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The varroosis in the Mediterranean region

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 21

1997
pages 77-86

Chemotherapy against *Varroa jacobsoni*: Efficiency and side effects

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SUMMARY - Chemical treatments have enabled a highly effective control of varroosis preventing great losses of colonies and allowing restoration of highly damaged apiaries to their former condition. Many acaricides of various chemical groups have been tested in several countries for their efficacy against *Varroa jacobsoni*. A range of substances have proved successful and have been approved for control in the infested countries with the appropriate modes of application. A reduction in the effectiveness of a widely used synthetic pyrethroid product, reported for the first time in Italy has been shown to be a consequence of the spread of *Varroa* drug resistant strains. This highlights the risk of basing control strategies on a single product and more generally on chemical treatment alone. Besides the required control effect, some of the drugs have also negative effects. The most important secondary effects include undesired effects on adult bees and brood and especially contamination of the beehive products. Among the hive products, the beeswax, as an effective residue store, has the central role in the residue accumulation process.

Key words: Chemotherapy, *Varroa jacobsoni*, acaricides, resistance, side effects, residues.

RESUME - *"La chimiothérapie contre* Varroa jacobsoni*: efficacité et effets secondaires". La chimiothérapie appliquée dans la lutte contre la varroose a permis d'éviter d'énormes pertes en colonies et de restaurer l'état sanitaire des ruchers endommagés. Plusieurs molécules acaricides de différents groupes chimiques ont été testées pour leur efficacité sur* Varroa jacobsoni. *Certaines de ces substances dont l'efficacité varroacide a été prouvée ont été enregistrées dans les pays infestés pour une mise en oeuvre dans le contrôle de l'acarien varroa selon les modes d'application les plus adaptés. L'apparition et la propagation de souches de varroa résistantes aux pyréthrinoïdes de synthèse, montre le risque qu'il y a à baser une stratégie de contrôle sur l'emploi continu d'une seule et même substance et plus généralement sur une lutte chimique exclusive. A côté des propriétés acaricides requises, certains produits peuvent avoir des effets secondaires néfastes les plus importants étant une toxicité pour l'abeille et l'accumulation de résidus dans les produits de la ruche. Parmi ces produits, le rôle de la cire, en tant que réserve de résidus est à considérer en premier.*

***Mots-clés** : Lutte chimique,* Varroa jacobsoni*, acaricides, résistance, effets secondaires, résidus.*

Efficiency of the chemical control of *Varroa jacobsoni* infestation

Following the invasion of western Europe by the disastrous mite pest *Varroa jacobsoni*, there has been an urgent need to develop and put into practice control measures. This caused a boost to research on varroosis control resulting in a tremendous amount of work and great efforts have been done in this field to find effective control methods. Now, since the apicultural practice showed that non chemical control methods in general are very labour intensive and often achieve a low efficacy, an organized fight against varroosis is almost possible with the help of chemotherapeutic measures (Ritter, 1981). The same situation will probably continue as chemical treatment will presumably keep on being more effective and less time consuming than biotechnical methods which must be at present combined with chemotherapy, including the use of natural substances.

Chemical treatments have enabled a highly effective control of varroosis preventing great losses of colonies and allowing restoration of highly damaged apiaries to their former condition. However, none of the methods used can eradicate the mites or stop them from spreading (Cavalloro *et al.*, 1988), because treatments with most acaricides are effective only when very little or no capped brood is present, a condition that occurs in colder regions during autumn and winter, but may never been satisfied in warmer regions. In addition, many products may be applied only over a given temperature, and this additional constraint is difficult to combine with the absence of brood (Milani and Barbattini, 1989).

Many chemicals (acaricides) have been tested all over the world for their efficacy against *Varroa jacobsoni*. A useful synopsis of products and active ingredients (a.i.) used for varroatosis control has been published by Wienands, (1988). Among these, a number of substances have proved successful.

