CONTROL OF THE MITE *VARROA DESTRUCTOR* IN HONEY BEE COLONIES

Klaus Wallner[1] from Universität Hohenheim in Stuttgart, Germany and Ingemar Fries[2] from the Swedish University of Agricultural Sciences in Uppsala, Sweden discuss the control of *V. destructor* in honey bee colonies by management methods and organic and traditional methods of chemical control

Introduction

What is varroa?

The honey bee mite *Varroa destructor* is an external parasite of the Asian honey bee (*Apis cerana*), where a balanced host-parasite relationship is established in the sense that the host fitness loss due to parasitism is limited because mite reproduction occurs in drone brood only (Peng *et al.*, 1987; Tewarson *et al.,* 1992). *V. destructor* was recently described when it was found that *Varroa jacobsoni,* the original mite described from *A. cerana*, was more than one species (Anderson and Trueman, 2000). *V. destructor* has adapted to European honey bees (*Apis mellifera*) and at least two different haplotypes are identified, the Korean haplotype and the Japan/Thailand haplotype (Anderson and Trueman, 2000). Possible differences in mite virulence between these groups of mites remain to be investigated but future research on *V. destructor*–honey bee relationships needs to pay more attention to the mite source than previously understood.

Life cycle and identification of V. destructor

Only adult female mites are found on the adult bees (reddish brown, approximately 1.1 × 1.6 mm, length × width (Figure 1). The mites feed on haemolymph by attaching themselves between the ventral abdominal sternites and piercing the intersegmental membranes. Reproduction can only occur with access to honey bee brood. With brood available, the mite leaves the adult bee and enters a brood cell within a day before capping of worker cells and within 2 days of capping of drone cells. Reproduction occurs only in the sealed brood cells and drone brood is preferred to worker brood, if available. When egg laying is initiated the female mite typically lays 4–5 eggs on worker brood and slightly more on drone brood at intervals between the eggs of about 30 hours. Normally, the first egg produced is unfertilized and develops into a male (arrhenotoky) and the remaining eggs into females (Figure 2). The development from egg to an adult male is slightly longer than 6 days whereas the females develop in about 5.5 days. This means that when the first female becomes adult, the male is ready to mate with her and subsequently with all his sisters thereafter.

The number of mated daughters produced within a cell is dependent on the duration of the postcapping period. Although the optimal production is much higher, the actual number of mated daughters produced in worker cells is often below 1 per cell invasion. This is due to infertility, mortality among the progeny or production of one sex only. In drone brood the reproductive success is considerably higher. The mother mite may enter cells for reproduction on repeated occasions but although the biological maximum is as high as 7–8 times, the actual average number is much lower because of mortality factors and depending on brood availability. Under optimal conditions with full access to

