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Varroosis

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Definition

Varroosis is a disease due to the mite *Varroa jacobsoni* Oudemans (Mesostigmata: Acarina). This ectoparasitic mite is haemophagous on both the adult and immature bee. Endemic on its natural host, *Apis cerana*, it causes an epizooty on its new host, *Apis mellifera*, after its recent transfer in the 1960's. A second species, *V. underwoodi* was described by Delfinado-Baker on *Apis cerana* and a third one, *V. rindereri* on *Apis koschevnikovi*.

General epidemiology

Varroa jacobsoni was first described as a parasite of *Apis cerana* in Java by Oudemans (1904). *Varroa jacobsoni* is endemic on this Asian bee species. Later, it was identified in 1944 in Singapore, about 1952 in the eastern coastal region of the USSR, in 1955 in Pakistan, in 1958 in Japan, and in 1959 in China. The introduction of *A. mellifera* to the Far East permitted these bees to become infested, probably when robbing *A. cerana* nests. In China, the first report of *V. jacobsoni* in *A. mellifera* colonies dates from 1960. After a hundred years of coexistence of both bee species in Japan and in Ussuria, the mite became destructive on *A. mellifera* between 1965 and 1970 in these areas.

The mite was introduced in 1967 to Bulgaria, and severe losses of bees occurred from 1974. In western Europe the mite was directly introduced from Pakistan into the German Federal Republic in 1977.

The parasite crossed the Mediterranean sea in 1975 by the importation of colonies from Romania to Tunisia, and also crossed the Pacific ocean by the importation of queens from Japan to Paraguay in the same year.

From the first focuses, this new epizootic disease became widely distributed on most continents in less than 20 years. At the present time only Australia and New Zealand are considered free of *V. jacobsoni*. Within a continent, neighbouring countries can easily become infested by swarms if apiaries are kept close to the borders.

The impact of this epizootic disease on colonies of *A. mellifera* is dramatic in the absence of efficient treatment. Some million colonies were lost in USSR between 1975 and 1985. The losses sometimes reached almost 100% when the mite was not recognised sufficiently early in an area.

Now beekeepers have effective acaricides at their disposal, but treatment is often not used or infrequently applied, because it is expensive. Populations of mites with resistance to some of the chemical acaricides have become established, complicating the therapy. Moreover, diseases associated with the parasite have become more frequent and severe.

Etiology

Biology

Classification

Although the classification of the acari is a difficult task, because of the lack of a phylogenetic system based on the analysis of fossils (Krantz, 1978), the general criteria used in the actual classification are based on: (i) the application of numerical taxonomic methods (phenetic classification); (ii) the development of formulised leg, palpal and idiosomal chaetotaxy and poroidotaxy (cladistic or phylogenetic classification); (iii) chromosomal mapping; and (iv) the use of behavioural and ecological data.

Varroa jacobsoni Oudemans belongs to the:

(i) Superorder Anactinotrichida: Characterised by Evans (1992), leg coxae are free and movable, body and leg setae isotropic, palp with ambulacrum represented by paired terminal claws or a movable subterminal, tined, claw-like structure, lost in ticks and some holothyroids. Idiosoma without dorso-sejugal furrow, podocephalic canal absent, chelicera typically with dorsal slit organ, free-living forms with subcapitular gutter or groove present and usually with associated tritosternum, the tarsi of legs II-IV with peripodomeric fissure associated with slit organs, apparently no anamorphosis and organs of Claparède and genital papillae lacking.

(ii) Order Mesostigmata (or Gamasida): Characterised by Evans (1992), venter of subcapitulum with a maximum of four pairs of corniculi, tritosternum with base and lacinia(e) rarely absent in parasitic taxa; anal valves usually nude, rarely with one pair of setae; gnathotectal process(es) usually present.

The simple or tined apotelic claw has a terminal, subterminal or basal position on the palpal tarsus. The hypostome serves only as a part of the floor of the gnathosoma and it bears a maximum of three pairs of setae, with two or three tined apoteles near the inner basal angle of the palpal tarsus. Tarsus I only rarely with a dorsal sensory pit. The palpal tarsal apoteles are never terminal. The epistome is present, roofing the gnathosoma. One or two pairs of stigmata, with or without peritremes, are commonly present in a ventrolateral position. Six setae on femur IV in deutonymphs and adults, a single epigynial shield with only one pair of epigynial setae in the intercoxal region. The external shield is usually entire and may be fused with the metasternal elements. The dorsum is generally covered by one or two shields, claws may or may not be present on tarsus I. The male genital aperture is either at the anterior margin of the sternal shield or within it. A spermadactyl may be present on the internal or ventral face of the movable chericeral digit. This order comprises twelve suborders.

(iii) Suborder Dermanyssina: Free-living females; pilus dentilis simple, setiform, inflated or absent; sperm access system unpaired or paired and opening by solenostomes in the region of coxae III and IV or on legs III, rarely on opisthogaster; male genital orifice presternal; movable digit of male chelicera with spermatodactyl; largest suborder with free-living and parasitic representatives.

(iv) Superfamily Dermanyssoidea: The Dermanyssoidea is a large and diverse group of free-living and parasitic mites, which appears to be derived from a basic free-living mite group (Hypoaspidinae), despite their morphological and behavioural heterogeneity. The specific characters of the Dermanyssoidae are:

- Sternal shield of the female normally developed, reduced or absent.
- Sternal setae generally present.
- Metasternal setae present or absent.
- Dorsal shield entire, divided or variously fragmented.
- Chaetotaxy of legs highly variable. Tibia I typically with six dorsal setae, genu IV nearly always with two al setae, and with one, or no, pv setae, palptarsal claw two tined.
- Male genital aperture anterior. Spermadactyl arises externally on the movable digit, free distally.
- Female podospermous.
- Larva without pygidial shield.

- Protonymphal pygidial shield broad, narrowed, or represented by a series of mesonotal scutella, entire shields with 4-8 pairs of setae either inserted on the shield or in the adjacent integument where the shield is reduced.
- Dorsal shield of deutonymph entire and generally incised, or retaining a separate small pugidial element posteriorly.

(v) Family Varroidae (Delfinado and Baker, 1974):

- Epigynial shield rounded, separated from triangular anal shield.
- Legs I-IV of comparable thickness.
- Tritosternum well developed, with lacinae.
- Sternal and anal shields variously developed, often reduced or expanded.
- Chelicerae various, dentate or edentate. Corniculi sclerotised or membranous.
- Palpotarsal claw present, 2 or 3 tined.
- Fixed cheliceral digit absent, with 2 pairs of hypostomal setae.
- Peritremes of female short, looped medially or terminally, confined to level of coxae III or coxae III-IV.
- Male may be without perimetres.
- Parasitic on bees.

Anatomy

A correct identification of the different developmental stages of *Varroa jacobsoni* is essential for research on its life cycle and population dynamics (Nannelli, 1986; Milani, 1987). See Fig. 1a-f, showing morphological characteristics of the different developmental stages of the mite described by Emmanuel *et al.* (1983) and Table 1 of Milani (1988).

