CHEMICAL CONTROL OF VARROA

Dr Max Watkins, Vita (Europe) Limited, Basingstoke, UK

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Abstract

The application of foreign substances to honeybee colonies may seem to some, unnecessary and incongruous with the perceived ethos of beekeeping. However, the relationship between the honeybee and its major parasite, *Varroa destructor*, is unusual, in terms of husbandry, and is a particularly dangerous one. To ensure the well-being of *Varroa*-infested honeybees, the beekeeper needs to understand the biology of the interaction and to be aware of the variety of tools available to assist his infected animals, including amongst other things, chemical control measures. The limitations and benefits of the use of the available chemical agents for the control of *Varroa* mites are discussed.

Background

Since humans started keeping animals, growing crops and clearing space for housing we have encountered animal or plant species which are either in the way or are bothersome, detrimental to our food sources or to our immediate environment. Attempts at controlling these pest animals or plants were probably not particularly effective and it was not until the 18th century that chemical methods of providing protection to humans and farmed plants and animals gained any importance. These methods were based initially on chance discoveries and trial-and-error. These “first generation” preparations were largely plant extracts, sulphur and heavy metal salts.

The “second generation” chemical methods, dating from the Second World War, consisted of synthetic organic and inorganic compounds. DDT for example, has saved millions of human lives and although it is no longer environmentally acceptable, the beneficial impact on our society today of compounds such as this should not be forgotten. The second generation products are often quite broad-spectrum in activity and have gradually been replaced by the “third generation” control agents which are more species-specific in medicinal or pesticidal activity - such as sophisticated nerve agents, hormone treatments, growth regulators, or the use of specific bacteria or viruses.

In pharmaceutical or agrochemical companies today, molecules synthesised by chemists are examined for biological activity in a well-defined sequence of tests. After each test stage, most of the candidates are rejected and only 1% - 5% are promoted to the next test stage. This elimination/promotion procedure is called “screening” and in order to select 1 single product for commercialisation around 20,000 molecules or more have to be tested. The whole process, of deriving one or two useful substances as control agents can take around 8 years. A cost of £100 million is probably conservative.

Development of a product based on this control agent may take a further two to three years, as substantial data on the product toxicology, efficacy, residues and manufacturing need to be generated before submitting the dossier for product registration. In Europe, honeybees are considered as livestock (food-producing animals) and any treatment administered must be registered as a veterinary medicine. After deriving a Maximum Residue Limit for the control agent (termed Active Pharmaceutical Ingredient or API) a product registration usually takes at least a further two years.

So, a new veterinary medicine, such as is used for control of *Varroa* mites on honeybees may take about 12 years from conception to availability.

Registration of Varroa treatments

The purpose of product registration is to ensure:

- the product safety - for the consumer (of honey and hive products), for the treated animal, (bees); for the user (beekeeper) and for the environment.
- that the product is manufactured to a high quality standard, does not deteriorate and has stability to last at least until the expiry date.
- that the efficacy of the product conforms with its label claims

These product development and regulatory stages have to be planned and overcome in order to have a viable, effective and legal product available for use.

It must be said that no molecule has ever been developed specifically with the control of *Varroa* mites in mind. Apiculture is miniscule, in relation to agriculture or other veterinary medicine sectors. Products for bees that have come to market over the past 30 years or so, have their origins as crop protectants or as treatments for more "significant" animal health targets, such as cattle or sheep. The molecules or products have been adapted for use in beekeeping only after success in these other sectors.

Rationale for controlling Varroa infestations in honeybees.

The relationship of the *Varroa* mite with the honeybee is particularly dangerous. Animals are often parasitized by other animal forms, such as tapeworms in cattle or ticks on dogs but unusually, the *Varroa* mite parasite is closely related to its host, the honeybee. The mite and the bee are both arthropods, sharing the same environment and similar biology; the reproduction of the mite is essentially synchronised with that of the bee. As the mites are secreted in the capped brood cells, feeding off the pupae and reproducing, they cannot be harmed or removed by the adult bees, nor can they be controlled by chemical treatment (with the exception perhaps of formic acid).