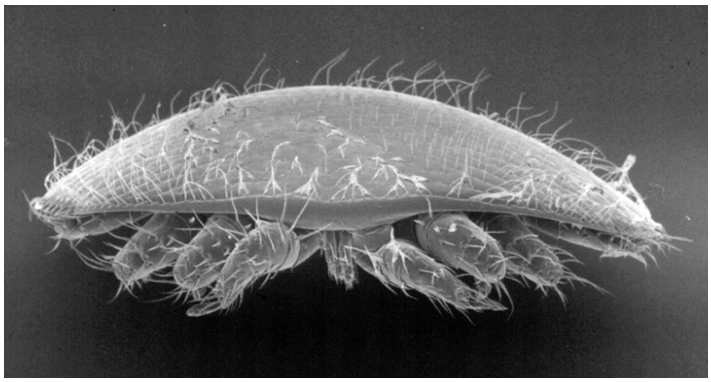

Figure 1. **Varroa mite female**

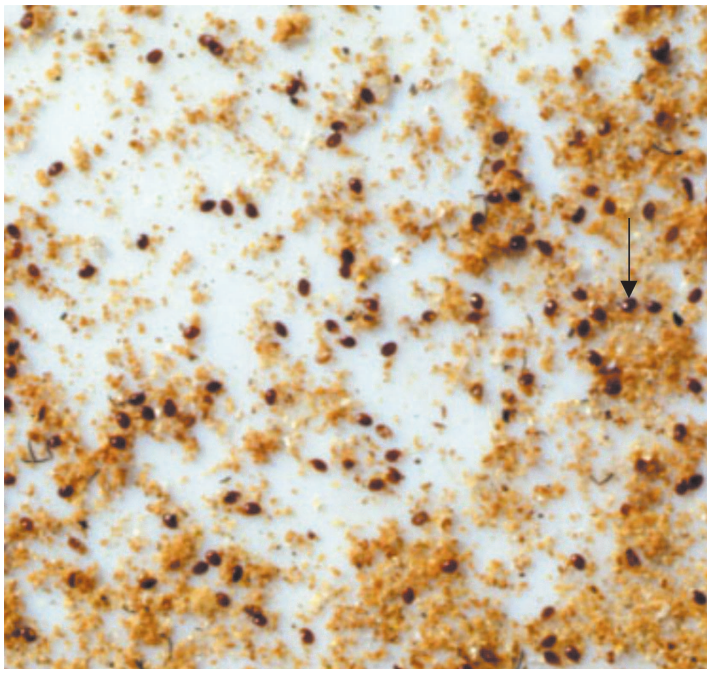

Figure 2. ***V. destructor* (arrow) in debris from a heavily infested bee hive.**

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DOI: 10.1039/b301510f

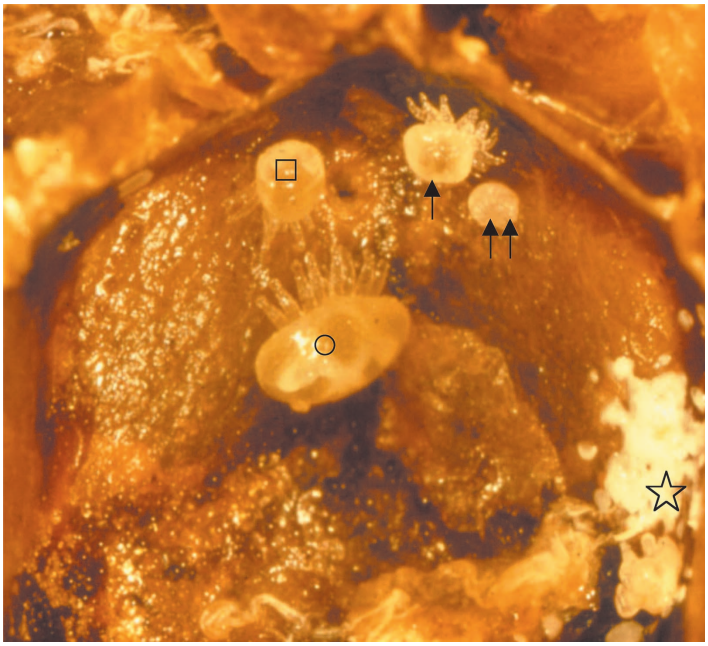

Figure 3. ***V. destructor* progeny in a sealed cell that has been opened. The mother mite and the bee pupa have been removed. Mite fecal deposits (star), female deutonymph (circle), female protonymph (square), male (arrow), first larval stage still inside the egg (double arrows).**

brood, during short periods of the year, the mite population may double in about 23–25 days under European conditions (see for example Fries, *et al.*, 1994; Calis, *et al.*, 1999, for more details.)

Because of the size of the adult female it is easily seen with the naked eye and, thus, it is easy to diagnose mite infestations. Nevertheless, the mite is difficult to spot on adult bees and within the sealed brood they remain unseen. The natural mite mortality is well suited to detection of mite infestations. Bee hives should be equipped with bottom board screens that allow collection of colony debris when this is wanted. The mites are easily spotted in the debris if this is not allowed to accumulate for more than a few days (Figure 3). The possibility to examine hive debris for mites serves more than a diagnostic purpose; it is also a rough indication of the size of the mite population, something essential when need based mite control is sought. In colonies where brood is emerging, one mite per day in the debris approximately represents a mite population of 120–150 mites in the colony (Liebig *et al.*, 1983)

Why do we need to control Varroa destructor?

The effects of this parasitic mite on European honey bees have been devastating with monumental colony losses world wide. The mite is now present on all continents except Australia and is newly found in New Zealand and South Africa. There are numerous methods for the control of *V. dest*ructor and in this short article we would like to look at the three main categories of control methods.

Management methods of control

Generally speaking management methods consist of apicultural techniques that limit or reduce mite population growth. Only very labour intensive methods can control the mite population sufficiently and such measures are most often regarded as complementary to other control options.

The preference of *V. destructor* for drone brood can be used to remove surprisingly large proportions of the mite population if sealed drone brood is removed from bee colonies (Rosenkranz and Engels, 1985). Removing drone brood alone will not control mite population growth but it should be an integrated part of any apicultural management scheme. Beekeepers who rely on pesticide use will benefit because the need for repeated pesticide applications, sometimes needed in areas with extended brood rearing periods, will be reduced. Beekeepers that want to avoid pesticides all together should use drone brood removal because it will reduce the need for extreme efficacy in other methods of mite control.