The morphology of the adult female is the most well-known, as it was described by Oudemans(1904), and later by numerous other authors. It is considered to be the type species of the *Varroa* genus. We rely on the description made by Nannelli (1986) of the female, that of Delfinado-Baker (1984) and Steiner (1995) of the nymphal stages and the male, and for more general aspects we refer to Ritter (1981).

The female

The body is ellipsoidal, its width being greater than its length (1600 µm wide and 1100 µm long). The dorsal shield is extremely sclerotised (sclerite dorsal), yellowish brown in colour darkening to dark reddish brown as the mite gets older. It has numerous feathery central setae and more robust marginal setae (dorsal chaetotaxy).

The ventral surface is formed by different shields, covered in sensory and protective setae, which are joined to each other by means of an interscutal membrane that enables the body to dilate during feeding and egg formation. These shields have different shapes and denominations according to their function: one central or opisthogenital sclerite, behind the genital opening. The genital opening is situated transversely between the third and fourth pair of legs, covered with a falciform genital plate. The sternal shield encircles the top segment of the legs. Two endopodal shields are present behind the fourth legs and two metapodal shields on the posterior sides. The anal sclerite with the anal opening, is on the posterior edge of the shield and is surrounded by setae.

The gnathosoma is situated anteroventrally and is visible through the dorsum. It forms the mouth parts which are made up of two sensory pedipalps and two chelicerae. The palps comprise four segments, whose setae follow a very precise pattern according to a particular formula for each different claw (palp chaetotaxy). The last segment possesses a thick bifid claw. The chelicerae have one fixed digit and one pointed, denticulate, movable digit. The salivary stylets are greatly developed and generally covered with sharp corniculi. The chelicerae, corniculi and salivary stylets form a structure which is particularly well-adapted to pierce the cuticle of the host.

Laterally, there are four pairs of robust, squat legs, formed by seven segments: coxa, trochanter, femur, genu, tibia, tarsus and pretarsus, with terminal membranous ambulacra without claw or

apotele. The ambulacra comprises an adherent sucker. All the segments, except the apotele, have numerous setae, which are particularly abundant on the tarsus. The Haller's organ is found on the tarsus of the first pair of legs.

The respiratory stigmata or spiracles are in a lateroventral position, between the third and fourth leg (mesostigmata), and are formed by a curved peritreme which continues with a tubular diverticle that connects with the trachea. This mechanism enables the adult female to breathe inside the capped cell where the quantity of oxygen is reduced and liquid is sometimes present (Richard *et al.*, 1990).

For descriptions of the structure of the nervous and genital systems, see the articles of Ionescu-Varo and Suciu (1979), de Ruijter and Kaas (1983), Akimov and Yastrebtsov (1984, 1985), Akimov *et al.* (1986), and Alberti and Haenel (1986).

The male

The male is yellowish white in colour, pear-shaped and is smaller than the female, measuring 750 µm long by 750-780 µm wide at the posterior end. It closely resembles the female nymphal stages in shape, size and colour. Its body is poorly sclerotised, apart from its legs, which are somewhat darker. The dorsal shield is finely covered with setae, very densely in the posterior part, but no differentiation exists between the marginal and central setae, unlike those of the female. The sclerotisation, chaetotaxy and the shape of the setae on the idiosoma are very similar to those of the deutonymph. However, they can be differentiated by: (i) the presence of the genital opening, situated in the sternogenital shield between the coxa II; (ii) the presence of a movable digit on the chelicerae, which have been transformed into a tubular structure used for sperm transfer; and (iii) the shape of the peritremes and tritosternum.

The salivary stylets are long and pointed. At the base of the gnathosoma 2 a series of 3 hypostomal setae are visible. The corniculi are triangular. Between the genital plate and the anus, there is a group of setae without any particular pattern. The legs are long and thin, the tarsus ending in a membranous ambulacra.

The egg and larval stage

The egg is pearly white with an elastic consistency and is ovoid in shape, measuring 600-630 x 500 µm. After 24 hours, an immobile, hexapod larva develops inside. The larva has a differentiated gnathosoma and unsegmented rudimentary legs. The unfertilised eggs follow a parthenogenetic development, producing haploid males (n=7) and the fertilised ones producing females (n=14). The larval stage lasts for a very short time, the protonymph rapidly appearing inside the body of the larva and breaking through the larval exuviae and then the egg shell, on emergence.

The protonymph

The protonymph is the first mobile stage. It is characterised by the presence of 4 pairs of legs, with membranous ambulacra at the extremity. It has a spherical, pearly white body which is unsclerotised. Although it is difficult to distinguish between the sexes, the male protonymphs are somewhat smaller than the females; the male is fairly ovoid in shape and has dimensions of 500-590 µm, whilst the female is spherical and is 530-750 µm in size. The chelicerae do not have a fixed digit, the movable digit being pointed and bidentate; small corniculi and the salivary stylets are present but are difficult to see. The dorsal cuticle has numerous small pointed setae, mainly concentrated at the front part of the body and in the posterolateral areas. The opisthogastric cuticle (posteroventral) has a few setae concentrated above the anal region. At the base of the gnathosoma there are 3 pairs of hypostomal setae which, like the three pairs of setae in the intercoxal region, form two linear series of 3 ventral setae. Palp chaetotaxy is 0,2,2, the setae of the palpal trochanter being absent. Stigmata are present and peritremes undeveloped (Fig. 1e).