Besides the physical and biochemical stress of a mite attack, the mite is known to actively suppress the immune response of the bee. (ref 10; 37).*Varroa* attack acts as a releaser for other viruses already present in the honeybee colony (ref 2; 7; 8;) and the combination of *Varroa* and heavy virus load within a colony is often fatal.

The parasite is highly mobile within the hive. They can run very quickly indeed and readily jump onto their hosts. Once attached to adult bees, as phoretic mites they become highly inter-colony mobile, being carried to new colonies during robbing or drifting by the bees. It is not unusual for a previously non-infested colony under a robbing attack to receive between 100 and 300 mites per day (ref 16).

Unlike most other host-parasite relationships, the *Varroa* mite kills its host, which is why the beekeeper has to intervene to help control *Varroa* infestations.

Risks & Benefits of chemical control

Safety margin
Honeybees are especially sensitive to chemical agents yet we need to control an arthropod pest living on the bee, within the beehive without affecting the bee itself.

Any treatment has to target the mite and have as large a safety margin as possible between affecting mites and affecting bees. It also has to be of low mammalian toxicity as we are introducing it to a food crop for humans (honey).

Residues
Chemical control substances, synthetic or natural, are either more lipophilic (attracted to fats or wax) or are more hydrophilic (attracted to water or in this case, honey). Whatever is introduced to the hive,

it is inevitable that an element of it will pass either into the wax or into the honey. More volatile substances, such as essential oils, usually dissipate through sublimation or evaporation over a short time. Some substances remain as the stable active ingredient, whilst other less stable actives break down into smaller molecules.

Residue levels of substances as a result of the use of authorised (registered) treatments do not usually have toxicological significance to honeybees or to humans. The rigorous tests which the molecules and products have been screened through prior to registration ensure that the toxicological profile of expected residue levels is accounted for.

However, medicines registered for use in beehives are often based on active ingredients already used in other veterinary or crop treatments. Improper and illegal use of crop or other animal treatments as hive treatment substances usually results in high residues accumulating in hive products.

Prolonged use of any treatment in honeybee colonies may result in some residue left behind but the risk is especially accentuated from the use of unauthorised products and may have sub-lethal effects on the adult bees, bee larvae or on the mites. Accidental pollutants such as drift from crop treatments or otherwise picked up by the bees whilst foraging may also affect the colony (ref 3; 9; 19; 21; 22; 27; 29).

With non-registered products, the dosage regimes and the kinetics of the release rate to the target are uncontrolled (ref 23), and such "artisanal" applications, although widespread in apiculture, lead to overdosing, residues in hive products (ref 19) and more speedy induction of resistance in the *Varroa* mites. (ref 11)

Benefits of chemical control

There are clearly disadvantages to using "chemicals" per se, as *Varroa* control agents but using properly-researched and developed formulations registered for use in beehives reduces much of the risk. The main advantage is that *Varroa* populations can be controlled through the use of such agents. Absolute elimination of mites with any one type of treatment is a false hope but effective products, especially when employed within an Integrated Pest Management system, can help reduce mite levels to below the threshold for economic and long-term damage to the colony.

In reducing the *Varroa* population, the beekeeper removes a major stress factor suppressing the immune system of individual bees and thereafter of the colony as a whole (ref 37; 38).

The spread and eruption of some of the many viruses affecting honeybees is known to be triggered by *Varroa* (refs 2; 20; 24). Transmission of bee-pathogenic viruses throughout and between colonies may be considered a covert stress to the bees and is a major contributor to the mortality of colonies. Virus infection, when combined with *Varroa* infestation can be a deadly combination (ref 2; 7; 8; 24; 39). Viruses may be transferred to bees or enter the honeybee colony from a variety of sources, including pollen, other pollinators (ref 25) or even through semen and eggs (ref 4).