Mites can also be trapped in worker brood. If the queen is successively trapped on three worker brood combs that are removed before the bees hatch, sufficient mite control can be achieved, at least under Nordic conditions (Fries and Hansen, 1993).

Another management system which can reduce mite population growth is colony divisions to make nuclei. When colonies are divided, the nuclei, the mother colony or both can be left without reproduction opportunities for the mites for some time depending on how the queen is managed (replaced by a queen cell, left in one of the units, or completely removed). Nucleus formation is no control method but divides the existing mite population and depending on systems for dividing colonies may allow the beekeeper to wait longer before a real mite control treatment is employed.

Organic chemical control

A range of chemicals that occur naturally in the honey bee colony environment and that are present in honey can be used to control *V. destructor*. The most commonly used so-called organic acaricides are formic, lactic and oxalic acids. It is believed that the acaricidial effect is based directly on a lowered pH which is less well tolerated by the smaller mite than the larger bee. Thymol, a substance found in high quantities in some types of honey, is also widely used.

Formic acid has been the most commonly used organic acid for varroa mite control. There are numerous ways of applying formic acid to control varroa. An advantage with formic acid is that there is also some miticidal effect on mites in sealed brood (Fries, 1991). Disadvantages include variation in efficacy and risks for the user handling concentrated acid. Formic acid fumigation is best used in the temperature range 12–25°C. Below this temperature the efficacy is reduced and above this temperature the bees may become agitated and leave the hive.

Lactic acid is applied in a 15% water solution sprayed directly on each comb side covered with bees. The treatment is very effective if repeated three times and well tolerated by the bees (Brødsgaard *et al.*, 1997). However, it is very labour intensive.

Oxalic acid has more recently proved to be highly

effective for mite control, both applied dissolved in sugar solution dripped onto the bees (Mutinelli, Baggio *et al.*, 1997; Nanetti and Stradi 1997) or as a fumigant applied after heating oxalic acid crystals inside the bee hive (Radetzki, 2001). Oxalic acid is only effective in broodless colonies and if applied in the late autumn problems with increased residues in honey are minimal.

Several registered commercial products containing thymol are available on the market such as ApiLife Var and Apiguard. Thymol is effective against *V. destructor* and have been increasingly used as the mite has become resistant to traditional acaricides. Thymol treatment requires long term application and is used most effectively in warm climates. Following the application recommendations for the products, thymol treatment can be used as an integrated part of a control programme. Correctly applied (after the honey flow) there is little risk of increasing thymol residues in honey, however, there are methods advocated with constant exposure of thymol in bee hives. Such methods should be avoided because residue levels become too high and because of increased risk of mite resistance.

Traditional chemical control

Water-soluble (hydrophilic) varroacides

Water-soluble active ingredients such as formic acid, oxalic acid and cymiazole endanger the quality of honey since these substances are readily dissolved in honey. Use of such substances during the nectar flow always results in considerable amounts of residues and should be avoided. Also, organic acids may introduce a false taste to the honey. However, volatile residues, such as formic acid, decrease in stored food and extracted honey over time (Capolongo *et al.*, 1996; Stoya *et al.*, 1986). Water-soluble ingredients have no negative long-term effect on beeswax quality since they are not soluble in beeswax.

Lipid-soluble (lipophilic) varroacides

These ingredients such as bromopropylate, coumaphos and fluvalinate, are stable and may successively accumulate in the wax comb over time. Fat-soluble ingredients are distributed throughout the colony by the bees' legs and bodies. All inner surfaces of the hive, which are walked on by bees such as the frames, bottom boards, and covers, are coated with a very thin layer of wax. Lipophilic substances have an affinity to this wax layer and may pass in measurable amounts into other bee products like honey, virgin wax and propolis. Contaminated wax samples in the laboratory given the same conditions as in the bee colony have clearly demonstrated such migration (Wallner, 1992). The greater the concentration in the wax, the more residues can be detected in honey in contact with the wax. Another negative effect is the contamination of wax particles in the honey (Wallner, 1995). Contaminated wax is a significant source of residues in honey because a natural degradation of varroacides in beeswax does not occur and there is an accumulation of lipophilic varroacides in beeswax with repeated applications. The effect of acaricide wax contamination on the mites, on the other hand, seems to be negligible unless extremely large amounts are present (Fries *et al.*, 1998). Beeswax has a large capacity to hold contaminants and the recycling of old wax combs into foundation does not change the concentration of the active ingredient to any significant degree. Also, the technical capabilities for cleaning of wax are limited (Vesley *et al.*, 1994; Wallner, 1995). Only the complete destruction of beeswax by, for example, burning the wax as a candle will destroy the stored ingredients (Wallner, 1998). In effect, fat-soluble ingredients, especially when they are stable and non-volatile, represent a great risk in apiculture of long-term residue accumulation.

