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Varroa destructor resistance to pyrethroid treatments in the United Kingdom

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Abstract

Varroa 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 75 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.

Key words: *Varroa destructor*, resistance, pyrethroid, monitoring.

Introduction

Varroa destructor (Anderson and Trueman, 2000) a major pest of the European honeybee (*Apis mellifera* L.) was first reported in the UK in 1992 in Devon. By September 2002 varroa was widespread throughout England and Wales and present in Scotland and Northern Ireland. The pyrethroid acaracides Apistan (fluvalinate) and Bayvarol (flumethrin) are the only registered varroacide treatments in the UK and have proved very effective in varroa control, relatively non-toxic to the bees and easy to use. Varroa resistance to these pyrethroid acaracides, detected due to high levels of colony losses, was first reported in Europe in 1992 (Watkins, 1997). 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).

The National Bee Unit (www.nationalbeeunit.com) (NBU) based at CSL runs the bee health programmes to control statutory notifiable bee disease in England and Wales on behalf of DEFRA (Department for Environment, Food and Rural Affairs) and NAW (National Assembly for Wales). Resistance of varroa to pyrethroid varroacides in the UK was detected as part of a routine national screening programme established as a contingency plan by the NBU in 2000.

Materials and methods

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.

Detection of resistance

The screening of colonies for resistance comprised a field kit monitoring system and confirmation of resistance with laboratory assays.The field 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. Approximately 400 adult bees collected from the brood chamber were placed in a lidded 400ml plastic drinks beaker in which the solid lid had been replaced with mesh and holes had been perforated in the sides to provide ventilation. The beaker was inverted and placed on a piece of sticky plastic to collect any fallen mites. An Apistan package bee strip (2.5% tau-fluvalinate) was hung through a slot inside the beaker for 4 hours to determine the number of susceptible mites dropping onto the sticky plastic. The number of mites remaining on bees (resistant to pyrethroid) 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).

Confirmation of resistance

Combs containing brood and mites for confirmation of resistance were sent to CSL by courier to minimise time between collection and assessment and no mites were used more than 3 days after collection. Mites were sampled from the submitted combs and confirmation of resistance was undertaken using the bioassay with a single dose of 200 mg/kg fluvalinate. The data were also compared with those of the field test kits to determine the comparability of field and laboratory data. In addition, mites for dose-mortality assessments for 5 varroacides (coumaphos, amitraz, fluvalinate, flumethrin and cymiazole) 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). For details of the laboratory assays see Thompson *et al*. (2002).

Results and discussion

Detection of resistance

By September 2002 a total of over 1000 colonies were tested in over 500 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 has shown showed 350 colonies with resistance in 75 apiaries by September 2002 but no resistance was detected outside this area (see figures 1 and 2).

Confirmation of resistance

A comparison of the results from laboratory and field resistance test results for the same colonies is shown in figure 3. 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=0.81). 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 15 samples from colonies confirmed as resistant in the laboratory the mean field efficacy using the 2.5% Apistan package bee strip was 11% (SD 12%) and the mean laboratory efficacy using fluvalinate at 200 mg/kg was 33% (SD 12%).

The dose-response curves for the mites from CSL, York and the resistant apiary in Devon are shown in figures 4-8. The susceptibility to fluvalinate of mites from colonies at York (LC_{50} 42.7 mg/kg) was 11 fold greater than those from the resistant apiary identified in Devon (LC_{50} 477 mg/kg) (figure 4). The 200 mg/kg discriminatory dose 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. For flumethrin, the mites from the York colonies (LC_{50} 0.47 mg/kg) were 13 fold lower more susceptible than the mites from the Devon colonies (LC_{50} 6.3mg/kg) (figure 5). The sensitivity of the mites from York and those from Devon to coumaphos is shown in figure 6. The curves are very similar and there was no difference in the LC_{50} between the mites from the two sources (York LC_{50} 14 mg/kg, Devon LC_{50} 11 mg/kg).