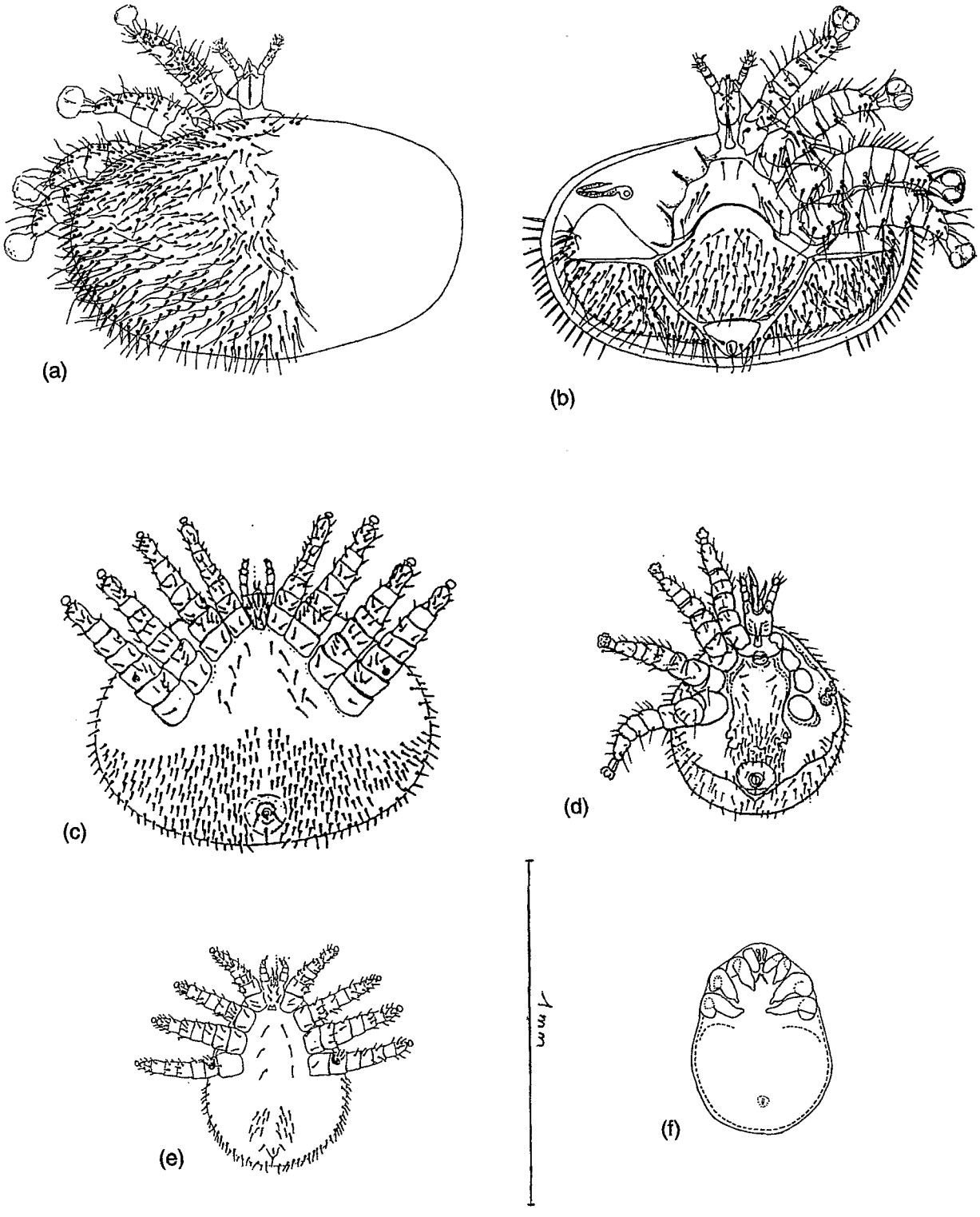

Fig. 1 *Varroa jacobsoni* Oudemans. (a) Dorsal face of the adult female. (b) Ventral face of the adult female. (c) Ventral face of the female deutonymph. (d) Ventral face of the adult male. (e) Protonymph. (f) Egg containing the hexapodal larva.

The deutonymph

The deutonymph has four pairs of legs, a gnathosoma like the adult, with two pairs of hypostomal setae and one pair of setae on the palp coxa. The cuticle of the intercoxal region has 5-6 pairs of setae. Both male and female deutonymphs can be distinguished from adults by the absence of the genital opening in the intercoxal region.

The female deutonymph is an active stage as regards feeding. It is spherical to ellipsoidal and its size varies between 750-1000 µm long and 800-1600 µm wide, according to engorgement; its body is pearly white; the dorsum does not have sclerotised areas and shows a marked hypertrichy. The male deutonymph is somewhat smaller than the female and is pear-shaped, 750-770 µm long and 750-800 µm wide. The gnathosoma is as in the adult. Chelicerae non-chelate, lacking a fixed digit; movable digit in the male deutonymph is a tubular bidentate appendage modified for sperm transfer. In the female, the chelicerae are similar to those of protonymphs and adult females, with a piercing structure. The corniculi are not well-developed, and are sometimes not symmetrical either in size or shape. In the female deutonymph, the salivary stylets are enlarged. The opisthogastric cuticle (posteroventral) of the female deutonymph has numerous setae densely covering the whole posteroventral region. The male deutonymph is similar to the male protonymph. Ambulacra of all legs lack claws.

Ontogenesis and population dynamics

Approximately 20% of the mite population in a colony is phoretic, preferentially parasitising the nurse bees. The mites, guided by larval pheromones, enter worker brood cells to feed on the L5 larvae 15 hours before capping, versus 45 hours for the drone cells. The stronger attraction of drone larvae partly explains the larger number of mites entering drone cells (5 to 8 times that of the worker cells), when drone brood is present in the colony. However, the attraction due to larval pheromones seems to be effective only at a very short distance because infested cells are randomly distributed and singly infested cells are the most frequently observed.

During the first five hours after capping, the bee larva consumes the remaining food in the bottom of the cell. The adult female (the mother mite) is released from the brood food, climbs on to the larva and feeds approximately every 1.6 hours (Donzé and Guérin, 1994). Afterwards, the larva, bearing the mother mite, spins a cocoon on the cell walls. About 35 hours post capping (hpc) in worker cells and 50 hpc in drone cells, the larva stretches out on its back, the head towards the cell capping and pupation starts. The mother mite remains and defaecates more frequently in the anal zone of the prepupa, building a faecal accumulation on the cell wall. When hungry, the mother mite leaves the faecal accumulation and moves to the feeding site, usually on the 5th abdominal segment of the immature bee. At 70 hpc whatever the bee sex, the mother mite lays its first egg on the cell wall, over the thoraco-abdominal junction. The other eggs are generally laid on the upper angles of the cells at an interval of 30 hours. Only eggs which have developed internally into hexapod larvae are viable. The first protonymph, which is a haploid male, hatches about 30 hours after oviposition and the following protonymphs, which are females (diploid), hatch after 26 hours. After a mobile phase of 42 hours and 23 hours for the male and the females respectively, the duration of the moult, leading to the mobile deutonymph, is about 20 hours for the male and 17 hours for the females. The mobile phase of the deutonymph lasts about 27 hours whatever the sex, preceding the imaginal moult, which lasts 35 hours for the males and 48 hours for the females. Finally, the first adults hatch at 224 hpc and 240 hpc for the male and the first female, respectively. If not feeding, the mites remain near the fecal accumulation, which facilitates mating. Since the adult male is hatched about 15 hours before the female, the male can feed and thereby produce sperm. The newly moulted female is mated for the first time about 15 min after its arrival at the faecal accumulation. Sperm transfer probably occurs when copulation lasts longer than 6 min. To fill the spermatheca completely, the female must be mated at least 4 times. The number of matings of the first daughter mite exceeds ten, but the frequency of mating decreases in relation to the rank of the daughter mite. In optimal conditions a male is able to fertilise 8 young females. After the brood cell is uncapped, the male and the immature female mites are unable to survive. The copulatory apparatus of mature but unmated female mite atrophies, making later matings unsuccessful. The phoretic stage of young females seems to be obligatory for maturation of the ovaries and the triggering of oogenesis, but its duration is still debated.

There is no significant difference between the development time of the mite on different brood types. By contrast, the mean number of eggs laid in worker brood and in drone brood is five and six

respectively. Considering the duration of cell capping in worker cells (12 days) and in drone cells (15 days), the sixth egg laid can reach maturity one day before emergence of the drone bee versus the fourth, before the emergence of the worker bee (Martin, 1994, 1995). The mean number of reproductive cycles per female mite is probably fewer than 2 (Fries and Rosenkranz, 1996) and the maximum number is seven (de Ruijter, 1987).

