

Acaricide (pyrethroid) resistance in *Varroa destructor*

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The infamous varroa mite has become one of the most serious pests of European honey bees, causing a worldwide loss of millions of colonies. As the eradication of the mite is impossible, beekeepers have had to rely heavily on a small number of acaricides as their main line of defence against the mite. However, the appearance and rapid spread of mites resistant to some of the most commonly used acaricides is causing a new wave of problems for beekeepers.

Introduction

Pesticide resistance is the ability of a pest to survive normally lethal rates of a pesticide. The ability to develop resistance to a wide range of pesticides is a widespread phenomenon among the mites,³ so it was almost inevitable that varroa would become resistant against the commonly used acaricides such as the pyrethroids, tau-fluvalinate (Apistan®) and flumethrin (Bayvarol®). Studies found that by comparing the concentration of a compound which causes 50% mortality in the test mite population (LC_{50}), resistant mites had increased LC_{50} values of 2-fold in Israel,¹⁶ 11-fold in the UK²¹ and from 36- to 440-fold in Italy.^{13,22}

Early detection is crucial to reducing both colony losses and spread of a resistant mite strain. However, as there are no physical differences between resistant and susceptible mites, the only way to distinguish the presence of resistant mites is to perform a resistance bioassay that has been developed.¹³ Unfortunately these are rarely performed by beekeepers and normally the first signs of a problem is the unexpected collapse of treated colonies, rather than beekeepers detecting a failure of the treatment. This has greatly helped the spread of resistant mites across Europe and possibly further afield.

Spread of resistant mites in Europe

Pyrethroid resistant mites were first detected in the Lombardy region in the north-west of Italy around 1991. This region was closely connected by a well-established movement of colonies to Sicily, where similar problems may have been occurring, but precise data is lacking, although resistant mites were later detected.²² Resistant mites quickly spread via bee movement into the neighbouring regions of southern Switzerland, Slovenia and

