5 à 554 mg/kg) (Sparaefico et al., 2001). Ceci confirme l’absence de résistance croisée et de multi-résistance au coumaphos chez les acariens des colonies résistantes au fluméthrine. En raison de l’instabilité de l’amitraze, on a utilisé la toxicité létale 50 (TL50) pour évaluer la toxicité de l’amitraze sous forme de bandelettes Apivar®. Les résultats au CSL, York et dans les colonies du Devon étaient semblables à ceux mentionnés par Faucon et al. (1995) (13 à 47 min), qui ont montré eux aussi qu’il n’y avait pas de résistance croisée entre le fluméthrine et l’amitraze. La toxicité du cymiazole pour les acariens du CSL, York et du Devon était semblable à celle enregistrée pour des populations sensibles d’acariens (Watkins, données non publiées). Comme la résistance a été détectée à un stade précoce, c’est la première fois que des données vraiment comparables ont pu être obtenues pour cinq varroacides différents pour des acariens issus des mêmes colonies. Ces données de laboratoire ont permis d’identifier le cymiazole, l’amitraze et le coumaphos comme témoins dans les tests de résistance au fluméthrine. L’utilisation de kits de terrain comme stratégie pour détecter la résistance à un stade précoce a permis de gérer la situation avant qu’elle ne soit largement répandue ou qu’elle ne provoque la mort des colonies.

Varroa destructor / résistance / pyréthrinoïde / surveillance


Die Giftwirkung von Flumethrin war vergleichbar zu der von Milani (1995) beschriebenen (0,28–0,36 mg/kg) Wirkung, aber etwas geringer als die 11,4–20,4 mg/kg bei resistenten Milben in Italien. Diese Ergebnisse zeigen eine Kreuz-Resistenz zwischen Fluvinate und Flumethrin.


**Varroa destructor** / Resistenz / Pyrethroide / Überprüfung oder Überblick / Rasterprüfung

### REFERENCES