By using a reliable chemical control measure to remove a high percentage of a *Varroa* population from a hive the expression of virus loads and that of other pathogens can be much reduced; the colony survives, continues with its essential role of pollination, produces more honey and continues for another season.

Varroa Treatments in Europe

At a meeting at the European Medicines Agency in London in December 2009, the *Varroa* treatments available in Europe were discussed (ref 5; 6). There are surprisingly few, as shown in table 1. Information about the various products and their mode of use is readily available by internet searches.

Product Name	Product form	Mode of Action	API
Apistan	Strip	Contact	tau-fluvalinate
Apiguard	Gel	Contact + Vapour	thymol
Apilife-Var	Briquette	Vapour	thymol + other oils
Apivar	Strip	Contact	amitraz
Bayvarol	Strip	Contact	flumethrin
Checkmite+	Strip	Contact	coumaphos
Ecostop	Pad	Vapour	thymol
Gabon	Strip	Contact	acrinathrin
Perizin	Liquid	Contact/systemic	coumaphos
Thymovar	Pad	Vapour	thymol
Varostop	Strip	Contact	flumethrin
Formic acid	Liquid	Vapour	Formic acid

Table 1. Anti-Varroa medicines registered in different European countries

A small percentage of honeybee colonies are left untreated every season. However, because of the danger posed by mite infestation, many beekeepers choose to treat but not to use a registered product. A great many honeybee colonies receive home-made concoctions based on agricultural, veterinary or other pesticidal preparations containing one or more of the following:

Control molecule	Control agent class
Amitraz,cymiazole, chlordimeform	Amidenes
Chlorfenvinphos, coumaphos, malathion	Organophosphates
Acrinathrin, flumethrin, tau-fluvalinate	Cyano-pyrethroids
Bromopropylate	Carbinol

Table 2. Unauthorised varroa control compounds.

Other more potentially hazardous treatments include chlorpyriphos, cypermethrin, rotenone and sulphur.

Essential oils and organic acids are used in a variety of methods and dosages. “Plant extracts”, chalk dust, sugar and vegetable oils have a limited effect but may also be used.

The most commonly used *Varroa* treatments are based on amidines, organophosphates, pyrethroids, or carbinols and all affect the nervous system of the target in one way or another (Fig 1).