Within the group of fat-soluble active ingredients, there are varroacides whose concentration can decrease in beeswax. This decrease is because they contain semi-volatile ingredients such as essential oils (*e.g.*, thymol, wintergreen oil), and other substances, which can decay into metabolites (*e.g.*, amitraz). During the application of the treatment, only some of the semi-volatile ingredients attach to the wax, while the majority evaporate because of the temperature in the hive (Imdorf *et al.*, 1995). As a result, the amount actually left in the wax is effectively reduced. Although, an accumulation of residues does not occur during years of application of semi-volatile substances, traces may remain detectable in honeycomb (Bogdanov *et al.*, 1998). The amount of semi-volatile ingredients can also be effectively reduced during the recycling of old wax into foundations, if the wax is not only liquefied but is also melted from the combs by the use of steam.

Problem of varroacidal residues

Residues in honey

With few exceptions, honey analyses in different laboratories have demonstrated that residues of stable, lipophilic substances can be found. Since 1988, long-term studies have been carried out at the University of Stuttgart-Hohenheim, in which up to 1000 honey samples are analyzed per year within the scope of general quality control. Up to now, the following varroacides have been detected in ppb levels in honey: bromopropylate, coumaphos, fluvalinate, malathion, diazinon, chlordimeform and cymiazole (Wallner, 1999).

For example, today, coumaphos (Perizin) is the most frequently used trickling solution in German apiaries for mite control. It is mainly used during the winter in colonies without brood, but sometimes it is also used in late summer as an additional treatment in colonies with brood. Coumaphos represents the most frequently detectable varroacide in German honey. In 2002, approximately 29% of examined honey samples were contaminated with levels between 3–15 ppb.

The synthetic pyrethroid fluvalinate is used for control of *V. destructor* worldwide. Impregnated carriers are inserted into the colony with amounts of fluvalinate in grams. In water solutions dripped onto the bees, the amount of ingredient required for efficacy is extremely low in comparison to other systemic varroacides (coumaphos, cymiazole). Residues in German honey are found only rarely when mistakes occur in the application and/or in preparing the trickling solution, and if residues in the wax are at a high level. In these instances, the substance migrates by diffusion from the comb into the honey. Fluvalinate can be found in

1% of honey produced in Germany with residue levels between 2–7 ppb. Higher amounts reaching 40 ppb were found in honey from Eastern Europe, and are reported from other countries, as well (Kubik *et al.*, 1995). Recently, fluvalinate resistant mites have appeared in several countries, showing that the continued use of the whole group of synthetic pyrethroids is in jeopardy world wide (Milani, 1995).

Residues in wax

Most of the fat soluble substances, with the exception of amitraz, are widely used as varroacides and can be found with ppm levels in beeswax. Since 1993, 300–1000 beeswax samples have been analyzed per year at the University of Hohenheim. Most of these samples were received directly from beekeepers. Up to now, the following varroacides have been detected in wax: bromopropylate, coumaphos, cymiazole, fluvalinate, flumethrin, thymol and tetradifon (Wallner, 1999).

For example, more than half of the international samples (58.0%) are contaminated with fluvalinate. As a fat-soluble, non-volatile substance, fluvalinate plays the chief role as a residue creating substance in beeswax, even if the substance cannot be used effectively today in many countries because of mite resistance. The same frequencies are also found in wax foundation on the international market, as beeswax is an internationally traded product.

In general, residues of varroacides in beeswax are not regulated. With the exception of the USA (fluvalinate 6 ppm), no official limits exist. Since large amounts of beeswax are processed for pharmaceutical purposes or for the food and cosmetics industry, pesticide residues are problematic. Several companies have created their own internal acceptable maximum limits of wax contaminants. Because of the easy migration of substances into honey, wax used for honey production should have low levels of contamination. To ensure that there is no measurable effect on the honey, residues in the wax should be lower than 1 mg kg^{-1} (Wallner, 1995).

Moderating factors in control of *V. destructor*

Using chemicals correctly

It is important to emphasise that the development of resistant populations of mites and the detection of chemical residues in honey and wax can, in many instances, be attributed to prolonged, frequent or incorrect use of the chemicals mentioned above, or to beekeepers making up their own treatments from related agrochemicals used in pest control. If beekeepers were to use these products as recommended and only when necessary the risk of substantial accumulation of chemical residues would be minimised.