Mite reproduction is not always completely successful. A primary reason is that bee emergence occurs before the last offspring reach maturity. Another reason is that some females do not produce males, which prevents mating in singly infested cells. The daughter mites are thus sterile or at least unable to lay female eggs. The same effect is observed when the male dies before adulthood. Juvenile mortality is also observed in female offspring. The rate of adult mite mortality is estimated at 0.6% in the brood, and 0.5% of the phoretic population per day during summertime.

It is possible to estimate the influence of these negative factors on the reproductive success of the mite. An essential criterion is the fertility ratio, defined as the proportion of mother mites producing progeny of both sexes versus the number of mother mites considered. For instance, in the tropical climate of Mexico, the mean ratio is 77.6% in European and Africanised bees, in contrast to the mean ratio of 43% in Africanised bees in Brazil. Correspondingly, the reproductive fitness, defined as the number of daughter mites having progeny of both sexes, compared to the number of mother mites, also has to be considered. When several adult female mites enter the same cell, their fertility ratio increases but conversely, their reproductive fitness decreases. However, mite aggregation in one cell favours outbreeding. The values of reproductive fitness range from 1.29 in a singly infested cell, 0.95 for 2 mother mites, to 0.62 for 3 mother mites (Vandame, 1996). In contrast, Donzé *et al.* (1996), using another means of calculation, found that the reproductive fitness increases when 2 or 3 mother mites enter the same cell.

Spread and transmission

Within a country, the spread of the mites generally follows the path of migratory beekeeping. Between and within apiaries, the mites are dispersed by foraging, robbing or drifting bees, by drones and also by swarms.

Factors affecting disease outbreaks and seriousness

The growth of the mite population depends on the biology of the honeybee and its tolerance to the mite. The climate influences bee colony development and thereby mite population dynamics; the presence of brood is essential for mite reproduction. In the warm Mediterranean climate, brood is generally present all year round, but may be absent in periods of drought or during certain nectar flows. In this latter situation, an imbalance can occur between the number of available brood cells and the number of phoretic mites, resulting in multiply infested cells. Such severe infestation is often followed by brood diseases which drastically weaken the colony.

A few hybrid strains or races of bee are partially resistant, or tolerant, to the mite. These include the Africanized bees in Brazil and Mexico, some strains of *A. mellifera intermissa* in Tunisia, and the bees in Papua New Guinea. However, in the latter case, there is some evidence that the mite has retained its adaptation to *A. cerana* and cannot reproduce in *A. mellifera*. At present, five hypotheses are proposed to explain the tolerance of certain strains of bee: (i) grooming behaviour, which means the removal of mites on adult bees by the parasitised bee itself or by other bees; (ii) the removal of parasitised brood; (iii) the attractiveness of brood to the mite; (iv) the infertility of mites; and (v) the post-capping period.

Pathogenesis

Physical effects

The weight of the mite is not insignificant in comparison with that of the adult bee or of a pollen pellet, respectively 0.3 mg, 100 mg and 15 mg. The energy expenditure of an infested foraging bee is thus greater and efficiency reduced.

Physiological effects

General criteria

Lifespan

In laboratory conditions 50% of unparasitised adult bees survive longer than 25 days, whereas only 25% of bees parasitised by 3 mites during their development survive for this length of time. In natural conditions, this reduction in lifespan of infested newly emerged bees is observed especially during late summer. However, it is hazardous to attribute the effect to the mite alone.

Body weight

Weight loss of newly emerged bees is correlated with the number of mites feeding on the developing pupa. The reduction in adult weight can be 30% if the immature bee is infested by more than 3 mites. The effect is less pronounced in drone than in worker bees (De Jong *et al.*, 1982).

Damaging effects

The mite regularly withdraws haemolymph from its host. On the immature bee, feeding episodes are hourly on the prepupa, every three hours at the pupal stage and every five hours near imaginal ecdysis, which corresponds to the end of egg-laying. However, the duration of feeding increases (from 2 to 15 min) as the interval between two feeding bouts increases. The mite also feeds frequently on the adult bee. The reduction in the haemolymph volume of infested pupae ranges between 15 and 40%. The reduction in the total protein concentration depends on the haemolymph volume withdrawn and on the quantity of depleted protein. The reduction in the total protein concentration can exceed 50%. A high molecular weight protein, arylphorin, is primarily depleted, jeopardising the supply of aromatic amino acids for the construction of adult tissues and the exoskeleton (Schatton-Gadelmayer and Engels, 1988). The low molecular weight proteins are also depleted. The synthesis of three or four new antigenic proteins in the haemolymph is specifically correlated with the parasitism. The qualitative composition of haemolymph cells is modified in both the drone and worker bee, but a reduction in the total number of cells is only observed in the drone. On the adult bee, in addition to proteins, the mite takes juvenile hormone III, which is indispensable for the development of the reproductive apparatus of the young female mite.

Mutilating effects

Internal organs or body parts are atrophied or deformed by mite parasitism. The most frequent injuries are a shorter abdomen and deformed wings. A reduction in the haemolymph pressure and a viral infection, both associated with the parasitism, have been suggested as a cause of the wing deformity.

Organs such as the hypopharyngeal glands and the fat body are damaged. Mite infestation causes a reduction in the total volume of these glands and in the size of the acini, which can exceed 30% for an infestation of three mites. The size of the fat body cells is reduced and the number of protein granules decreases. The depletion of protein reserves may increase the susceptibility of the bee to pesticides.

Interactions with other pathogens

Fungi

In several countries, an increase in the incidence of ascosphaerosis (chalkbrood) has been correlated with mite infestation. Although the cuticule of the adult bee and mite can be contaminated with fungal spores, the transmission of the fungus to larvae, by the mite, has not been demonstrated.

Bacteria

The mite can also be superficially contaminated with the spores of *Paenibacillus larvae*, but experiments have failed to demonstrate the ability of the mite to trigger the disease, probably because the bacterial spore germinates only in the larval gut. Similar observations are true for *Melissococcus pluton*. If symptoms resembling those of American or European foulbrood are found in cases of varroosis, they are not necessarily due to bacterial pathogens. In contrast, septicaemic infections of the immature or imaginal bee stages are due to opportunistic bacteria (*Serratia* spp., *Pseudomonas* spp., *Bacillus* spp.) injected directly into the bee haemolymph during mite feeding. The mite is not only contaminated on its body surface, but can also harbour these bacteria in its haemolymph, salivary glands and gut.

Viruses

According to Ball and Allen (1988), the mite is able to transmit a number of viruses (acute paralysis virus, chronic paralysis virus, slow paralysis virus, Japan strain of Egypt bee virus, renamed deformed wing virus, and cloudy wing virus). However, young pupae are most susceptible to infection by injection. In the absence of the mite, only CPV causes disease outbreaks (hairless black syndrome), whereas the other viruses were rarely found to be responsible for mortality in nature before the appearance of *V. jacobsoni*. In particular, acute paralysis virus is only detected at low levels in uninfested colonies, but the virus is found in large amounts in dead brood and adult bees in severely infested colonies (Ball, 1985). The death of young larvae in such colonies may be due to virus secreted in the food by infected nurse bees. The mite acts not only as a virus vector, but also as an activator of the multiplication of viruses already present in the bee as latent infections.