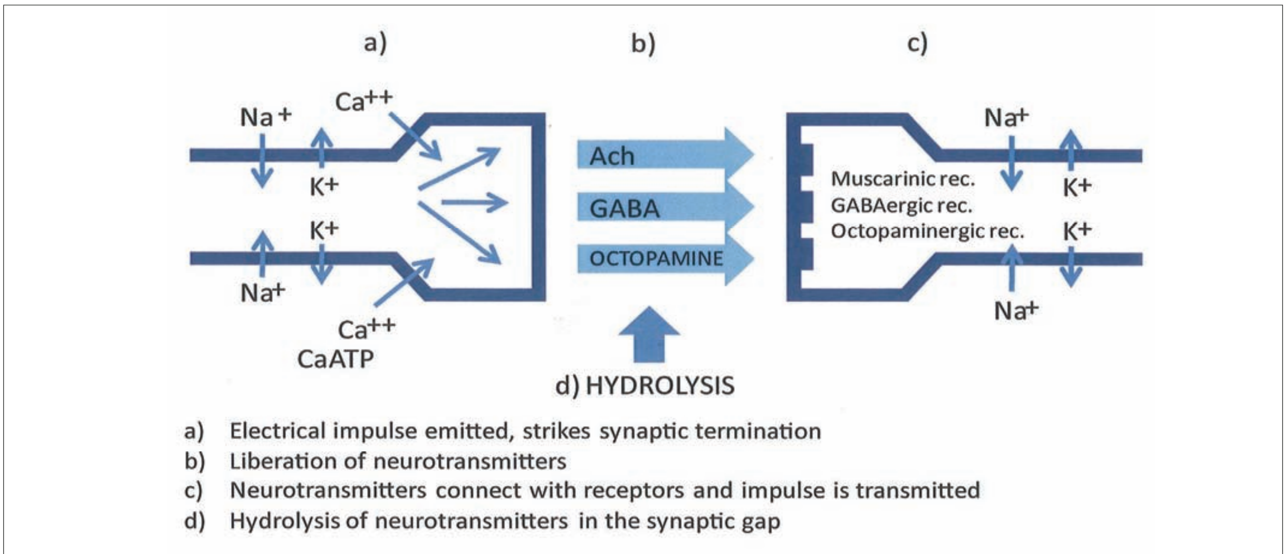

Fig. 1. Nervous system function in mites.

Nervous system function depends on ion flow into and out of nerve cells, liberation, transmission and degradation of neurotransmitters across nerve synapses. The classes of substance mentioned above all affect one or more of the processes in nervous conduction, each class of compound targeting a different site of disruption. As the mode of action of the different control agents is so specific, they can be highly effective. However, if even a small proportion of mites are able to avoid or to detoxify such a substance which acts on a specific site within the nervous system, resistant strains can develop (Fig 2).

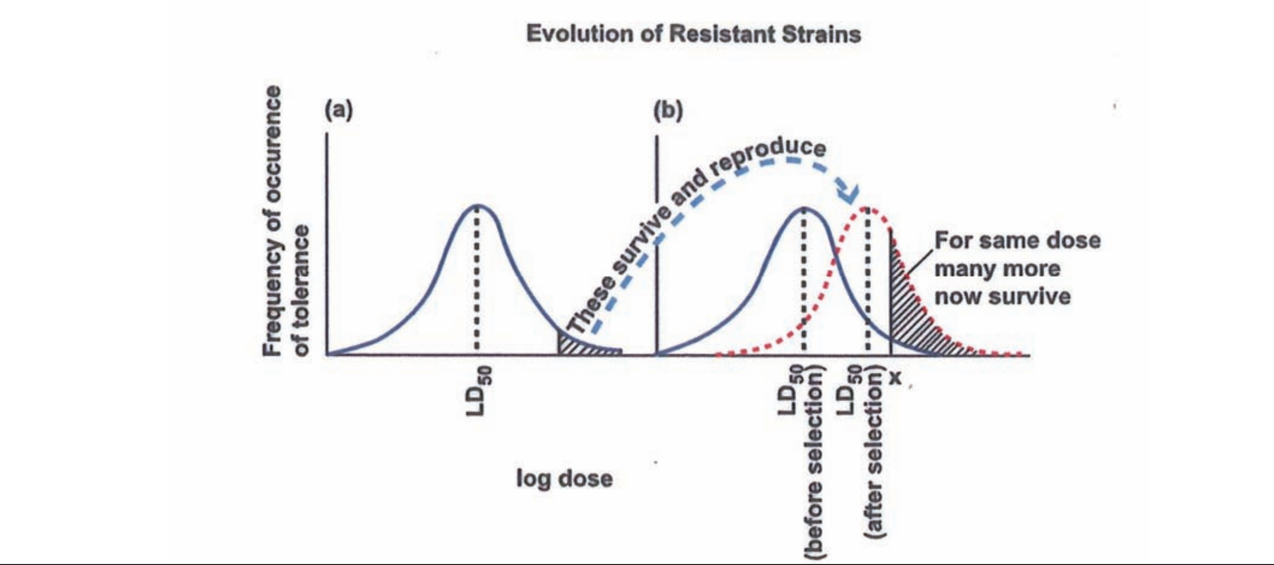

Fig. 2. Bell curve showing evolution of resistant strains. (After Hassell, K. A. 1966, Scient. Hort. 18.103-115).

