VARIATION IN THE EFFICACY OF TREATMENT WITH APIGUARD® (VITA EUROPE) ON THE BASIS OF THE VOLUME OF AIR AROUND THE PRODUCT

by Marco Lodesani, Manlio Pugliese[1], Cecilia Costa, Simone Franceschetti, Eleonora Bergomi
All authors from CRA-Istituto Nazionale di Apicoltura, Bologna
Except [1] Associazione Apicoltori Reggio Emilia e Parma

INTRODUCTION

After years of relative tranquillity in the battle against varroatosis via administration of evaporates *(ApiLife Var®)*, the summer of 2005, with its meteorological changes outside the norm (temperatures in the month of August closer to autumn values than summer ones), created some difficulties in the buffer treatment strategy for late summer. The low temperatures recorded for a consistent period of time caused the sublimation of the *ApiLife Var®* tablets.

The implementation of the 2006 health plan resulted, for the *Associazione di Reggio Emilia e Parma*, in some changes not related to the active ingredient as such (thymol), but rather to the adoption of various administration techniques that saw the integration of the traditional *Api Life Var* with a new product (*Apiguard®*), which is also a thymol-based product but has characteristics that allow it to operate satisfactorily where temperature ranges are greater.

The collaboration between the *Associazione* and the National Apiculture Institute (INA) envisaged, *inter alia*, the monitoring of the natural death of the varroa mites, which was undertaken using "sentry" hives located at various points within the territory of the province; these provided information about the natural death of the varroa mites: the periodic counts of numbers of dead varroa mites on the sheets placed in the trays provided guidance as to the changes in infestation.

Among the practices for biomechanical control, one of the methods also recommended, especially during the month of July, was the removal of some sealed brood frames so as to reduce the level of infestation of the hives.

In this experiment, we wanted to verify whether and how the "air space" left around the gel influenced the efficacy of the treatment. Indeed, in the experiments performed to date, no consideration has been given to how the "air space", ie the space left between the upper bars of the combs and the apex of the crown board, influences the efficacy of the product.

MATERIALS AND METHODS

TECHNICAL INFORMATION RELATIVE TO THE PRODUCT USED

Apiguard® is a thymol-based (25%) acaricide product made by Vita (Europe) Limited; the specific gel formulation allows gradual release of the active ingredient.

The gel acts as a sponge tablet in which the mesh structure thickens or thins out with changes in temperature. When the temperature increases, the volatility of the thymol increases but the mesh structure of the gel thickens at the same time, thus regulating the release of the active ingredient. Because of the particular composition of the product, in which the thymol is "imprisoned" in the mesh structure of the gel, it is necessary for it to be transported via physical contact among the bees, which

then spread it throughout the nest. The product must therefore be highly accessible. The other action occurs via sublimation: the thymol is spread through the hive via ventilation by the bees in their attempts to get away from the vapours, thus directing it towards the entrance to the hive.

Each hive was administered 50 g of gel previously spread uniformly on the cardboard provided (12 x 9.5 cm). In total, two applications were performed, each with a duration of two weeks.

The manufacturer recommends use of the product when the mean daily temperature varies between a minimum of 15°C and a maximum of 40°C.

DOSES AND METHOD OF ADMINISTRATION

In this test, we used the product in the 3 kg tub presentation with the following doses:
• 2 applications of 50 g each, two weeks apart, on cardboard (12 x 9.5 cm) provided by the company.

Vita Europe confirms that the applications of 50 g guarantee the presence of a greater quantity of thymol vapour at any temperature and therefore greater efficacy. Indeed, the company has also indicated another dose (2 applications of 25 g each, one week apart), but this is indicated for use where the maximum daily temperature is greater than 25°C.

Given the impossibility of predicting weather changes and having seen the meteorological data for the past few years, which are characterised by particularly variable Augusts with drops in mean daily temperatures of as much as 10° compared to the mean values, it is preferable to opt for administration of 50 g.

METHOD OF ADMINISTRATION: VOLUME OF THE AIR SPACE

The manufacturer recommends a space of at least 0.5 cm between the layer of gel and the crown board; this corresponds to a total space of 1 cm (0.5 cm height of the gel layer + 0.5 cm of free space) between the upper part of the combs and the crown board. This space cannot be obtained by positioning the crown board in the normal way; this distance is in fact on average 6.5 mm (the so-called “bee-space”).