The sensitivity of the mites from York and those from Devon to cymiazole is shown in figure 7. Again the curves are very similar and there was no difference in the LC_{50} between the mites from the two sources (York LC_{50} 0.9 μg/mite, Devon LC_{50} 0.8 μg/mite). The sensitivity of the mites from York and those from Devon to amitraz is shown in figure 8. The time to knockdown was very similar and there was no difference in the LT_{50} between the mites from the two sources (York LT_{50} 35 mins, Devon LC_{50} 39 mins).

The toxicity of fluvalinate to the susceptible mites from CSL, York was slightly higher than that reported by Milani (1995) 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 scale of resistance in Italy reported by Milani (1995) or Trouiller (1998). The 200 mg/kg discriminatory dose for detecting resistant mites is the same as that identified by Trouiller (1998).

Devon was also the site of the first reported case of varroa in the UK in 1992 and therefore pyrethroid treatments may have been used 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 data reported here clearly demonstrate the cross-resistance to fluvalinate and flumethrin but no resistance to coumaphos, amitraz or cymiazole. Detection of resistance in the early stages has allowed truely comparable data to be 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 and Special Treatment Authorisation has been obtained from the Veterinary Medicine Directorate, DEFRA, so they can be used by Appointed Bee Inspectors to treat affected colonies.

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 kits and laboratory confirmation will continue 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. In addition, we are investigating the whether mutations exist in the sodium channel gene of pyrethroid-resistant varroa mites. This will allow the development of a rapid and sensitive DNA based test enabling early detection and development of a resistance management strategy.

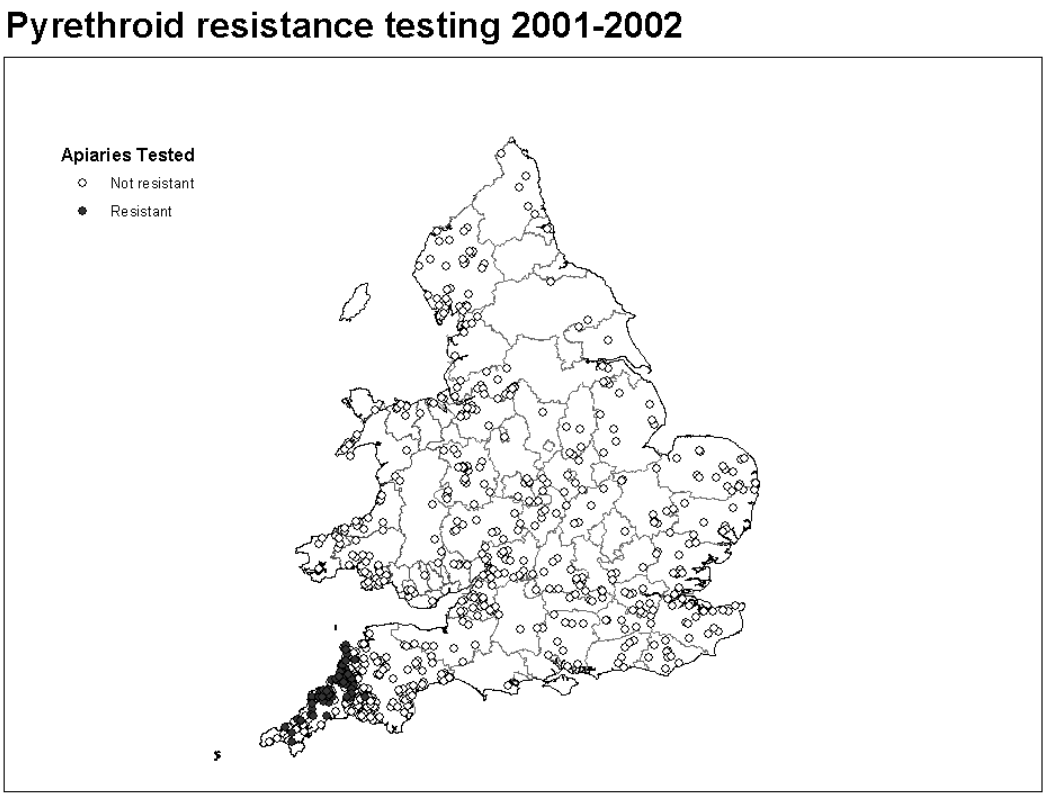

Figure 1. Distribution of varroa resistance testing in England and Wales to September 2002.