Varroa resistance

Apparent lower efficacy of a treatment is often attributed too quickly to resistance. *Varroa* mites are highly mobile. A honeybee colony successfully treated may suddenly suffer huge re-infestation pressure (ref 16), depending partly on treatment (or not) of neighbouring apiaries. This can result in high mite loads appearing in the newly-treated hive – which the beekeeper may mistakenly attribute to low treatment efficacy. Field efficacy tests alone cannot demonstrate with certainty, the presence or absence of resistance. A sensitive laboratory bioassay must be used in conjunction and the two sets of results compared and related.

Since the 1990's, *Varroa* treatments based on cyano-pyrethroids, amidenes or organophosphates have been used extensively, for the major part in home-made preparations. The massive selection pressure exerted on the mites eventually lead to the emergence of strains of *Varroa* mite resistant to these classes of compound (refs 13; 15; 17; 26).

Bioassay & Resistance Monitoring

In the early 90's pyrethroid efficacy against *Varroa* seemed to be less regular than expected. The bioassay used in Germany at that time to determine *Varroa* susceptibility to control agents, showed no resistance when testing mites taken from populations that had survived hive treatments. The resistance bioassay was proved to be not sensitive enough so a new method was developed at the University of Udine, Italy (Fig 3).

Once the resistance bioassay was established, laboratories across Europe were equipped with the materials and training. A substantial campaign of resistance monitoring clarified the spread of the resistance (refs 1; 12; 14; 26; 28; 30; 31; 32; 33; 34; 35; 36) and alternative treatments and practices could be advised.

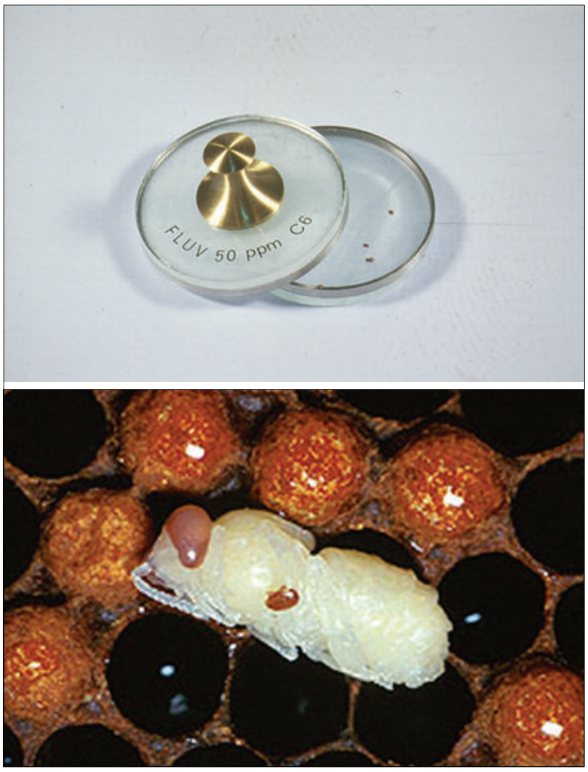

Fig. 3. Development of bioassay at Udine.