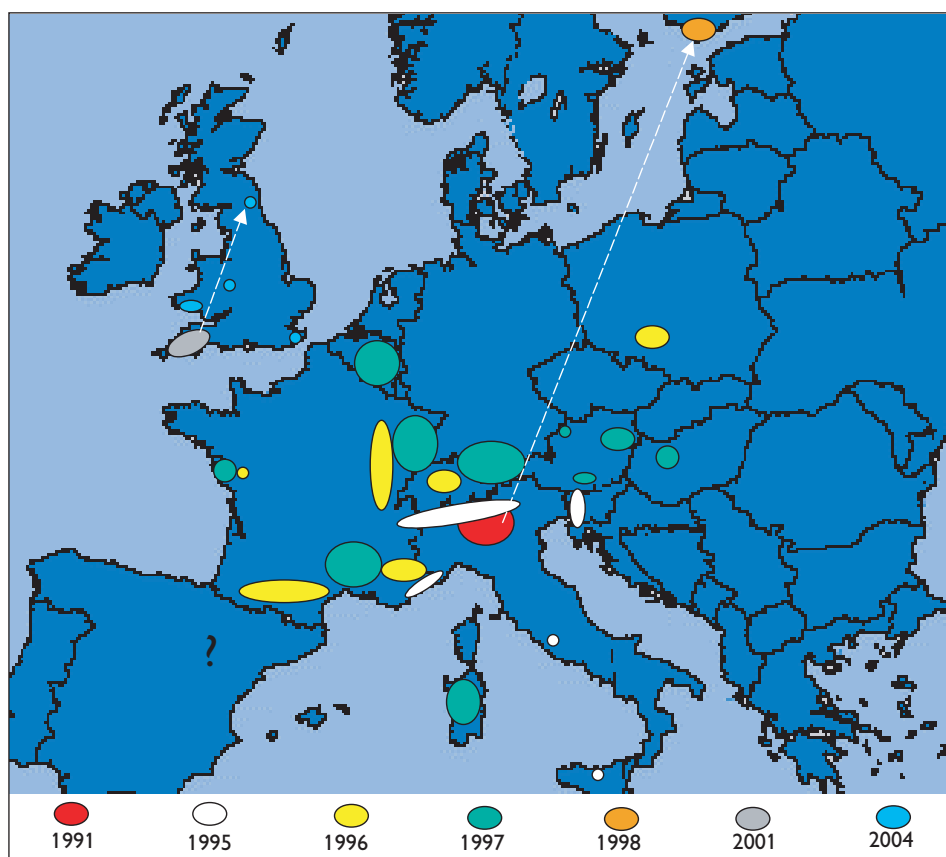


FIG. 1. The spread of pyrethroid resistant mites throughout Europe. The areas indicated are regions where resistant mites have been confirmed. However, resistant mites are much more widespread than indicated here. The arrows indicate probable movement of resistant mites.^{11,21,22}

southern France. From there it continued its spread throughout Europe following established colony trade routes in France²² finally reaching Germany in 1997, Finland via possible bee movement from Italy in 1998 and UK in August 2001 (fig. 1). In the UK the first outbreak discovered was limited to only 25 apiaries²¹ but by June 2004 it had already spread to around 140 apiaries, some of which

are many hundreds of kilometres from the original source (fig. 1). The pattern of spread of resistant mites throughout mainland Europe and UK was very similar to the pattern of spread exhibited by the 'original' varroa mites. This is a slow local spread by flying bees moving mites between colonies, with irregular long-distance jumps caused by beekeepers moving infested colonies.

Although during the original spread of the mites (non-resistant) they appeared in many areas that could not be clearly linked to the movement of bees. However, since mites can only move attached to bees, the movement of bees is the only possibly explanation to explain the pattern of spread, since mites cannot spontaneously appear in a new area. A similar dispersal pattern of resistant mites to non-resistant mites suggests that the main underlying reason (bee movement) is similar. However, in cases where no obvious bee movement is known, it is often assumed that resistance has arisen independently,²¹ normally without any other supporting evidence.

Spread of resistant mites outside Europe

In 1997 pyrethroid resistant mites were detected in the USA. Although the first reports of resistance were from South Dakota,¹ it was quickly established that this was linked to bees moved from Florida where tests confirmed that pyrethroid resistant mites were also present.⁶ Incidentally, Florida was also the place where the original varroa mites entered the USA in 1987.¹⁹ Florida is a major bee breeding and distribution centre with bee packages sent to all over the USA. This has aided the long-distance spread of both the original varroa and now their pyrethroid resistant sisters. In only seven years resistant mites have spread nationwide in the USA with almost all states reporting problems due to resistant mites (P Elzen, personal communication). How varroa was originally introduced into Florida remains an enigma, but it remains a possibility that the pyrethroid resistant mites have used the same or a similar route. Outside the USA and Europe pyrethroid resistant mites have also been reported in Israel¹⁶ and Argentina.⁷

How many times has resistance appeared?

Did resistance appear once and spread or has resistance appeared independently several times? The definitive answer will depend on the determination of the precise resistance mechanisms and the genes involved. Laboratory bioassays found that the resistance mechanisms of some mites share some similarity, indicating a possible identical determinism,¹⁰ but further work is needed. Although some accidental movements of resistant mites are documented, in most cases beekeepers are naturally cautious about revealing their colony movements.

The initial spread of varroa demonstrated the ineffectiveness of international quarantine laws¹⁷ and revealed the extent that movement of honey bees, both locally and globally played in this process. The spread of pyrethroid resistant mites has followed a similar pattern,