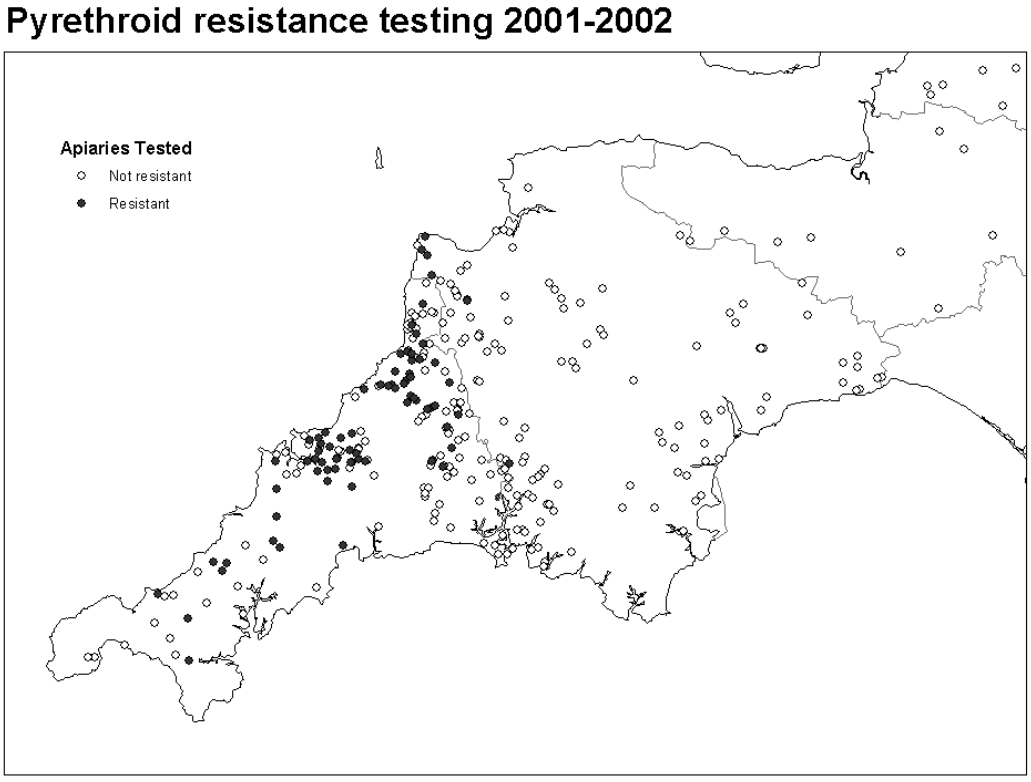

Figure 2. Distribution of resistant apiaries in Devon and Cornwall to September 2002.