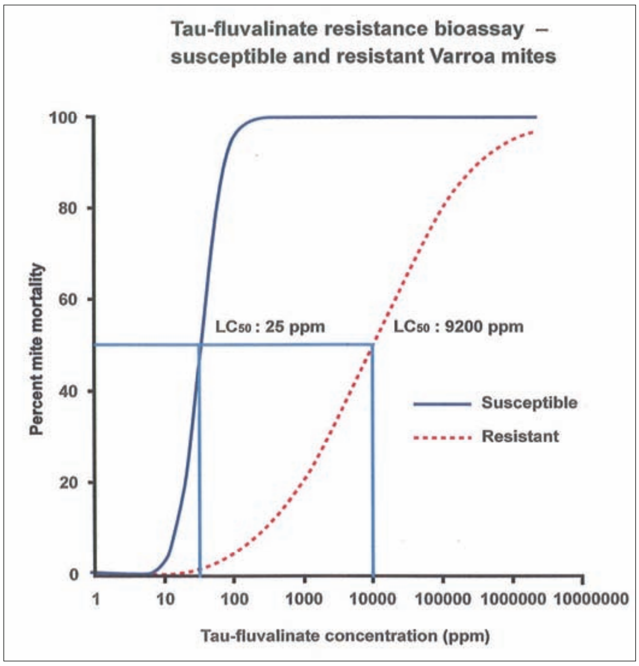

Fig. 4. Resistance in varroa mites to pyrethroids. The LC_{50} of [normal] sensitive mites is 25 mg/kg and for the resistant strain it is around 9000 mg/kg.

The pattern of resistance of *Varroa* to pyrethroids such as tau-fluvalinate was always the same in Italy (Figure 4), whatever region the samples were taken from (ref 17). Indeed, through monitoring in different countries, the exact same pattern was seen, which suggests that the resistance mechanism arose once, and then spread. Paths of probable resistant mite diffusion can be readily traced along migratory beekeeping routes and the mobility of the bees and phoretic mites ensured that the resistance moved rapidly.

Positive correlation between results from a resistance field test kit and the laboratory bioassay at Udine meant that a reliable estimate could be made of [pyrethroid] product efficacy in the hive (Fig 5), (ref 35).

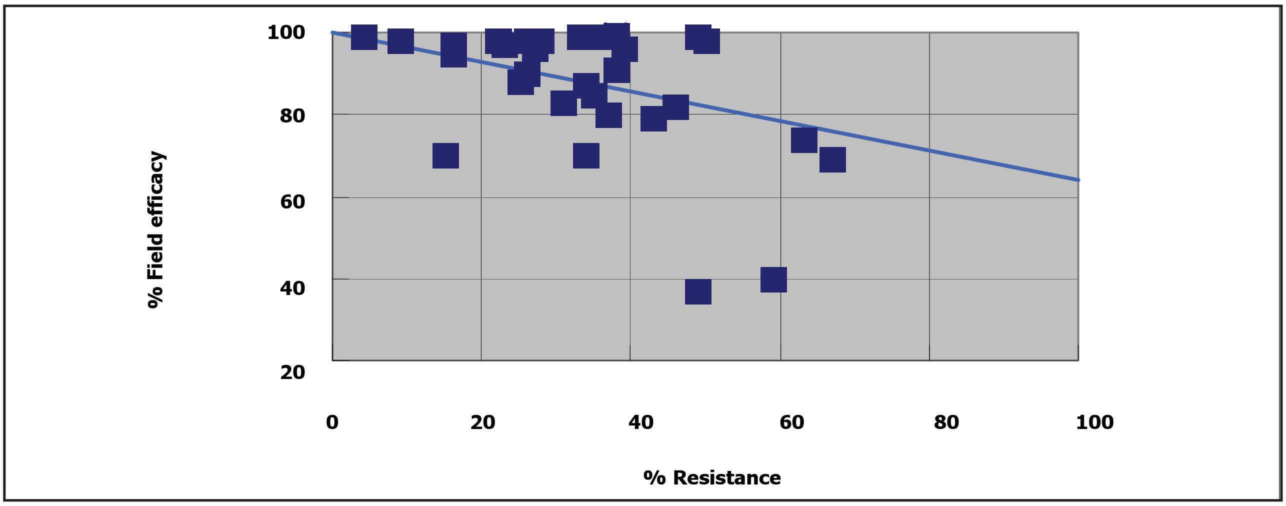

Fig. 5. Correlation of field efficacy with laboratory resistance bioassay.