Beside an optimal efficiency against *Varroa*, products administered into hives should also achieve the following important characteristics; they have to be: (i) well tolerated by the bee; and (ii) non toxic for man, consumer of the hive products and manipulator of varroacide agents. The efficiency of a given acaricide is varying depending on many factors such as bee colonies, climates, bee races, the time of treatment, modes of application, etc. The highest efficiency is reached when the active ingredient is evenly distributed into the hive so that each *Varroa* mite is exposed to a lethal dose. It is than possible to eliminate 90% or more of the mites born by adult bees, which result is remarkable in the apicultural practice (Colin and Gonzalez-Lopez, 1986). Most medications, whatever is the way of administration, do only hit mites parasitizing adult bees, while those reproducing inside the sealed brood are not reached.

Modes of application of chemotherapeutic agents

The early methods of chemotherapy of varroosis were mainly traditional pest control methods. Spraying, dusting, fumigation and aerosol were typical modes of application used in the "first generation" of control methods for *Varroa jacobsoni*.

Dust on

Currently used in Greece and Rumania, this mode is applied to treat colonies in activity before and after the collecting period. Active ingredients (Malathion, naphthalene, thymol, chlorobenzylate, etc.) are mixed with a vehicle which can be glucose, marble dust, kaolin or cellulose. About 50 gr are necessary for one application. The main disadvantage of this method is the contamination of the hive products either by the *a.i.* and the excipient.

Evaporation

Evaporating agents which are volatile compounds have been widely used for the control of the tracheal mite, *Acarapis woodi.* Treatment with these agents can be carried out without disturbing too much the bee colonies; it necessitates doses ranging between 0.5 to 50 gr of a.i. and a period of application spreading from one to several weeks. It is difficult to control the daily evaporated quantity of the product since this quantity depends on the external temperature. In addition, beeswax absorbs, reversibly or not, a non-negligible amount of the volatile compounds leading to accumulation problems. The main substances used are menthol, thymol, formic acid, naphthalene, etc.

Spray

Spray products were very common. Spray treatment however is very time consuming as combs have to be taken out and treated individually, constraining therefore the manipulator to spray during daytime only (Ritter, 1983). The main products which have been used by spray application were Kelthane and Tactik (a.i. = amitraz).

Fumigation

Treatments using fumigation have replaced evaporation in the control of the tracheal mite *Acarapis woodi.* Active substances incorporated to the fumigant strip must have a good stability since the molecule has to sublimate into the hive near a source of heat (heat emitted by nitrate combustion in some cases). In order to reduce the important irritation caused to the bee colony when treated by fumigation, artificial means like enlargement of the beehive are used. Main substances used in such a way are, dicofol, phenothiazine, bromopropylate (Folbex V.A.®), etc. Bromopropylate was shown to have an adequately good effect against *Varroa* mites (Klepsh *et al.*, 1983) while in the same time it is well tolerated by the bees and the brood.Treatments applied by fumigation led to residue accumulation problems.

The further development of chemical varroa control methods brought a change in the application method. Modern methods include:

Systemic treatments

The first systemic acaricide used for *Varroa* control was chlordimeform (Ruttner *et al.* 1980) which was abandoned because of residue problems. Later, Perizin® (Ritter 1985) was developed and commercialized as a precursor of the "second generation" of varroosis treatments. Fed to bees and biologically 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 easy and fast: the medicinal syrup is sprinkled into the bee ways. A specific problem with systemic acaricides is that the bees which take up the administered syrup absorb a so high amounts of acaricides up to the level of toxicity that some initial bee mortality has to be accepted (Koeniger and Chmielewzky, 1986).

Heated aerosol treatments

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

The warmth of the aerosol breaks up the cluster of bees when the brood is absent allowing treatment when ambient temperature is low; in addition the efficacy of the acaricide is improved by the heat. The production of a heated aerosol necessitates special devices not always at beekeeper's disposal.