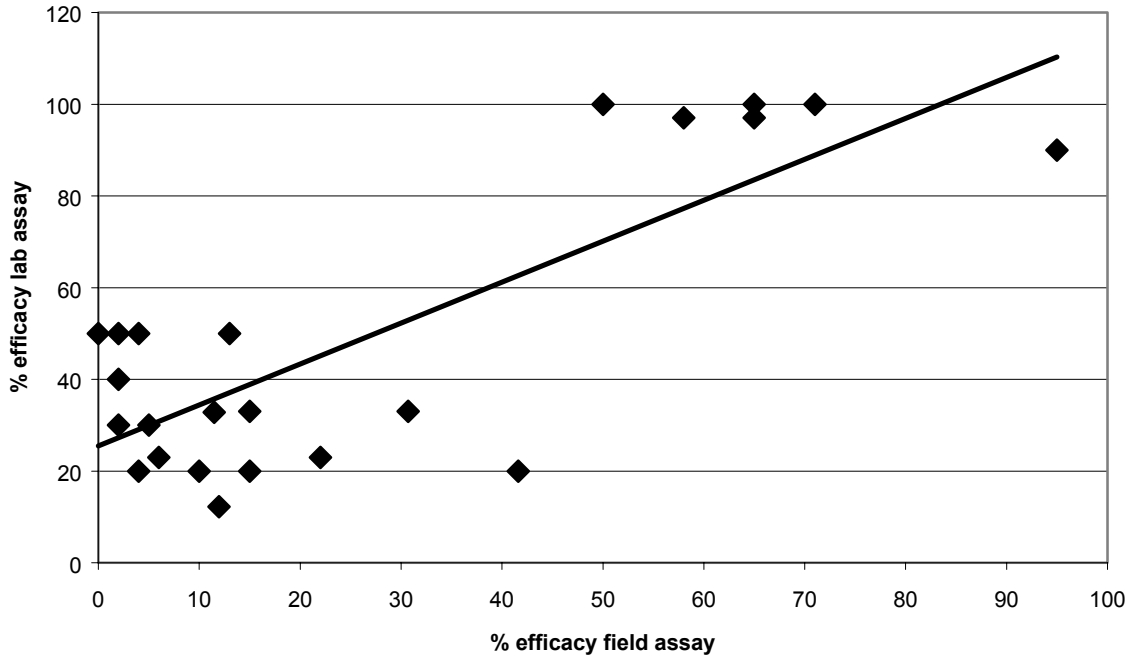

Figure 3. Correlation between field assay efficacy results using Apistan package bee strips and results of laboratory assays using a 200ppm dose of fluvalinate.

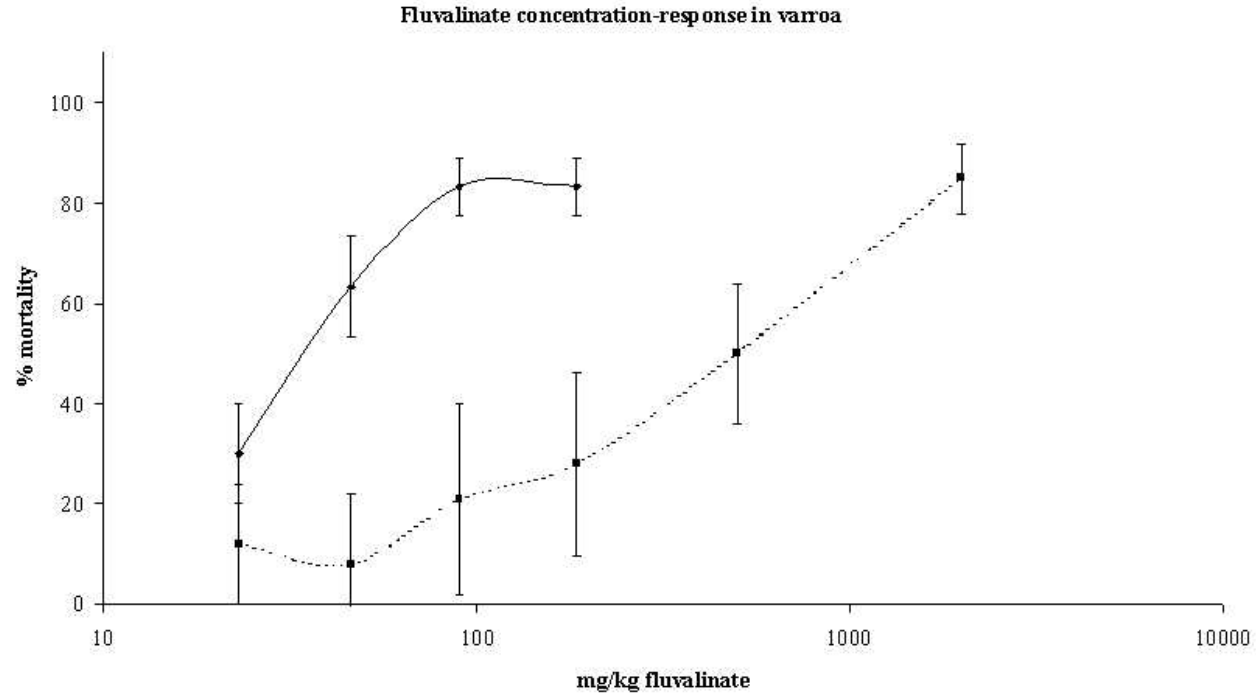

Figure 4. Mortality curve for varroa mites exposed to fluvalinate (mean percent mortality ± SD) with mites from CSL, York (solid line) and resistant apiaries in Devon (dotted line).