Fig 5 - The relationship between field treatment efficacy and the data obtained in the laboratory assay showed that the test was able to detect resistance before any lack of efficacy could be observed in the field. According to the regression curve, an efficacy of 60% in the laboratory corresponds to 85% efficacy in the field.

The ability to accurately detect resistance can help prevent colony losses. Where mites have become resistant to one type of treatment, a different method of control must be chosen. The selection pressure on the mites can be removed by not repeating treatments with the same class of compound for years on end. Treatment alternation and Integrated Pest Management strategies should be followed.

Resistance reversion

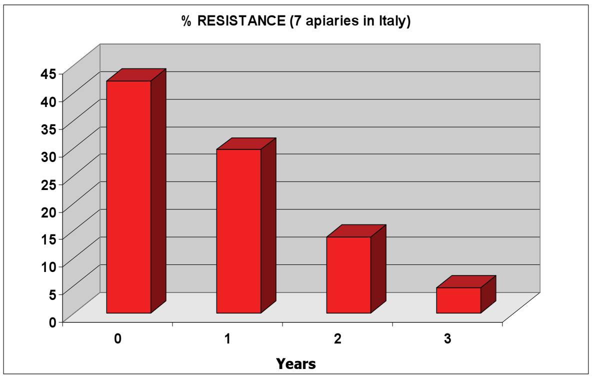

Fig. 6. Pyrethroid resistance reversion in varroa.

In the absence of pyrethroid treatments, the proportion of resistant mites within a population declines naturally. Honeybee colonies containing resistant mites were kept in Northern Italy, in an area free from pyrethroid treatment and also in commercial colonies. As can be seen from Figure 6 (ref 18) the mite population became more susceptible even within a year and very susceptible within 3 years. At the start of the experiment, the resistance level was 50%, and two years later, it was 8%, corresponding to a field efficacy value of 96%.

In this study the percentage of resistant mites decreased by a factor of two from year to year, which indicates that being resistant bears some cost to the mite. With the selection pressure removed, it is more advantageous for the mite to revert to the susceptible form. Reproductive success is often more restricted in resistant strains.

Dearth of Varroa treatments

There is currently little variety in the legal treatments available for the control of *Varroa* mites. This is partly due to the high cost and time required for development and the difficulty of designing or discovering a suitable molecule and formulation. The regulatory position has to be considered along with the cost of the final product to the beekeeper. A product may work very well but if it were too expensive to manufacture, it would not be viable for the beekeeper. On registration in some countries, these products are classified under Veterinary or Pharmacy prescription only. This adds to the cost and restricts the availability to the beekeeper.

Due to the small size of the beekeeping sector and its complexity, very few companies consider developing or registering molecules or products for use in honeybee colonies.

The future for the chemical control of Varroa

It is hoped that new breakthroughs and developments will eventually occur, yielding new types of treatment, but of course, there are no guarantees of this happening, especially in the short-term. More frequent monitoring of honeybee colony health and *Varroa* levels and practising good husbandry are ever more important. Educating beekeepers on the dangers of using haphazard chemical mixes and instead applying regulated treatments is vital, to protect bees for the future.

The few chemical treatments that are currently available in Europe have been registered and well-used because they have been proven to work. There is no perfect product or solution and resistance could be a factor in lower efficacy in some areas. Monitoring resistance levels can be key in determining an effective control strategy. The presence or level of mite resistance will vary greatly from area to area and regular monitoring of resistance levels can help save honeybee colonies. Where resistance levels are low, it may be possible to use the products and methods at hand within an Integrated Pest Management system. Following product label directions and simultaneous treatment of hives within an area to reduce the risk of re-infestation are basic, yet crucial steps. If we are to win this battle, or at least to put up a good fight against the voracious *Varroa* mite, we need to ably use every means at our disposal, with minimum risk to our cherished bees.

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Max Watkins

Vita (Europe) Limited, Vita House, London Street, Basingstoke, Hampshire, RG21 7PG, UK.
Email: max.watkins@vita-europe.com