The hives tested have therefore been subdivided into three groups with different volumes of upper air space (the space between the apex of the combs and the upper closure) inside which the product is placed; this product requires adequate ventilation and contact by the bees in order for its action to occur in the correct manner (explanation of experimental groups in Fig. 1).

Fig. 1

Group 1: “BEE-SPACE” without any additional spacing, 6.5 mm space between the apex of the combs and the crown board, equivalent to an air space of 1,181 cm3 (6.5 mm H x 385 mm L x 472 mm D)

Group 2: HIGH BEE ESCAPE FRAME 22 MM placed between the nest and the crown board (equivalent to a standard inverted crown board), 28.5 mm of space between the apex of the combs and the crown board, equivalent to an air space of 5,179 cm3 (28.5 mm H x 385 mm L x 472 mm D)

Group 3: HIGH INVERTED CROWN BOARD (60 MM H INTERNAL SECTION), 66.5 mm of space between the apex of the combs and the crown board, equivalent to an air space of 12,084 cm3 (66.5 mm H x 385 mm L x 472 mm D)

NUMBERS, PERIOD AND TEST LOCATION

The test was performed in the province of Reggio Emilia, in two separate apiaries located in Coviolo and San Rigo (the foothills). The treatments were commenced in August 2006 using the following timetable:

- 10 August: first treatment with 50 g of *Apiguard*® gel spread over cardboard placed on the frames;
- 24 August: second treatment with *Apiguard*® and caging of the queen;
- 6 September: caging of the queen;
- 14 September: control treatment (50 ml of dripped oxalic acid) without brood sealed and receptive to the mite.

The placing of the queen in the cage, where she was then left suspended between the central combs until release, is necessary to prevent her laying; in this way, at the time when the control treatment with oxalic acid is applied eight days after her release, all of the mites will be on the adult bees and therefore in a "receptive" state, and easily accessible by the treatment. The hives (Dadant Blatt hives with ten frames) were naturally infested with varroa mites and had not undergone any treatment since that applied in the winter of the previous year.

In the two apiaries in which the test was performed, from the 31 initial hives (16 + 15), half were selected following controls to assess the uniformity of the hive (quantity of brood, adult bees and honey stock) and of the infestation, assessed via the natural death of the varroa mites in the week preceding the commencement of the test. In the end, excluding all the cases of incongruity, the test was performed on 10 + 10 hives.

Table 1. Efficacy values obtained following treatment on the basis of the air space around the product and associated variability indices; se: standard error of mean; **C.V.**: coefficient of variation of the mean; different letters in the significance column indicate significant differences between the groups.

METHODS	mean (%) (± se) [min-max]	SIGNIFICANCE	CV(%)
BEE-SPACE - volume: 1,181 cm	78.3 (± 4.62) [52 -88.1]	a	15.62
BEE ESCAPE FRAME - volume: 5,179 cm3	87.6 (± 2.45) [80.7 - 97.2]	ab	7.42
HIGH CROWN BOARD - volume: 12,084 cm	92.4 (± 0.91) [89.8 - 96.8]	b	2.62

The allocation of the hives to the three groups subject to analysis was done via drawing lots.
Other hives - in variable numbers in the two options - were kept as a control group relative to the parameters for development of the hives. The reading of the numbers of mites killed on the adhesive sheets placed in the boxes under the screened bottom boards was taken every 2-3 days in the first week of treatment and every 4-5 days during successive periods. The hive entrance constitutes the only point of aeration of the hive. The efficacy of the treatment was calculated according to the following formula:

E% = (VT x 100) / (VT + VC),

where:
E% = Efficacy
VT = varroa mites killed during the treatments with thymol.
VC = varroa mites killed during control treatment with oxalic acid.

The coefficient of variation (CV) used for comparison between the treatments was calculated according to the following equation:

CV= sd x 100/mean

where:
sd = standard deviation.
In general, the design of the experiment was in line with the provisions of the "European Group of integrated varroa Control" (Anonymous, 1999).
The climatic data were read from the nearby centre of the ARPA meteorological service.