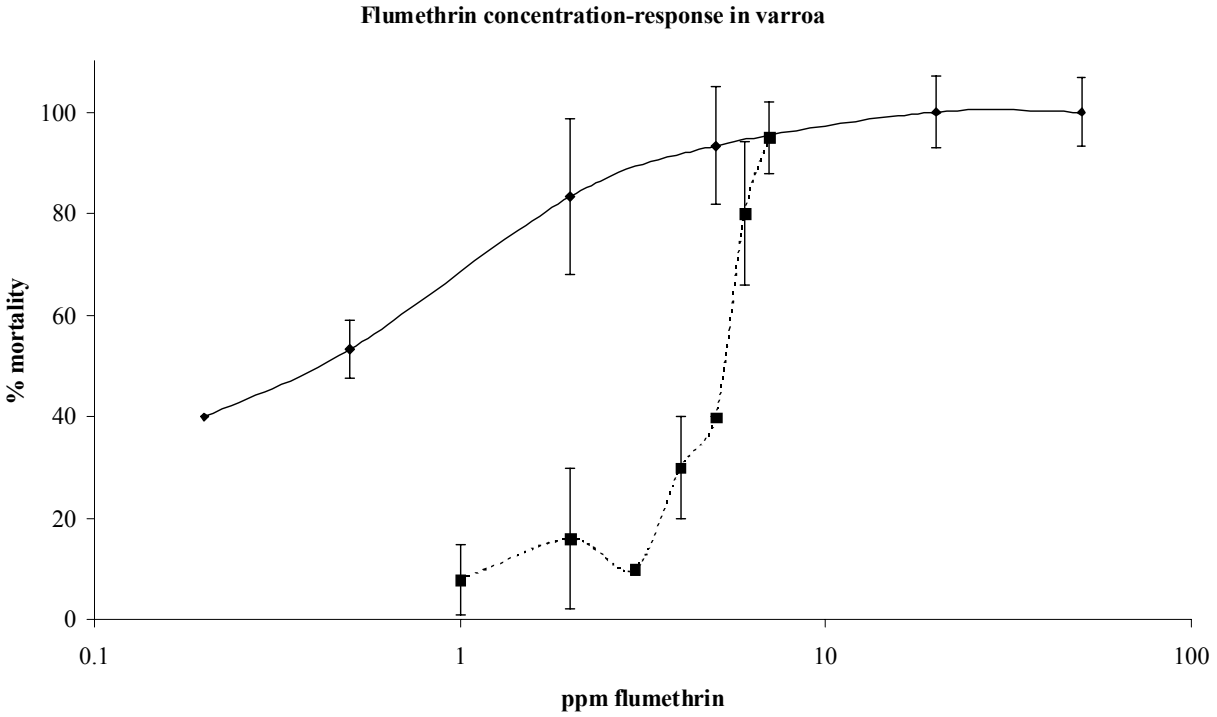

Figure 5. Mortality curve for varroa mites exposed to flumethrin (mean percent mortality ± SD) with mites from CSL, York (solid line) and resistant apiaries in Devon (dotted line).