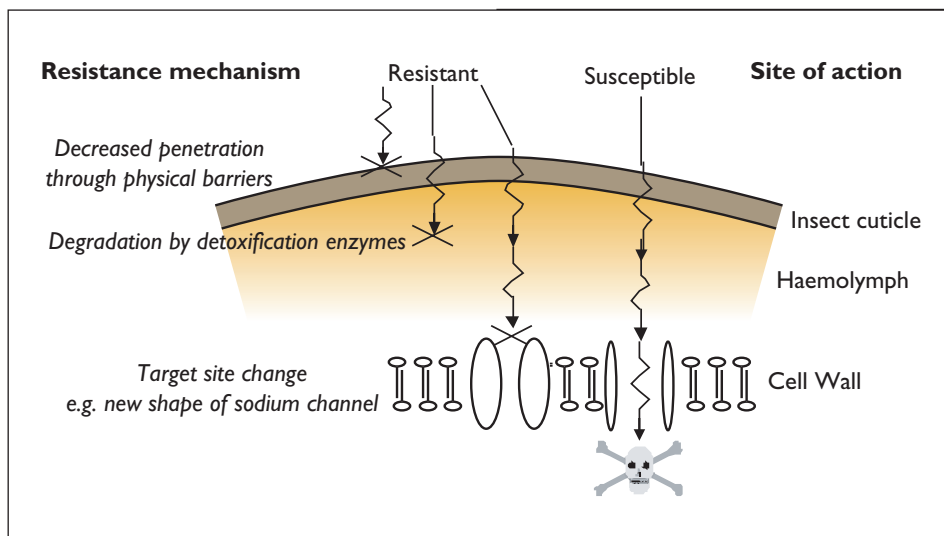


FIG. 2. The three major resistance mechanisms against pyrethroids and their general site of action.

i.e. following well-established colony movement routes, which suggests that resistance did in fact arise only once, probably in Italy, and then spread throughout Europe²² and possibly even further a field. Resistance in Italy arose after only four years of pyrethroid use,¹⁴ although Klartan/Mavrik may have been used for many more years than this, whilst in the USA, most of Europe and the UK, pyrethroids have remained effective for over 10 years. In most arthropod populations resistance does not normally arise independently in many different geographic regions, but appears in one area and then the resistant strain spreads elsewhere because of its high selective advantage.¹⁴ Furthermore, the similar shape of the dose response curve between resistant mite populations from different areas does not support the idea of many independent origins.²² Cross-resistance between tau-fluvalinate, flumethrin and acrinathrin was expected due to the extremely close chemical similarity between these compounds.

Resistance to non-pyrethroid acaricides

Resistance to acaricides such as organophosphorous coumaphos (Perizin[®]) has been reported in Italy²⁰ and Switzerland. Resistance to amitraz has been reported in Croatia,⁵ is suspected in France, and now is confirmed in the USA (P Elzen, personal communication). No resistance to amitraz, cymiazole or coumaphos has so far been detected in the UK mite population.²¹

Pyrethroid resistance mechanisms in varroa

Pyrethroids are manufactured chemicals that are very similar in structure to the pyrethrins, which occur naturally in some

chrysanthemum flowers. Pyrethrins were recognized in 1800 to have insecticidal properties but break down quickly in the environment, especially when exposed to sunlight. Pyrethroids are toxic to insects, as well as to mammals, and last longer in the environment. Pyrethroids, like DDT, kill the individual by prolonging the opening of the cells' sodium channels, which leads to paralysis and death.

The three main non-behavioural mechanisms by which animals have become resistant to a wide range of pesticides (fig. 2) are to modify the dose by reducing its penetration, improving its detoxification by enzymes (metabolic resistance), or by changing the shape the target site itself, i.e. the sodium channel, so it becomes less sensitive to the pesticide.²⁵

Currently there are no studies that have investigated if mite-resistant populations are able to reduce or prevent the penetration of pyrethroids by changes to physical properties of the cuticle. In Europe and Israel varroa resistance may be partly explained by metabolic resistance as there is an increased detoxification (break down) of pyrethroids by enzymes, in particular monooxygenases, and possible by a small (non-significant) increase in esterase activity.^{10,16} However, data from the USA indicated that these metabolic pathways were not involved in their mite resistant population.²

In the USA pyrethroid (fluvalinate) resistance in varroa was associated with four new point mutations in the sodium channel gene.²³ It is already known that similar point mutations in a sodium channel gene, which by changing the shape of the proteins in the cell wall associated with the transport of sodium ions,

reduce the cells' sensitivity to pyrethroids.^{23,24} Ongoing research²⁴ is attempting to determine if any of these resistance-associated mutations are indeed involved in tau-fluvalinate resistance in varroa. These sodium channel gene mutations found in the USA mite population need to be investigated in the European mite resistant population. This is because if different resistance mechanisms were shown in the European and USA mite populations this would be the best evidence for the appearance of two independent strains of the resistant mites.