RESULTS AND DISCUSSION
OVERALL TEST CONSIDERATIONS

The temperatures recorded during the test period were in compliance with those prescribed by the manufacturer, except for several hours during the first treatment where maximum values of 42°C and minimum night-time values of below 15°C were recorded, with mean values around 25°C. During the second treatment (late August / early September), the temperatures dropped noticeably, as frequently occurs during this period, with night-time minimum values of 7°C but with mean values for the period still above 20°C. With respect to rainfall, during the first treatment, there were two afternoon sessions that were not particularly intense, and the same again during the second treatment, with the last one heavy and long-lasting; during the treatment with oxalic acid, there were three days with summer weather. Considering the period, the change in rainfall is considered to be average for recent years and not prejudicial for the outcome of the test.

In no case were side effects observed other than a greater exodus of bees in the hours immediately following administration of the product, and this only occurred in some hives. All of the queens survived the period of enforced confinement, until resumption of spring in the following year. From the end of treatment until the winter period, the production of brood and the honey stocks (although these were not measured using exact quantitative methods) were not different in the groups with different spacings and in the control hives treated with different products.

Considering the data as a whole, the change in the numbers of varroa mites killed during the two successive treatments reflects the values traditionally seen with sublimating products, with a gradual loss of acaricide power with the passage of time down to a minimum killing power of a hundred or so mites at the end of the first treatment and a rapid resumption of the death rate on placement of the second gel treatment, followed then by a fall to a few mites/day at the end of the two treatments. The cumulative percentage death rate shows a rectilinear development except for a slight kink towards the end of the first treatment: the linearity of the change demonstrates that there were no particular events to disturb the normal course of the test.

SUBDIVISION OF THE RESULTS BY APIARY AND BY TREATMENT

In the Coviolo apiary, on average 2,188 (± 677 standard error) mites were killed per hive, for all treatments combined (*Apiguard*® + oxalic acid), while in the San Rigo apiary, the mean was 3,732 (± 716 standard error) mites. The differences in infestation between the two locations are probably due to the fact that in spring, in the Coviolo apiary, 1-2 combs of sealed brood were removed per colony for the formation of nuclei.

In each case, the absence of statistically significant differences in the values for total deaths obtained in the two different apiaries, both for overall data (F = 1.97; P = 0.178) and in the interactions with the various treatment groups (F = 0.52; P = 0.60), makes it possible to consider the data obtained in the two apiaries together, grouping together the results for the colonies subject to the same experimental variables.

In total, at the end of the first treatment, on average 1,603 (± 305 standard error) mites died, after the second 948 (± 162 standard error) and with the control treatment 409 (± 101 standard error), thus providing an overall efficacy for the treatment with *Apiguard*® of 86.18%, which is in line with the mean data obtained by Baggio et al. (2004), and slightly lower than those for Nanetti *et al.* (2005), in excess of 90%.

For the first gel treatment, on average 57.9% (± 2.9 standard error) of the mites died compared to the overall total for both gel treatments. On average, the data on efficacy of the first treatment were lower than those reported by other authors (Marinelli et al., 2001: 65% in the first treatment, 70% in the second; Baggio and Mutinelli, 2002: 66.9%); this is probably due to a greater quantity of brood hatched in the second treatment, which in turn was the result of an abundant collection of honeydew in the weeks immediately preceding the commencement of the test.

COMPARISON OF THE RESULTS FOR THE EXPERIMENTAL GROUPS

The overall efficacy of the treatments in Group 1 was 78.3% (± 4.62 standard error), for Group 2 this was 87.6% (± 2.45 standard error), and for Group 3 this was 92.4% (± 0.91 standard error). The differences in efficacy between Groups 1 and 3 were significantly different (Bonferroni test P = 0.027) while those between Groups 1 and 2 were not (P = 0.20) and neither were those between Groups 2 and 3 (P = 0.568) (Table 1) (Fig. 7-8).