Minimising the risk from residues

Observing sensible colony management procedures such as harvesting honey before the application of treatments, not using combs from the brood nest area in honey supers and annual replacement of a proportion of all combs in a colony, can do much to minimise any risk.

Conclusions

V. destructor in managed honey bee colonies must be controlled for beekeepers to stay in business. Application of synthetic acaricides inside bee colonies is the most common means of controlling the mites, although management methods and some organic acids also can be used effectively for this purpose. Lately, alternatives to synthetic acaricides have received increased attention because of fast spreading resistance in mite populations to several of the products used.

The treatment of colonies against *V. destructor* influences the quality of bee products in many countries of the world. Depending on the chosen varroacide, varying levels of residues can be found in honey, beeswax, and propolis.

To limit acaricide contamination of bee products, the use of synthetic, lipophilic varroacides in colonies should be minimized, and the use of organic acids or essential oils increased. It is also necessary to change the practice of recycling wax into foundation. Old combs that are contaminated should not be used for the production of foundation. Instead, foundation should be made from virgin wax and wax cappings. With an increased production of virgin wax in colonies, an efficient acaracide application system, and a system to separate contaminated combs from the wax recycling process, residue levels in bee products can be maintained below the detectable limits and far below the maximum residue levels.

In some areas where mites have been present for decades with access to large feral populations of bees the need to control the mite population in managed colonies seems to have diminished. In South America (africanized bees; Rosenkranz, 1999) and in North Africa (*A. mellifera intermissa*; Boecking and Ritter, 1993) bee colonies survive mite infestations without treatment. To what extent this reflects a natural selection process is not documented but epidemiological considerations (Fries and Camazine, 2001) suggest that a highly virulent mite will be retained as long as beekeepers remove the natural selective pressures on the mite by treating infested colonies with effective acaricides. This does not mean that beekeepers should not control the mite populations, most bee colonies at least in Europe would probably die otherwise. But perhaps control strategies need to be worked out that allow a more balanced host-parasite relationship to develop.

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Dr. Klaus Wallner works on analysis of residues in bee products at the Landesanstalt für Bienenkunde at the University of Hohenheim in Germany. He is a leading expert in Europe on the effects from disease control in bee colonies on the quality of bee products. His extensive analytical work has lead to a better understanding in the beekeeping industry for the need to reduce or avoid certain disease control methods in the beekeeping practice.

Dr. Ingemar Fries is responsible for the reference laboratory for bee diseases in Sweden and has worked extensively on varroa mite biology and control. His focus has continuously been directed towards use of ecological methods for disease control and recently he organised a European network on integrated varroa control in Europe, financed by the European Commission ("Coordination in Europe of research on integrated varroa control", Concerted Action 3686).

ARS AWARD FOR BEE SCIENTIST

Molecular geneticist Jay D. Evans has been named an "Outstanding Early Career Scientist of 2002" by the Agricultural Research Service, the chief scientific research agency of the U.S. Department of Agriculture for his studies of genes that influence honey bee development, pest resistance, and other traits.

Evans joined ARS' Bee Research Laboratory at Beltsville in 1998, and within his first 3 years had authored or co-authored 12 manuscripts, including a paper in the *Proceedings of the National Academy of Sciences* that examined the interplay of the hive environment and genes in determining whether honey bee larvae become queens or workers.

Evans' genomics research also extends to honey bee parasites, insect pests, and pathogens. Using an approach called molecular phylogenetics, for example, Evans and colleagues established South Africa as the original source of U.S. introductions of the small hive beetle, a pest that infiltrates bee hives to feed on pollen and honey. His development of "genetic markers"– specific regions of beetle DNA that distinguish it from other insects–has given federal and state regulatory agencies an important surveying tool for tracking the pest's U.S. migration.

Evans' lab also is finishing up work to sequence DNA in the mitochondria, or cellular "power plants," of *Varroa* mites, parasites that feed on honey bee blood. One aim is to study genetic variation in the DNA so that the *Varroa's* taxonomic status can be clarified. Another goal is to develop genetic markers that could be used to track the *Varroa's* migration patterns, check for re-introductions of the parasite, or screen mite populations for pesticide resistance.

Evans has served on the Honey Bee Genome Project Consortium; scientists are expected to finish sequencing the entire honey bee genome, an estimated 16,000 genes. For more information on the Bee Research Laboratory see http://www.barc.usda.gov/psi/brl/USDA