Symptoms, differential diagnosis

If an infested colony is imported into a mite-free country, the newly infested colonies will go through an asymptomatic period of two years. In the early stages of infestation, mites are very difficult to see on the thorax or the abdomen of an adult bee, during normal colony inspections. The symptoms become most evident after an intense nectar flow, because the brood area is considerably reduced, which forces the phoretic mites to infest a high percentage of the brood cells near capping time. Some weeks later, a large proportion of the newly emerged bees are deformed and unable to undertake normal hive activities or foraging flights, which causes the sudden collapse of the colony. At this time, the adult female mites collect on the bees and on the combs. Brood diseases are often present.

When varroosis is firmly established in a country, a severe infestation can be suspected from the presence of adult bees with deformed wings. Brood or adult bee diseases are also often evident. The application of an acaricide is then useful to estimate the infestation level. Under low power magnification, the genus Varroa can easily be recognised.

Sample collection

Methods of extraction

Honey

50g of honey is diluted with 200 ml of distilled water and this solution is filtered through a Buchner funnel apparatus for vacuum filtration (Chmielewski, 1995), the mites and other residue thus being retained in the filter paper. These are then examined under a stereoscopic microscope, isolated with a fine brush and stored in Oudemans fluid until their identification. Oudemans conservative fluid contains 5 parts glycerine, 87 parts alcohol (70°) and 8 parts glacial acetic acid.

Pollen (Garcia Fernandez's methodology)

If the pollen sample originates from a comb, a section of 5x10 cm should be taken, and transported to the laboratory in wide-topped flasks. They are then stored in the freezer until being processed.

The sample is processed by covering the whole piece of comb or grains pollen with alcohol (40°) and stirring it for 30 minutes, then sieving the resulting solution and proceeding as in the case of honey.

Detritus from the bottom of the hive (Garcia Fernandez's methodology)

Take the detritus which has collected at the bottom of the hive over a certain time (generally one month) and weigh it. Take a certain quantity (10 g), dilute it in alcohol (40°) by stirring it, and then filter it through a sieve with three different mesh sizes (100 µ, 300 µ and 500 µ). Each sieve is then observed under the microscope and the number of mites per gram/detritus is calculated.

Preparation and mounting to identify the mites

Clearing

Various clearing mixtures can be used, such as lactophenol clearing mixture with the following composition: 50 g lactic acid; 25 g phenol crystals; 25 g distilled water.

Clearing can also be carried out with pure lactic acid (85%). Other methods are described in laboratory manuals (Locquin and Langeron, 1978).

Mounting

A provisional mounting can be made on a cavity slide with lactic acid as the mounting medium. With this method the specimen can be oriented by moving the cover slide and leaving only half the cavity slide covered. With the help of a pin, the sample can be moved until it is in the desired position.

Permanent mounting may be made in aqueous mounting media or resins soluble in oil. In the former, clearing is not necessary but as the medium is hygroscopic, the cover slide must be sealed with an insoluble protective lacquer.

Aqueous mounting media: Medium of Berlese with chloral hydrate; a variant is Hoyer's liquid (50 ml distilled water; 30 g gum arabia; 200 g chloral hydrate; 20 ml glycerine).

Leave the mixture to mature until it clears.

Identification

Follow the classification and the identification as indicated in Section on Biology and see the Chapter Tropilaelapidosis on *Apis mellifera* for differential diagnosis.

Treatment

Chemical control of *Varroa jacobsoni*

Generalities

Following the introduction into western Europe of the damaging mite pest *V. jacobsoni,* there has been an urgent need to develop and to put into practice control measures. This led to increased research

effort on varroosis control and tremendous advances have been made in the search to find effective methods. Apicultural practice has shown that non-chemical control methods are, in general, very labour intensive and often of low efficacy. However, now, with the help of chemotherapeutic measures, effective control of varroosis has almost been realised (Ritter, 1981). This situation will probably continue as chemical treatment will presumably remain more effective and less time consuming than biotechnical methods. Such management practices must at present be combined with chemotherapy, including the use of natural substances.

Chemical treatments have enabled a highly effective control of varroosis, preventing the loss of numerous colonies and restoring severely damaged apiaries to their former condition. However, none of the methods used can totally eradicate the mites, or stop them from spreading (Cavalloro, 1988), because treatment with most acaricides is effective only when very little or no capped brood is present; a condition that occurs in colder regions during autumn and winter, but which may never be achieved in warmer regions. In addition, many products may only be applied above a given temperature, and this additional constraint is difficult to combine with the absence of brood (Milani and Barbattini, 1989).

Worldwide, many chemicals (acaricides) have been tested for their efficacy against *V. jacobsoni*. A useful synopsis of products and active ingredients (a.i.) used for varroosis control has been published by Wienands (1988). Among these, a number of substances have proved very successful.

In addition to optimal effectiveness against *Varroa*, products applied in hives should also have the following important characteristics. They have to be: (i) well tolerated by the bee; and (ii) non-toxic for man, as a consumer of hive products and manipulator of varroacide agents. The efficacy of a given acaricide varies depending on many factors, such as the size of the bee colony, climate, bee race, the time of treatment, mode of application, etc. The greatest efficacy is achieved when the active ingredient is evenly distributed in the hive so that each *Varroa* mite is exposed to a lethal dose. It is then possible to eliminate 90% or more of the mites on adult bees; a remarkable result in the context of general apicultural practice (Colin and Gonzalez-Lopez, 1986). Most medications, whatever the means of application, are effective only against mites parasitising adult bees, those reproducing inside sealed brood cell are not affected.

Modes of application of chemotherapeutic agents

The early methods of chemotherapy were based on traditional pest control methods. Spraying, dusting, fumigation and aerosol were typical modes of application used for the "first generation" of agents for the control of *V. jacobsoni*.

Dusting

This method is currently used in Greece and Rumania to treat active colonies before and after nectar flows. Active ingredients (Malathion, naphthalene, thymol, chlorobenzylate, etc.) are mixed with a bulking agent which can be glucose, marble dust, kaolin or cellulose. About 50 mg of active ingredient is sufficient for one application. The main disadvantage of this method is the contamination of hive products either by the a.i. or the carrier.

Evaporation

Evaporating agents, which are volatile compounds, have previously been widely used for the control of the tracheal mite, *Acarapis woodi*. Treatment with these agents can be carried out without too much disturbance of the bee colony. Doses between 0.5 to 50 g of a.i. and a period of application ranging from one to several weeks, are necessary. It is difficult to control the quantity of the product evaporating daily since this quantity depends on the external temperature. In addition, beeswax may irreversibly absorb a significant amount of the volatile compound, leading to problems of accumulation. The main substances used are menthol, thymol, formic acid, naphthalene, etc.

Spraying

Products applied by spraying were very common. However, this method is very time consuming as combs have to be removed and treated individually and application is also limited to the day time only (Ritter, 1983). The main products which have been used for spray application are kelthane and Taktik® (a.i. = amitraz).