Cost of resistance

Often, resistance in populations of arthropods can be costly, e.g. by the over-expression of certain detoxification enzymes. This, in the absence of the pesticide, leads to reduced fitness of resistant individuals due to lower reproductive ability or less efficient metabolism than their susceptible counterparts.^{4,9} For example, some resistant strains of mosquitoes have only one quarter of the reproductive potential of susceptible strains in the absence of an insecticide,⁸ although this probably represents an extreme case. Conversely the disadvantage associated with resistance can be small¹⁸ and in some cases zero.

It was suggested that pyrethroid resistant varroa do have a lower fitness compared to susceptible mites, but no data were presented.²⁵ However, a detailed study of the reproductive ability of resistant and susceptible mite populations in the USA revealed no differences between the two populations.¹² In Italy no disadvantage or only a small consistent one (reduction in fitness) associated with fluvalinate resistance was found in varroa, which is consistent with observations on insects when resistance is due to monooxygenases.¹⁵ Furthermore, if the resistance mechanism is due to point mutations²³ the metabolic load on resistant mites may again be very small and so differences in mite reproduction would not be expected.

Conclusion

All the current evidence (i.e. we do not have any evidence to the contrary) suggests that mite resistance to pyrethroids has arisen only once or twice and that the major cause for the rapid spread of resistant mites is the movement of bee colonies by beekeepers. While positive selection only takes place as a consequence of the use of an acaricide (removing non-resistant mites from the population so allowing the resistant mites to thrive), the mutations that give rise to the resistance occur independently from

exposure, as they are chance events. There is no evidence that the misuse of treatments selects more quickly for resistant mites than correct treatments. Therefore, the occurrence of resistant mites is not associated with the misuse of treatments as has often been claimed, since the same end result will occur irrespective if the treatment has been used correctly or not.

The appearance of coumaphos resistant mites is a worrying trend, and it needs to be established quickly if a similar mechanism to that used by pyrethroid resistant mites is been employed. Molecular biology will help us understand the resistance mechanisms and the costs associated with resistance, which in turn will help in the development of the most effective control strategy. For example, if the resistance mechanisms employed by varroa do in fact have no or a small cost then rotational use of different acaricides, i.e. using a pyrethroid one year then coumaphos in the next year followed by a pyrethroid in the next and so on, will be ineffective.