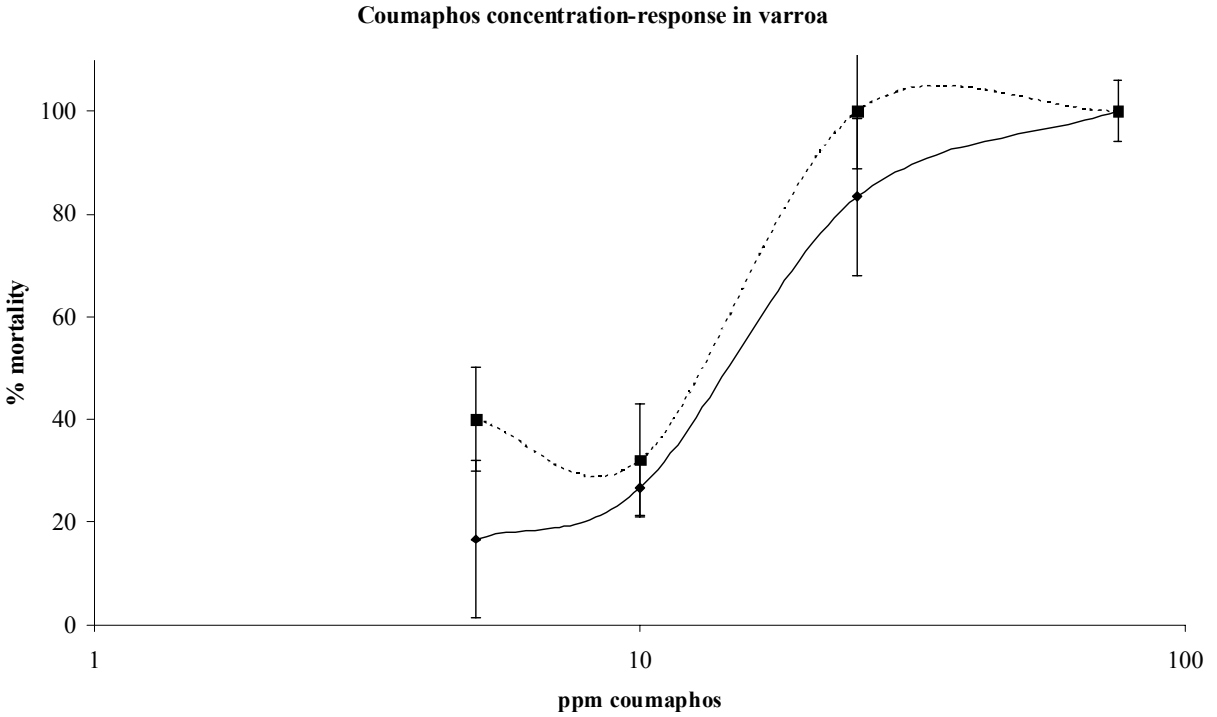

Figure 6. Mortality curve for varroa mites exposed to coumaphos (mean percent mortality ± SD) with mites from CSL, York (solid line) and resistant apiaries in Devon (dotted line).

