Apidologie, 1988, **19** (2), 131-138

APPLICATION OF CONTACT ACARICIDE AGAINST *VARROA* MITES WITH CONTAMINED PROTEINACEOUS FOOD

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SUMMARY

In an effort to find new ways to control varroatosis, malathion was mixed with dry pollen substitute in a concentration of 50 ppm. The preparation was put in a sickle-like arrangement in empty cells in the central area of the combs. These combs were put into 15 bee colonies during the period from November 1985 to March 1986. This kind of treatment was quite satisfactory to control Varroa mite without disturbance of bee colonies during the late season when the winter cluster was formed. The position of contaminated comb (s) within the cluster was an important factor for the effectiveness of the acaricide. About 50 % of the dead mites were found on the bottom of the hive during the first day and 80 % during the first three days after the application of the preparation. The toxicity of malathion was masked but not lost 10 days after the introduction of contaminated comb in the bee colony.

The brood and the adult worker bees were not affected. All colonies overwintered successfully and no queen loss was observed.

INTRODUCTION

The condition that is most important for the application of an acaricide against varroatosis is the absence of sealed brood in the bee colony. It is very laborious to achieve this condition during the spring or summer. On the other hand, the effectiveness of most chemical agents is negatively affected by the low air temperature when applied during the broodless period (winter). Lastly, almost all methods of chemotherapy of Varroatosis involve repeated applications of the drug.

To overcome, to some extent, these disadvantages we tested a new method involving the application of contact acaricide late in the season after the cluster is formed.

MATERIALS AND METHODS

Dry pollen substitute (90 % soybean flour and 10 % pollen powder) was contamined with malathion to a concentration of 50 ppm and then was put in empty cells of combs. These combs were inserted in colonies during winter period when the cluster was formed.

Treated combs were put in the colonies at the beginning of the experiment and remained there till the end of the treatment. The contaminated comb area constituted about 1/5 of the whole comb area had a sickle-like shape facing the bottom of the frame and occupied a position slightly over the center of the comb. Each comb contained about 200 g of the preparation.

The number and the position of contaminated combs placed in the colonies varied as follows :

a) One comb was put in the center of the winter cluster in 3 colonies.

b) One comb was put at each side of the winter cluster in 3 colonies.

c) One comb was put in the center and two others, each at each side of the cluster in 3 colonies.

d) Combs with and without contaminated preparation were put in alternative places in 6 colonies.

e) Combs with uncontaminated pollen substitute were put as in case d) in 3 colonies (control).

The rate of *Varroa* infestation was determined before and after application by collecting 3 samples each of 100-150 worker bees from 3 different frames of each colony, as suggested by PAPPAS and THRASYVOULOU (1986). The number of *Varroa* mites for each sample was found as described by DE JONG and GONCALVES (1981).

By using hive traps (IFANTIDIS, 1981), the daily number of dead bees for 10 successive days before and 13 days after the introduction of contaminated comb (s) was recorded for each colony. The possibility for a harmful effect of this treatment against adult honey bees was also studied by marking sixty worker bees immediately after their emerging. These bees had been fed with contaminated food during their larval and also during early adult stage. The daily number of dead mites was also recorded in the same traps.

This work was done at the apiary of Aristotle University of Thessaloniki during winter of 1985. The average temperature during the months of experiment was 10.3 °C with range of 0.0-21.4 °C.

RESULTS AND DISCUSSION

Some hours after insertion of the contaminated comb(s) in the experimental colonies, the bees were observed removing excess proteinaceous food out of the completely full cells. Their effort continued until they leveled the food to the usual depth that is observed in cells occupied by naturally deposited pollen. As a result of this, large amounts of the preparation were found on the bottom of the hive on the first day of application and progressively smaller amounts on the following days.

In their effort to remove the excessive food from their cells the bees used their mandibles and their legs. One could see a slight covering of the proteinaceous food on the pollen brushes of a great number of worker bees. The mites could come in contact with the acaricide as they were carried on the contaminated bees and probably as they walked for short periods on the contaminated area of the combs.