Fumigation

Treatment by fumigation replaced evaporation for the control of the tracheal mite *Acarapis woodi*. Active compounds incorporated into the fumigant strip must be stable since sublimation of the molecule should only occur in the hive, when heated (by nitrate combustion in some cases). In order to reduce the irritant effect caused to the bees by fumigation, additional space is provided within the colony. The main substances used as fumigants are: dicofol, phenothiazine, bromopropylate (Folbex V.A.®), etc. Bromopropylate was shown to be adequately effective against *Varroa* mites (Klepsh *et al.*, 1983) while at the same time being well tolerated by the bees and brood. Treatments applied by fumigation led to problems with the accumulation of residues. Further development of chemical control methods brought a change in the mode of application. Modern methods include:

Systemic treatments

The first systemic acaricide used for *Varroa* control was chlordimeform (Ruttner *et al.*, 1980), but this was abandoned because of residue problems. Later, Perizin® (Ritter, 1985) was developed and commercialised as a precursor of the "second generation" of varroosis treatments. Fed in syrup and distributed by trophallaxis in the colony, the acaricide passes into the haemolymph of the bees and kills the mites feeding on them. The treatment of colonies is simple and fast, the medicinal syrup being sprinkled into the bee ways. A specific problem with systemic acaricides is that the bees that take up the syrup absorb such large amounts of acaricide it is toxic and some initial bee mortality has to be accepted (Koeniger and Chmielewski, 1986).

Heated aerosol treatments

An aerosol is a suspension of microdroplets in a volume of air heated at 35-40°C. These fine particles, evenly distributed through the hive, settle on all surfaces, including bees and mites (Colin and Faucon, 1983).

The warmth of the aerosol breaks up the cluster of bees when brood is absent, allowing treatment when the ambient temperature is low. In addition, the efficacy of the acaricide is improved by heat. The production of a heated aerosol necessitates special devices which are not always available to beekeepers.

Slow release methods

Another means of effectively distributing acaricide throughout the colony is by using the mechanism by which the bees continuously distribute the queen substance within the population (de Ruijter and van den Eijnde, 1989). The active ingredient of this type of drug is released slowly from a plastic strip and is actively dispersed by the bees themselves by contact. This new approach to chemotherapy has brought considerable improvement in controlling varroosis. The use of impregnated inserts with prolonged effectiveness has evident advantages in comparison to previous methods of treatment; once applied these products are effective over a long period, mites are killed when they leave brood cells and reinfestation is prevented as long as the product remains active. The inserts are prefabricated plastic strips which are hung directly between the frames in a honeybee colony. Amongst these products, polyvinylchloride strips containing fluvalinate (Borneck and Merle, 1989) quickly became one of the most widely used products throughout the world. Similar strips made with polyethylene and containing flumethrin, another pyrethroid, (Koeniger and Chmielewski, 1986; Koeniger and Fuchs, 1988) are commercially available in some European countries. Recently, amitraz, a chemical belonging to the

formamidines group and known for its efficacy against *Varroa* has been incorporated to plastic strips and commercialised in France under the name of Apivar®.

Acaricides used for *Varroa* control

The choice of acaricide and mode of application differs from one country to another (different regulatory authorities have responsibility for decisions on chemotherapeutic beekeeping products). Acceptable levels of post-therapeutic residues of many substances have not been determined or standards agreed. All of these factors have led to a great diversity in the treatments being used in different areas. However, if the efficiency of control and the safety for human consumers are taken into account, the number of proposed treatments will decrease appreciably.

Table 1 (modified after Ritter, 1988) shows the main products being used in Europe and some Mediterranean countries, their a.i., form of application, means of administration, dosage, etc.

Mite resistance

Selection of mites resistant to pesticides is a well known phenomenon. The first cases of resistance to organophosphates were reported in 1947. According to Delorme and Dacol (1989), all crop pests may develop resistance to chemicals used in control strategies. This is especially true for mites which, in general, have short life cycles and particularly for *Varroa* which may have twelve generations per year. There is an increased probability of *Varroa* mites developing resistance as a result of prolonged contact with an acaricide. This is one of the reasons that manufacturers of products for controlling *Varroa*, advise users to limit the frequency and period of application. Despite precautionary measures, it is now evident that acaricides such as fluvalinate (Lodesani *et al.*, 1992) accumulate in the wax creating conditions for prolonged contact with *Varroa, especially inside brood cells where they reproduce.* The likelihood of *Varroa* developing resistance was particularly high, as many other mesostigmatid mites had already been reported to be resistant to synthetic pyrethroids, including fluvalinate. The ineffectiveness of fluvalinate has been reported in Italy and in France. The use of this chemical for varroosis control has become widespread over the last few years because it is easy to apply and relatively cheap. The product consists of polyvinylchloride (PVC) strips impregnated with the a.i. at a concentration of 10%, under the commercial name of Apistan®. This has proved to be highly effective against *Varroa* (Borneck and Merle, 1989: 99.38-99.72% control; Llorente Martinez *et al.*, 1989: 99.33% control). Nevertheless, the use of Apistan® has often been replaced by wooden strips impregnated with the same active ingredient, made by the beekeepers themselves using Klartan® and Mavrik®. Both products contain fluvalinate but are not authorised for use in beekeeping and difficulties in controlling the dose have led to problems of effectiveness.

Studies conducted in Italy (Lombardy) to determine if there was some resistance of *Varroa* to fluvalinate, showed variation in effectiveness between individual colonies and apiaries (Lodesani *et al.*, 1995). These observations provide evidence for the presence of populations of *V. jacobsoni* resistant to fluvalinate in apiaries where the treatment was unsatisfactory.

To assess the susceptibility of *Varroa* mites to fluvalinate and other pyrethroids, Milani, (1995) developed a laboratory bioassay which was used to test populations of *Varroa* believed to be susceptible and resistant to Apistan® treatment. The median lethal concentration (LD_{50}) of a.i. required to kill mites originating from areas where treatments with fluvalinate were no longer effective was about 25-50 times higher than that of susceptible mites. The (LD_{50}) of flumethrin and acrinathrin for mites surviving Apistan treatments increased 10-60 times. In a similar laboratory study, Colin *et al.* (1997) found great differences in the resistance level values calculated for *Varroa* populations originating in the South of France. These values ranged between 1.92 and 182.7.

Resistance to other acaricides, belonging to different groups of chemicals, has also been reported. Resistance to bromopropylate and to chlordimeform was shown to be favoured by underdosing and this has been verified in laboratory tests (Ritter and Roth, 1988). The presence, in different countries, of *Varroa* populations simultaneously resistant to different pyrethroids, highlights the risk of basing control strategies purely on chemical treatments, particularly when the substances used belong to the same chemical family.