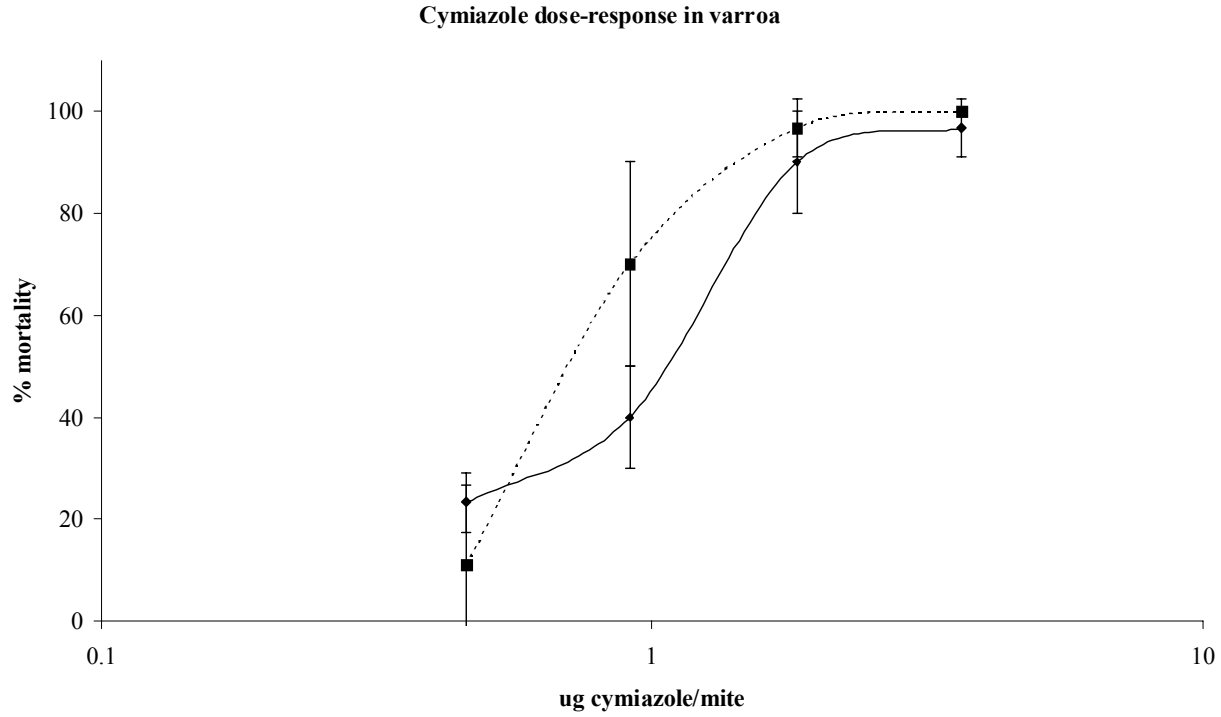

Figure 7. Mortality curve for varroa mites exposed to cymiazole (Apitol) (mean percent mortality ± SD) with mites from CSL, York (solid line) and resistant apiaries in Devon (dotted line).

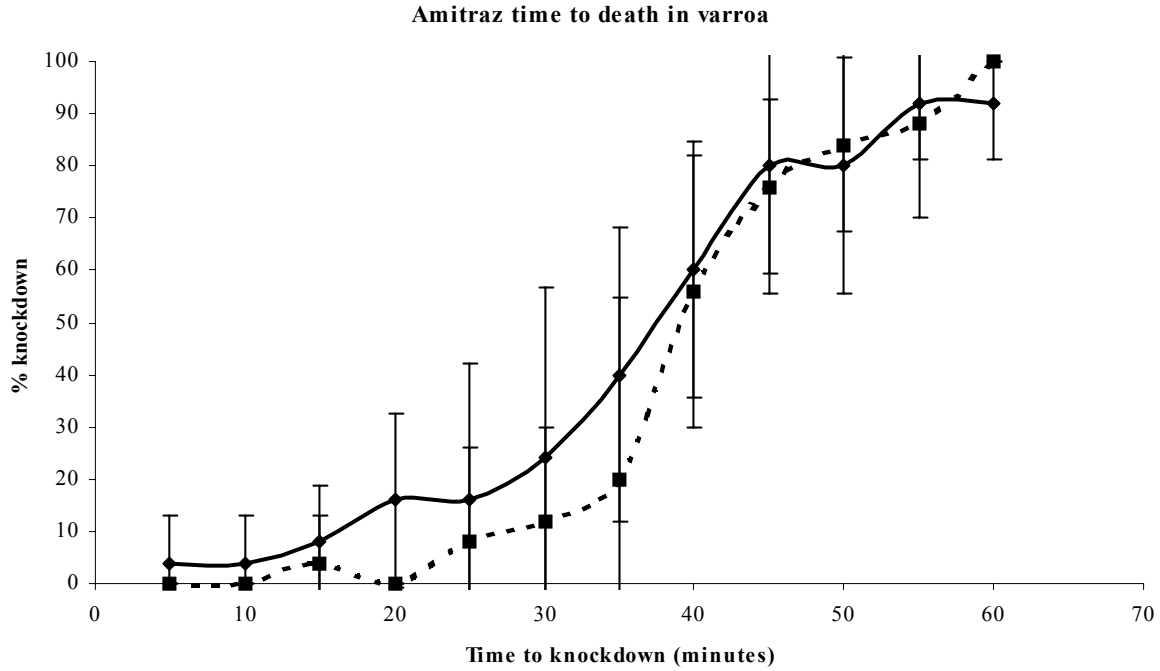

Figure 8. Time to knockdown for varroa mites exposed to amitraz (mean time to knockdown ± SD) with mites from CSL, York (solid line) and resistant apiaries in Devon (dotted line).

Acknowledgements

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