In addition to the result in itself, which is already enough to express a positive opinion on the need for a greater space around the gel, it is interesting to compare the variability of the data obtained for the three tests on the basis of coefficients of variation (CV). In the bee-space group, the CV was 15.62, in the bee escape frame group it was 7.42 and in the high crown board group it was 2.62 (Table 1).

There was therefore an inverse change between the volume of air and the variability of efficacy. In particular, the CV obtained with the largest air space was six times lower than that for the minimum space, while the intermediate volume of air achieved an intermediate CV value; this indicates, in the case of the maximum volume of air available for the product and the bees (high crown board), a great degree of uniformity of the efficacy data, which is what the beekeeper wishes to obtain when administering a drug: that it is equally effective in all hives treated.

In this respect, we should note that a few mm difference in height translates into significant differences in volume (eg 0.5 cm more in height = 909 cm3 more volume). The differences between a low inverted crown board (or bee escape frame) and a high one can therefore be in the order of 7,000 cm3, with more than double the volume available for the evaporate.

The efficacy data for *Apiguard* in the bibliography are highly variable and no reference is made to the volume of the air space but rather only to whether the crown board has been inverted. Baggio et al. (2004) noted a limited acaricide action using Apiguard in conditions where colony activity was low, while other authors (Papachristoforou et al., 2004) in Greece obtained values greater than 90% with various types of administration and during various periods of the year (90.30%-93.91% in spring, 95.09%-96.59% in summer). In some works (Veca & Colombo, 2006), a “normal” crown board (2-3 cm) was used, obtaining an efficacy value of 74.6%, while in other works (Marinelli et al., 2001; Marinelli, personal poster) performed with higher crown boards (approximately 5-6 cm), the efficacy

was noticeably greater (94.3% in 1999, 96.5% in 2000), but these works often do not provide specific details of how much space was left above the product.

In an analysis by Esquijarosa (2003), the efficacy obtained with treatment was very much lower (56.3%): one of the factors that could have influenced these data, according to the author, related to the insufficient space between the gel and the crown board.

The data therefore conclude that the presence of a large air space around the gel administered on cardboard is essential for obtaining a satisfactory therapeutic efficacy of the product that is, on average, proportionally greater with the increase in the space above the gel.

This is probably due to the better circulation of air and therefore a more constant and complete sublimation of the active ingredient enclosed in the gel, which makes it possible to optimise the spread and action of the thymol.

BIBLIOGRAPHY

Anonymous, 1999. Recommendations from the CA3686. The evaluation of a treatment against Varroa jacobsoni. Concerted Action “Coordination in Europe of Research on integrated control of varroa mites in honey bee colonies”. Meeting Merelbeke, Belgium, 13-14 November 1999.
Baggio A. and Mutinelli F., 2002. Principi attivi per la lotta alla varroasi, esperienze di campo in Atti del Convegno finale “Il ruolo della ricerca in apicoltura”, Bologna, March 2002: 99-103.
Baggio A., Arculeo P., Nanetti A., Marinelli E., Mutinelli F., 2004. Field trials with different thymol-based products for the control of varroasis. American Bee Journal 144(5): 395-400.
Esquijarosa E.M., 2003. Apilife Var and Apiguard: evaluation of two organic treatments against varroasis and acariosis of the honey bee. In www.apimondia.org/apiacta/slovenia/en/esquijarosa.pdf
Marinelli E., DePace F.M., Ricci L., Persano Oddo L., 2001. Impiego di diversi formulate a base di timolo per il trattamento estivo antivarroa in un ambiente mediterraneo. L'Ape nostra amica 23(5): 6-10.
Nanetti A., Bacandritsos N., Papanastasiou I., Saitanis C., Papadoulis G.T., 2005. Studio comparative su sei formulate contenenti timolo nel controllo di Varroa destructor (Anderson & Trueman) in Grecia. Apoidea 3(2): 126-133.
Veca M. and Colombo M., 2006. Efficacia del timolo nel controllo di Varroa destructor. L'Apis N. 9: 11-17.
Papachristoforou A., Xonis K., Mariphoglou P., Panagiotou P., Kostadinidou E., Felekidou P., 2004. Prove di campo per la verifica dell'efficacia di Apiguard nel controllo della varroasi. Apitalia 7-8: 37