Slow releasing methods

Another way of distributing the active ingredient with the help of the bees themselves is by using the mechanism by which the bees distribute the queen substance throughout the colony continuously (de Ruijter and Van Den Eijnde, 1988). In this type of drug the active ingredient 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 varroatosis. The use of impregnated inserts with a prolonged effectiveness has evident advantages compared to the previously practised treatments; once applied these products are effective for a long period of time, mites being killed as they gradually leave brood cells and reinfestation is avoided as the product remains active. Inserts are prefabricated plastic strips which are ready to be hung directly between the frames in a honeybee colony. Among these products, polyvinylchloride strips containing fluvalinate (Borneck and Merle, 1987) quickly became one of the most widely used products over the world. Similar strips made with polyethylene and containing flumethrin, another Pyrethroid, (Koeniger and Chmielewsky, 1986; Koeniger and Fuchs, 1988) are commercialized in some European countries. Recently, amitraz, an *a.i.* belonging to the formamidines group and known for his high efficiency against *Varroa* has been incorporated to plastic strips and commercialized in France under the name of Apivar®.

Acaricides used for Varroa *control*

The choice of acaricides and their mode of administration changes from one country to another; the making of rules dealing with beekeeping chemotherapeutic products is not the same everywhere depending on wether beekeeping is considered as an animal or a vegetal production. Post-therapeutic residue levels of many substances have not been fixed by specific norms. All these factors led to a great diversity in the treatments being practised in different areas. However, if efficiency and safety for human consumer 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, way of administration, dosage, etc.

Table 1. Authorized acaricides used in Europe for the control of *V. 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	
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 gr granules in 100 ml water or dependent on colony size	0,04 gr (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 26 days	2 times	3-4 times	1 time for 26 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 10 °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 1/2 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
Literature	Ritter and Perschil (1983)	Wachendörfer *et al.* (1985)	Ritter (1986)	Colin *e al.* (1983)	Richez and Le Conte (1995)	Ritter (1985)	Santas *et al.* (1981)	Borneck and Merle (1987)	Ritter (1988)	Koeniger and Chmielewksy (1988)

Chemical control, resistance of *V. jacobsoni* to acaricides

Selection of resistant mites to pesticides is a well known phenomenon. The first cases of resistance to organophosphates have been reported since 1947. According to Delorme and Dacol (1989), all crops enemies may develop resistance to pesticides used in control strategies. This possibility is especially greater as mites have in general short life cycles particularly *Varroa* which perform twelve generations per year. It also becomes more probable to see *Varroa* mites developing resistance as a result of a prolonged contact with an acaricide. That is one of the reasons why manufacturers of products for controlling *Varroa*, advise users to limit the frequency and period of use. In spite of all, it is now proved that varroacides such as fluvalinate (Lodesani *et al.*, 1992) accumulate in wax creating conditions for a prolonged contact with *Varroa* especially inside the cells alveoles where it reproduces (Vandame *et al.* submitted). It is likely for *Varroa* to develop resistance more especially as many other mesostigmatid mites have already been reported to be resistant in particular to synthetic pyrethroids including fluvalinate.

Ineffectiveness of fluvalinate in Italy

This active ingredient for varroatosis control has become widespread over the last few years because it is easy to use and relatively cheap. A specific product for varroatosis control consists in polyvinylchloride (PVC) strips impregnated with the a.i. at a concentration of 10% under the commercial name Apistan®. This product have been proved to be highly effective against *Varroa* (Borneck and Merle, 1990: 99.38-99.72%; Llorente Martinez *et al.*, 1989: 99.33%; Llorente Martinez *et al*, 1989: 99.33%). Nevertheless, Apistan® strips have often been replaced by operators on their own initiative with another product containing the same active ingredient. Klartan® and Mavrik® are a not authorized products for use in beekeeping but they are widely administered using especially wooden inserts where the fluvalinate content cannot be controlled. leading to problems of effectiveness.

Studies conducted in Italy (Lombardy) to investigate if, in fact, there was some resistance of *Varroa* to fluvalinate, showed individual colony variation and differences in the average effectiveness obtained in the apiaries. These observations provide evidence for the presence of strains of *Varroa jacobsoni* resistant to fluvalinate in the apiaries where treatments were unsatisfactory.