Table 1. Authorised acaricides used in Europe for the control of *Varroa jacobsoni* infestation (modified after Ritter, 1988)

Commercial name	Folbex VA	Formic acid plates (IMP)	Perizin	Anti-varroa Schering	Apivar	Apitol	Malathion	Apistan	Bayvarol (liquid)	Bayvarol (strips)
Manufacturer	Ciba-Geigy	Klinger	Bayer	Schering	Biové	Ciba-Geigy		Zoecon	Bayer	Bayer
Active ingredient	Bromopropylate	Formic acid	Coumaphos	Amitraz	Amitraz	Cymiazol hydrochlorid	Malathion	Fluvalinate	Flumethrin	Flumethrin
Form of application	Fumigant strips	Evaporating plates	Systemic functioning liquid	Aerosol	Plastic strips	Systemic solution	Powder	Plastic strips	Systemic functioning liquid	Plastic strips
Mode of application	Fumigation	Evaporation	Trickled on	Aerosol steam	Contact	Trickled on	Dust on	Contact	Drop on	Evaporation and contact
Dosage per treatment and colony	1 strip	1 plate per super (20 g formic acid)	1 ml liquid in 49 ml water	20 ml liquid in 1 l water	2 strips	2 g granules in 100 ml water or dependent on colony size	0.04 g (5%) powder per hive	2 strips	1 ml liquid in 49 ml water	4 strips per occupied chamber
Frequency or period of use	4 times	4 times for 12 hours	2 times	2 times for 1 to 2 minutes	1 time for 56 days	2 times	3-4 times	1 time for 56 days	2 times	1 time 4-6 weeks
Interval between applications	4 days	7 days	7 days	7 days		7 days	7 days		7 days	
External temperature limits	Min 8°C	Min 12°C – max 25°C	Min 5°C	Min 8°C		Min 10°C	Min 15°C – max 30°C		Min 5°C	
Time of day for treatment	Evening	All day preferably evening	All day	Morning or evening	All day	All day	All day	All day	All day	All day
Special preparations	Insert strips through hive entrance or in empty frame, close flight entrance for ½ hour	Plates are placed above frames on bottom board	Solution is trickled on bees between combs	Introduce aerosols through hive entrance or from top, gasmask should be worn during application	Strips are placed between combs	Solution is trickled on bees between combs	Dust is scattered between combs	Strips are placed between combs	Solution is trickled on bees between combs	Strips are placed in 2 beeways per chamber, 2 strips in each beeway

Side effects

Effects on bees

Many publications on the chemical control of varroosis make observations on the tolerance of bees to acaricide products. Some papers compare the toxicity to bees and to mites, which is more significant than the absolute LD_{50} for bees (Milani, 1992). All acaricides have weak insecticide characteristics, thus, the clinical signs of an eventual toxicity for bees will not be expressed in an acute form but, most probably, in a chronic form. This is particularly relevant for the queen which has the greatest longevity in the colony. Some acaricide products have been shown to cause bee mortality immediately after use (Kilani *et al.*, 1981), others influence queen viability and fertility or have an appreciable toxicity for the brood which is more susceptible to acaricides than adult bees (Marin, 1977).

Residue problems

The risk of residues in bee products such as honey, wax and propolis is increasing as lipophilic pesticides are used more frequently in hives. Since most insecticides and acaricides are, to some degree, lipophilic they accumulate in wax and may then be slowly released into honey. The presence of residues in hive products is detrimental to their image as natural and unpolluted. Contamination has become a serious problem necessitating checks for residues of the acaricides applied. Although studies of interactions between acaricides and each hive product are still required, beeswax has a central role as an effective accumulator. The highest levels of insecticides used for agricultural purposes are found in old beeswax, indicating that residues accumulate in combs over the years (Gayger and Dustmann, 1985). Analysis of more than five hundred samples of wax showed that in all countries where acaricides are used, uncontaminated beeswax is rarely found. Often several pesticides can be detected in one sample (Wallner, 1995). The accumulation of acaricide residues in wax has negative implications, especially for the consumption of contaminated honey and the use of this wax in the cosmetic and pharmaceutical industries. Another important consideration is the induction of acaricide resistant *Varroa* populations as a consequence of the presence of the active ingredient in wax at sublethal levels and for prolonged periods of time.

For certain acaricides which have low affinity for wax, like coumaphos (Perizin®), bromopropylate (Folbex VA Neu®) and fluvalinate (Apistan® - 10% a.i., or Klartan® and Mavrik® - 24% a.i), the higher the concentration in the wax, the greater the amount of residue that can be detected in the honey. With these products, a concentration of only 1 mg kg^{-1} in wax is sufficient for their detection in honey. By contrast, acaricides known to have an extremely high affinity for wax (e.g. flumethrin) and consequently a weak tendency to leach out, are not detected in honey even when present at a concentration of 400 mg kg^{-1} in wax (Wallner, 1995). When treatment is carried out properly, according to the directions for use, the level of residues of legally registered products is very low. Residue levels may exceed the limits permitted by current regulations when beekeepers do not conform to the directions for use by either applying a greater number of treatments than required, or by using higher doses than necessary. The use of other agricultural products, not suitable for use in bee colonies, leads to the same problems.

Productive beekeeping in the presence of varroosis has been achieved with chemotherapy, but problems with the accumulation of residues in hive products and the establishment of acaricide resistant populations of mites further emphasises the risk of dependence on chemical treatments for control.

Natural products for the control of varroosis

To avoid some of the consequences of reliance on chemical control, the use of natural products is helpful, although more time-consuming. Three categories of these products can be identified in the area of varroosis control: (i) the molecules produced by animal metabolism; (ii) the extracts of edible plants; and (iii) methods or products permitted in organic farming.

Products of animal metabolism

The organic acids

Formic acid and lactic acid are naturally present in hive products or in animal organisms. The study of Sabatini *et al.* (1994) gives the different levels of lactic and formic acid in monofloral honeys harvested in

Italy. The maximum level of lactic acid detected was 686 mg kg^{-1} in honeydew honey and 273 mg kg^{-1} of formic acid in chestnut tree honey. Thus, if treatment is undertaken outside a nectar flow, no residue problems can occur. Because the details of the treatment given by Imdorf *et al.* (1996) are for Swiss conditions, some adaptation of the method is needed under Mediterranean conditions to obtain the same quality of result. Whatever the country, the corrosive property of the acids requires the beekeeper to be well protected with rubber gloves, mask and glasses when preparing the solutions of acid or using them for treatment.

Formic acid: The first report of treatment with formic acid is given by Kunzler *et al.* (1979), but in field conditions it was damaging to the bee brood and to the queen. The primary problem is to regulate the evaporation of acid in the hive, independent of the temperature and at an appropriate concentration to kill the mite (Bolli *et al.*, 1993). Today, although no ideal solution is available to beekeepers, Imdorf *et al.* (1996) propose two means of application in hives: (i) the short-term; and (ii) the long-term treatment.

(i) The short-term treatment consists of evaporating small amounts of formic acid. A volume of 30 ml is released over 6 to 10 hours within the colony. When applied above the combs, 60% formic acid is used, whereas 85% is necessary when applied from below. Two or three applications are made within a week and repeated twice, at an interval of one month. The effectiveness of the short term treatment can reach 95%. This value was confirmed in Italy by Mutinelli *et al.* (1996), when using sponges impregnated with 60% formic acid. However, the loss of queens or interruption of egg-laying may result.