Table 1 shows the results of the effectiveness of the preparation against *Varroa* in connection with the number and the position of contaminated combs in the winter cluster. It appears that the position of the contaminated combs is a more significant factor for the effectiveness of the acaricide than their number. Using only one comb in the center of the winter cluster (treatment a) the decrease of infestation after the application was higher than the case of using two combs at the outside of the cluster (treatment b). In treatments a, c, and d the infestation level was reduced to values near zero regardless of the initial level of infestation as a result of the combined effect of position and number of the contaminated combs.

TABL. 1. — *Effect of pollen substitute contaminated by malathion (50 ppm) against* Varroa *mite* [1]

Treatment [2]	Varroa infestation (%) on adult bees		Mean change
	before treat.	after treat.	
a	9.65 ± 3.37	0.47 ± 0.79	9.18
b	9.06 ± 4.03	5.50 ± 2.52	3.56
c	7.50 ± 3.54	0.50 ± 0.50	7.50
d	10.15 ± 3.72	0.30 ± 0.21	9.85
e	7.23 ± 3.37	5.10 ± 2.96	2.13

(1) The period of experiments was from : 29/11/85 to 9/1/86 for treatments a, b, c, e ; 29/12/85 to 10/3/86 for treatment d.

(2) As indicated in materials and methods.

The slight reduction of infestation rate in control colonies could be attributed mainly to the natural death or loss of the mites (treatment e).

Figure 1 shows the daily mortality of *Varroa* in treated colonies. About 50 % of dead mites were found on the bottom of the trap during the first 24 hours and 85 % during the first 3 days.

The number of dead mites observed approached zero about 10 days after application. This was an indication that the mite infestation was also approaching zero and was confirmed by worker bee sampling (Table 1).

The mortality of mites carried on adult bees, although extensive, may allow a few of them to reach and enter the brood cells of the appropriate age. The small percentage of surviving mites and the restricted area of brood in which they could be protected should not significantly decrease the effectiveness of this method of treatment. We verified this by opening sealed cells of 4

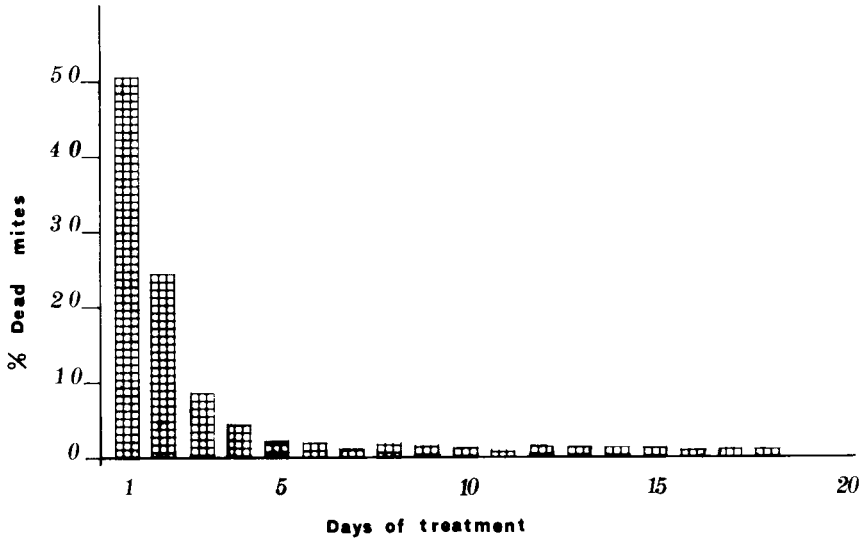

FIG. 1. — *Distribution of daily* Varroa *mite mortality in six broodless colonies treated with contaminated pollen substitute with 50 ppm malathion for the period of 29-12-85 to 10-3-86*

Total number of trapped dead mites : 5859.

colonies, where we found 0.0, 0.3, 2.0 and 3.0 % infestation, while the infestation in a control colony was 13.5 %. By converting these percentages to absolute numbers, on the basis of the mean amount of sealed brood of all treated colonies, the resultant number was about 25 mites/colony in the brood cells.

To see whether malathion maintains its effectiveness in the cells for a period longer than that of the initial mortality of parasites (Fig. 1), a contaminated comb was transferred to a new colony after it had been used for two weeks in another colony. On the day following introduction the number of dead mites was small and not significantly different than that observed before the introduction of the comb. When the « packed » contaminated food was dug out of the cells with forceps and was then put back in the same colony, the effectiveness of malathion against *Varroa* was again visible. It appears that the toxicity of the acaricide was not lost but it was « masked » 2 weeks after the introduction of the comb due to the restricted contact of the bees and mites with it in the semifilled cells.