References

- BAXTER, J; EISCHEN, F A; PETTIS, J S; WILSON, W T; SHIMANUKI, H (1998) Detection of fluvalinate-resistant *Varroa* mites in US honey bees. *American Bee Journal* 138: 291.
- BELL, J R; GLOOR, S; CAMAZINE, S M (1999) Biochemical mechanisms of fluvalinate resistance in *Varroa jacobsoni* mites. *American Bee Journal* 139: 308–309.
- CROFT, B A; BAAN, H E VAN DE (1988) Ecological and genetic factors influencing evolution of pesticide resistance in tetranychid and phytoseiid mites. *Experimental and Applied Acarology* 4: 277–300.
- DENHOLM, I; ROWLAND, M W (1992) Tactics for managing pesticide resistance in arthropods: theory and practice. *Annual Review of Entomology* 37: 91–112.
- DUJIN, T; JOVANOVIĆ, V; SUVAKOV, D; MILKOVIĆ, Z (1991) [Effects of extended use of amitraz-based products on the formation of resistant strains of *Varroa jacobsoni*.] *Veterinarski Glasnik* 45: 851–855 (in Croatian).
- ELZEN, P J; EISCHEN, F A; BAXTER, J R; ELZEN, G W; WILSON, W T (1999) Detection of resistance in US *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate. *Apidologie* 30: 13–17.
- FERNANDEZ, N; OMAR, G (1997) [New indications of decrease in the efficiency of the active ingredient fluvalinate.] *Boletín del Colmenar* 4: 10–11 (in Spanish).
- GEORGHIOU, G P; TAYLOR, C E (1977) Genetic and biological influences in the evolution of insecticide resistance. *Journal of Economical Entomology* 70: 319–323.
- GEORGHIOU, G P; TAYLOR, C E (1986) Factors influencing the evolution of resistance. In Committee on Strategies for the Management of Pesticide Resistant Pest Populations, National Research Council (eds) *Pesticide resistance: strategies and tactics for management*. National Academy Press; Washington, DC, USA; pp. 157–169.
- HILLESHEIME, E; RITTER, W; BASSAND, D (1996) First data on resistance mechanisms of *Varroa jacobsoni* (Oud.) against tau-fluvalinate. *Experimental and Applied Acarology* 20: 283–296.
- LONDZIN, W; SLEDZINSKY, B (1996) Resistance of honey bee parasitic mite *Varroa jacobsoni* to varroacide preparations containing tau-fluvalinate. *Medicina Veterinaria* 52: 526–528.
- MARTIN, S J; ELZEN, P J; RUBINK, W R (2002) Effect of acaricide resistance on reproductive ability of the honey bee mite *Varroa destructor*. *Experimental and Applied Acarology* 27: 195–207.
- MILANI, N (1995) The resistance of *Varroa jacobsoni* Oud. to pyrethroids: a laboratory assay. *Apidologie* 26: 415–429.
- MILANI, N (2001) Management of the resistance of *Varroa* mites to acaricides. In Webster, T C; Caron, D (eds) *Mites of the honey bee*. Dadant & Sons Inc; Hamilton, IL, USA; pp. 241–250.
- MILANI, N; DELLA VEDOVA, G (2002) Decline in the proportion of mites resistant to fluvalinate in a population of *Varroa destructor* not treated with pyrethroids. *Apidologie* 33: 417–422.
- MOZES-KOCH, R; SLABEZKI, Y; EFRAT, H; KALEV, H; KAMER, Y; YAKOBSON, B A; DAG, A (2000) First detection in Israel of fluvalinate resistance in the *Varroa* mite using bioassay and biochemical methods. *Experimental and Applied Acarology* 24: 35–43.
- OLDROYD, B P (1999) Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends and Research in Evolutionary Ecology* 14: 312–315.
- ROUSH, R T; DALY, J C (1990) The role of population genetics in resistance research and management. In Roush, R T; Tabashnik, B E (eds) *Pesticide resistance in Arthropods*. Chapman & Hall; New York, USA; pp. 97–152.
- SANFORD, M T (2001) Introduction, spread and economic impact of *Varroa* mites in North America. In Webster, T C; Caron, D (eds) *Mites of the honey bee*. Dadant & Sons Inc; Hamilton, IL, USA; pp. 149–162.
- SPREAFICO, M; EORDEGH, F R; BERNARDINELLI, 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.
- THOMPSON, H M; BROWN, M A; BALL, R F; BEV, M H (2002) First report of *Varroa destructor* resistance to pyrethroids in the UK. *Apidologie* 33: 357–366.
- WANG, R; LIU, Z; DONG, K E; ELZEN, P J; PETTIS, J; HUANG, Z Y (2002) Association of novel mutations in a sodium channel gene with fluvalinate resistance in the mite, *Varroa destructor*. *Journal of Apicultural Research* 40(1–2): 17–25.
- WANG, R; HUANG, Z Y; DONG, K E (2003) Molecular characterization of an arachnid sodium channel gene from the varroa mite (*Varroa destructor*). *Insect Biochemistry and Molecular Biology* 33: 733–739.
- WATKINS, M (1996) Resistance and its relevance to beekeeping. *Bee World* 77: 15–22.

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