(ii) The long-term treatment was first proposed by Krämer (1982). Fibre plates impregnated with formic acid, are sealed in plastic bags (Krämerplatte, Illertissner Milbenplatte). Before application, a calculated number of 1.5 cm diameter holes are opened in the plastic, the number of holes varying according to the hive type, the colony strength and the climate. The plate, isolated from contact with the cover and the top bars of the frames, is inserted for 7 days initially and then for 14 days, one month later. "The efficiency of this method of treatment depends on the formic acid concentration in the air of hive and on the duration of the treatment ... If there is an evaporation of more than 7 g of formic acid per day, a treatment efficiency of more than 95% may be expected, at 10 g per day it will exceed 97%" (Imdorf *et al.*, 1996). To obtain this level of control under Mediterranean conditions, it is necessary to adapt the Swiss method to regulate the evaporation rate of the formic acid to between 7 and 10 g per day, taking into account temperature variations (Mutinelli *et al.*, 1996).

Lactic acid: Lactic acid, sprayed directly on the adult bees covering the comb, has an efficiency of approximately 80%, if the colonies are broodless. Colonies in countries around the Mediterranean sea have a very short broodless period and at least 4 applications per year are needed. All the combs covered with adult bees must be finely sprayed with 5 to 8 ml of 15% lactic acid per side. Losses of bees can be expected if doses are exceeded.

Oxalic acid: Although oxalic acid is not of animal origin, it is also used by spraying adult bees with a concentration of 3% (w/v) and at a dose of 3-4 ml per comb side. In broodless colonies, the efficiency of the treatment is approximately 98%, with little variation between colonies.

Drone brood extracts

Arculeo *et al.* (1989) tested a commercial formulation of semiochemicals involved in the attraction of *V. jacobsoni* to drone larvae (Varroutest®). After spraying defined brood areas, the authors concluded that the mite infestation was 42% higher in sprayed areas than in unsprayed ones. However, the use of the product in trapping combs was not successful (Otten and Kraus, 1988).

Aromatic or oleaginous plant extracts

Essential oils

Essential oils are obtained by steam distillation of the whole plant or particular parts of the plant. Their presence in hives was reported by Lejeune *et al.* (1984), who asserted that propolis contains 5% to 10% of essential oils. Consequently, the reintroduction into the hive of essential oils extracted from plants attractive to bees, seemed to be a natural means of control of the parasite. Colin (1990) tested a mixture

of two essential oils: *Thymus vulgaris* L. (thymol type) and *Salvia officinalis* L. The main active molecules in thyme are para-cymene and thymol and, in sage, camphor, alpha thujone and eucalyptol. Aqueous solutions of 1% thyme extract and 0.5% sage extract formulated with an emulsifier, were administered to the hive via the entrance in aerosol form, by means of a "Phagogene®" apparatus. After four 60 s applications of the aerosol at 4 day intervals, the effectiveness of the treatment in colonies containing brood was 95.4% (s.d. 3.6%, n=10). No detrimental effects were observed during or after the experiment. Oil residues in honey were below the detection limit.

Vegetable oils

In the laboratory, Brodsgaard *et al.* (1994) tested the effectiveness of aqueous solutions of oils of rape or soybean, containing an emulsifier approved for foodstuffs. Mite mortality levels greater than 95% were obtained with 50% oil concentrations. However, such concentrations of oils were damaging to the bees.

Thymol

Thymol was one of the first molecules used in the effective control of varroosis in Eastern Europe (Mikitiouk and Grobov, 1978). Administered by evaporation, sublimation from crystals (Lodesani *et al.,* 1990) or diluted in vegetable oil (Gal *et al.,* 1992), the efficacy is very variable but can be about 80% depending on the amount evaporated, which itself depends on the external temperature. Bee mortality increases when the temperature increases. In order to minimise the drawbacks, Chiesa (1991) sprinkled powdered thymol crystals onto the top bars of combs occupied by bees in the late fall, when the diurnal temperatures were lower than 12°C. The doses were varied according to the strength of the colony; 0.5 g of thymol per comb completely filled with bees. Treatment consisted of 4 identical applications carried out at 2 day intervals. The effectiveness was greater than 99% in broodless colonies, the lowest value (i.e., 94.99%) being due to the presence of some brood throughout the treatment period (Chiesa, 1991).

The combination of thymol, menthol, camphor and eucalyptol

Mixtures containing various proportions of these aromatic molecules were first tested in Tunisia by Popa (pers. comm.), with encouraging results. A few years later, a quaternary mixture (Apilife-Var®) was registered in Italy. Approximately 20 g of the mixture (76% thymol, 16% eucalyptol, 4% camphor, 4% menthol) was used to impregnate a small tablet of vermiculite. According to the experiments of Imdorf *et al.* (1994, 1995), it is more effective to place the tablet on top of the brood combs and to replace it with another one 3 or 4 weeks later. Under optimum conditions of application, the efficacy of the treatment was greater than 92% in Switzerland and in the South of France. However, in the Mediterranean climate it is essential to take several precautions: (i) the tablet must be above, but removed from the broodnest; (ii) the diurnal temperature must be over 25°C; and (iii) some races of bee are particularly sensitive to the treatment and may leave the hive. The problem of post-therapeutic residues can occur causing an alteration in the taste of the honey at application rates of 1.1 mg kg^{-1} (Imdorf *et al.*, 1994), but this poses no problems in terms of human toxicology.

Methods or products permitted in organic farming

Thermotherapy

Grobov (1977) reported that a temperature of 41°C kills the mites on adult bees within 5 minutes. According to Le Conte *et al.* (1990), the reproductive success of female mites in brood cells is significantly reduced at a temperature above 36.5°C. The use of thermoregulated chambers to kill the mites either on the adult bees or in the brood, has been suggested.

Copper salts

Considered as natural compounds in organic farming, Guiraud *et al.* (1989) suggested that these salts can, directly or indirectly, block the respiratory system of the mite. Cupric gluconate, which is less dangerous to bees than to the mites, was given to the colony in sucrose syrup or in sugar candy at a

dose of 2.5 g per colony. In a field experiment, Bruneau (1990) estimated the efficiency of mite control at 47% and consequently advised beekeepers against using it for treatment.

Concluding remarks

In conclusion, the authors, who have experience in the use of volatile substances for the treatment of mites, agree that the efficacy depends on the local conditions and that some care is needed. In the Mediterranean region, the optimal conditions are a high and stable external temperature and the absence of worker brood (the drone brood being cut out in spring). The main precautions consist of avoiding reinfestation and robbing (Nazzi *et al.*, 1995), in treating outside periods of nectar flow or queen rearing and in verifying the efficacy of the treatment (Milani, 1990). The alternative to chemical control is thus more than the simple use of a vegetable or animal extract in place of a synthetic acaricide. It requires an additional effort from the beekeeper to manage the bee colonies, which is more time consuming. For this additional investment, this type of treatment could alternate with chemical control every other year and effectively prolong the useful life of current acaricide products whilst also minimising the accumulation of residues.

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