In a previous paper we found that the proteinaceous food containing 50 ppm malathion was not harmful to honey bee brood (IFANTIDIS *et al.*, 1986). In the present paper we examined the effect of this food on 60 young adult (marked) bees because of the intensive consumption of pollen during the first

TABL. 2. — *Effect of pollen substitute contaminated by malation (50 ppm) on brood and adult bees*

Treatments [1]	Sealed brood area in/dm [2]		Mean change of sealed brood [2]	Daily number of dead bees		Mean change of dead bees [2]
	Before application	After application		Before application	After application	
a	1.40 ± 1.39	1.93 ± 1.05	+ 0.53	5.2 ± 4.65	1.6 ± 0.60	− 3.6
b	1.40 ± 1.68	1.65 ± 0.35	+ 0.25	2.5 ± 0.72	2.4 ± 1.31	− 0.1
c	1.50 ± 2.19	1.26 ± 0.97	− 0.24	1.80 ± 1.21	4.5 ± 3.64	+ 2.7
d	0.15 ± 0.18	1.93 ± 0.81	+ 1.78	0.71 ± 0.80	2.53 ± 1.87	+ 1.82
e	1.50 ± 0.67	2.03 ± 0.67	+ 0.53	2.53 ± 1.75	2.06 ± 1.55	− 0.47

(1) As described in materials and methods.

(2) Data on brood area and on bee mortality were taken for 10 days before and 13 days after malathion application.

weeks after their emergence. We found no differences between these bees and control bees as far as their longevity is concerned.

Table 2 shows the area of sealed bee brood and the daily number of dead bees before and after application. In most cases the production of brood during the treatment was slightly increased. The brood was compact and no dead larvae or pupae were found in their cells. On the other hand the daily number of dead adult bees, that were trapped at the bottom of the hive, remained at the same negligible level before and after the introduction of the contaminated combs. All colonies overwintered successfully and no queen loss was observed.

The treatment of varroatosis tested in this research showed that *Varroa* can be destroyed to a very satisfactory extent in winter when the cluster has been formed. The toxicity of malathion applied with this method seems not to be effected by the temperature probably because the bee cluster itself keeps the temperature high enough. The disturbance of the colonies was not serious. In addition, no negative effects were detected on brood. From the practical point of view, it should be emphasized that the beekeeper needs to open the colony only once during the period of the treatment. The contamination of honey and other bee products was not serious and will be reported on another paper.

Received for publication in September 1987.
Accepted for publication in March 1988.

RÉSUMÉ

ADMINISTRATION D'ACARICIDES DE CONTACT POUR LUTTER CONTRE L'ACARIEN *VARROA* PAR MÉLANGE DE LA SUBSTANCE ACTIVE À LA NOURRITURE PROTÉIQUE DE L'ABEILLE

Dans l'optique de mettre au point de nouveaux moyens thérapeutiques contre le varroatose, on a mélangé du malathion, à la concentration de 50 ppm, à un succédané de pollen. Avec cette préparation on a rempli des cellules vides. La surface du rayon ainsi traité représentait environ 1/5^{e} de la surface totale, avait une forme de croissant ouvert vers le bas et occupait une position légèrement au-dessus du centre du rayon.

Les rayons traités ont été placés dans la grappe hivernale et y sont restés jusqu'à la fin de l'expérience. Chaque rayon renfermait environ 200 g de préparation. Les essais ont été menés à Thessalonique de novembre 1985 à mars 1986. A cette période la température atmosphérique journalière varie de 0 à 21 °C. Outre l'efficacité de la préparation contre le parasite, on a également testé sa nocivité éventuelle vis-à-vis du couvain et des abeilles adultes. On ne présente pas dans ce travail de données concernant la pollution du miel engendrée par cette méthode ; elles feront l'objet d'une publication ultérieure, en cours de préparation.