To assess the susceptibility of *Varroa* mites to fluvalinate and other pyrethroids, Milani, (1995) has developed a bioassay which was tested on strains of *Varroa* believed to be susceptible and strains of mites surviving Apistan® treatment and thus supposed to be resistant. The median lethal concentration (L_{50}) of mites originating from areas where treatments with fluvalinate are no longer effective was about 25-50 times higher than that of susceptible mites. The (L_{50}) of flumethrin and acrinathrin on mites surviving Apistan treatments increased 10-60 times. In a similar study, Colin *et al.* using another laboratory test found great differences in the resistance level values calculated for *Varroa* populations originating from geographically distinct regions of France. These values range between 1.92 and 182.7.
Resistance have also been reported to other acaricides belonging to different chemical groups. Resistance to bromopropylate and to chlordimeform was shown to be favoured by underdoses and this has been verified with laboratory tests (Ritter and Roth, 1988)

The presence in different countries of *Varroa* strains simultaneously resistant to different pyrethroids, points out the risk of basing the control strategies on chemical treatments only, particularly when the substances used belong to the same chemical family.

Chemical control of *Varroa*, adverse side effects

Effects on bees

Many papers on the chemical control of varroatosis make observations on bee tolerance of varroacide products. Some papers compare the toxicity to bees and to mites, which is more interesting than the absolute DL_{50} for bees (Milani, 1992). All acaricide substances have weak feeble insecticide characteristics, thus, the clinical signs of an eventual toxicity for bees will not express themselves under an acute form but rather under a chronic form essentially for the queen which has the greatest longevity in the colony. Some varroacide products have been shown to cause some bee mortality immediately after the treatment (Kilani *et al.*, 1981), others have an influence on viability (queen loss) and fertility of the queen or an appreciable toxicity for the brood which is more susceptible to acaricides than adult bees (Marin, 1977).

Residue problems

The risk that residues can be detected in bee products such as honey, wax and propolis is as high as lipophilic pesticides are more frequently introduced into hives. Since most insecticides and acaricides are more or less lipophilic, they are accumulated in wax and then may be slowly released into honey. The presence of residues is detrimental to the image of the beekeeping which products are well-known to be natural and unpolluted. Contamination of the hive products has become a serious problem entailing checking for residues of the acaricides employed. Though studying interactions between varroacides and each hive product is necessary, the beeswax, as an effective store, has the central role. Higher levels of some insecticides used for agricultural purposes are usually found in old beeswax indicating that residues accumulate in combs over the years (Gayger and Dustmann, 1985). Tests with more than five hundred wax samples showed that in all countries where varroacides are used, uncontaminated beeswax can hardly be found. Often various pesticides can be detected in one sample (Wallner, 1995). The residues accumulation of acaricide products in wax has negative implications especially the consumption of contaminated honey and the use of this wax in the cosmetic and pharmaceutical industries. Another important consideration is the induction of drug resistant *Varroa* strains as a consequence of the presence of the active ingredient in wax at sublethal levels and for long periods of time.

For certain varroacides which are weakly bound to wax, like coumaphos (Perizin®), bromopropylate (Folbex VA Neu®), fluvalinate (Apistan® - 10% a.i. or Klartan® and Mavrik® -24% a.i) the higher the concentration in the wax is, the more residues can be detected in the honey. With these products, concentration in wax of only 1mg/kg is sufficient to detect them in honey. In contrast, active ingredients (flumethrin) known as having an extremely high affinity for wax and consequently a feeble tendency to migrate are not detected in the honey even with a wax concentration of 400 mg/kg (Wallner, 1995). When treatments are carried out properly according to the directions for use, the levels of residues of a.i. of products legally registered are rather low. These residue levels overstep the limits allowed by current regulations when beekeepers do not conform to the directions for use either applying a greater number of treatments than required or using doses higher than necessary. The use of agricultural products, not suitable for use in the beehive leads to the same problems.

Cohabitation with varroosis has been achieved owing to chemotherapy, but problems either of residue accumulation in the beehive products or *varroa* drug resistant strains highlight the risk of carrying control strategies based only on chemical treatments.

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