Il résulte de ces expériences que l'efficacité de la méthode dépend plus de la position des rayons traités que de leur nombre dans la colonie. Un rayon situé au centre du nid à couvain est 2 fois plus

efficace que 2 rayons placés sur les bords de la grappe (Tabl. 1, traitement a). Lorsque des rayons traités sont placés alternativement avec des rayons non traités, le niveau d'infestation s'approche de zéro quelque soit le niveau initial d'infestation (Tabl. 1, traitement b). De même, le traitement comportant un rayon traité au centre et 2 autres, chacun à une extrémité de la grappe, a une efficacité accrue (Tabl. 1, traitement c).

Au cours du 1er jour on a dénombré 50 % de l'ensemble des acariens tués et 85 % au bout des 3 premiers jours (Fig. 1). Les acariens survivants ont été retrouvés à la fin de l'expérience dans des cellules de couvain operculé et sur des abeilles adultes. Leur nombre est si faible qu'il ne risque pas d'exercer de dégâts sensibles sur l'hôte. Aucune action néfaste sur la colonie n'a été mise en évidence à la fin du traitement. La production et le développement du couvain, ainsi que le nombre d'abeilles mortes avant et après le traitement, ne sont pas influencés (Tabl. 2).

ZUSAMMENFASSUNG

DIE ANWENDUNG VON KONTAKT-AKARIZIDEN GEGEN DIE *VARROA*-MILBE DURCH MISCHUNG DER WIRKSUBSTANZ IN DAS PROTEIN-FUTTER DER HONIGBIENE

In dem Bestreben, die Varroatose durch neue chemotherapeutische Methoden unter Kontrolle zu bringen, wurde Malathion in einer Konzentration von 50 ppm in Pollenersatzmittel gemischt. Mit dem Präparat wurden leere Wabenzellen vollständig gefüllt. Die mit dem Gemisch gefüllten Zellen bildeten eine kompakte, sichelförmige Fläche, die der unteren Leiste des Wabenrahmens gegenüberstand ; sie machte ca 1/5 der gesamten Wabenfläche aus und lag etwas höher als das Wabenzentrum.

Die präparierten Waben wurden in die Wintertraube der Bienenvölker gehängt und blieben da bis Ende des Versuches. Jede Wabe enthielt ca 200 g des Präparates. Die Versuche wurden in Thessaloniki zwischen November 1985 und März 1986 durchgeführt. Die Tageswerte der Lufttemperatur schwankten in dieser Periode zwischen 0° und 21 °C. Neben der Effektivität des Präparates gegen den Parasiten wurde auch nach einer möglicherweise schädlichen Wirkung auf die Brut bzw. auf die adulten Individuen des Bienenvolkes gesucht. In der vorliegenden Arbeit werden keine Angaben über Honigverunreinigung wegen der Anwendung dieser Methode präsentiert. Sie werden der Inhalt einer in Vorbereitung stehenden Publikation von uns sein.

Es wurde herausgestellt, dass die Position der präparierten Waben eine positivere Wirkung bezüglich der Effektivität gegen die Milbe hat, als ihre Anzahl pro Versuchsvolk. Eine Wabe im Nestzentrum war effektiver als zwei solche an den Seiten der Wintertraube (Tab. 1, Behandlung a und b). Wenn präparierte Waben abwechselnd mit normalen in das Bienenvolk gehängt wurden (beginnend mit einer präparierten Wabe an der zweiten Stelle der von Bienen besetzten Waben) näherte sich der Varroabefall dem Nullwert am Ende des Versuches unabhängig vom Anfangs-Niveau des Befalls (Tab. 1, Behandlung d). Ebenso war die Behandlung effektiver mit einer Wabe im Zentrum und je einer an den Seiten der Wintertraube (Tab. 1, Behandlung c).

Am ersten Versuchstag fielen ca 50 % der insgesamt getöteten Milben ab ; innerhalb der ersten drei Tage stieg dieser Prozentsatz auf mehr als 85 % (Abb. 1). Überlebende Milben wurden am Ende des Versuchs in verdeckelten Brutzellen gefunden — wenn solche in den Versuchsvölkern entstanden sind — wie auf adulten Bienen ; ihre Anzahl war so niedrig, dass eine merkbare Schädigung des Wirtes bis zum nächsten Winter nicht zu erwarten war. Es wurde keine negative Wirkung auf das Bienenvolk am Ende der Behandlung festgestellt. Die Produktion bzw. Entwicklung von Bienenbrut, bzw. die Anzahl toter Bienen vor und nach der Behandlung blieben unbeeinflusst (Tab. 2).

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