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Original article

The effectiveness of systemic agents used to control the mite, *Varroa jacobsoni*, in colonies of the honey bee, *Apis mellifera* depends on food distribution patterns

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Summary — A common means of combating the mite *Varroa jacobsoni* is to use systemic agents assumed to be spread by trophallactic interactions between honey bees within a colony. Colonies of *Apis mellifera* with age-marked bees were used to analyse distribution patterns of these agents. Methylene blue dye was used as a tracer in place of a systemic agent in order to determine possible effects of the method of application on distribution patterns within a colony. Applying a sugar solution by sprinkling a colony was found to cause a faster and more even distribution among the colony members than applying the agent in a feeding-jar. However, the effectiveness was lower and the contamination of combs and top bars was higher. Results show that with lower concentrations methylene blue is a less accurate tracer for food transfer than has been reported in the literature. It is concluded that trophallaxis is of minor importance for obtaining an even distribution of a systemic agent in a bee colony.

***Varroa jacobsoni* / acaricide / Perizin / food distribution patterns / trophallaxis / tracer**

INTRODUCTION

In most temperate and tropical regions beekeeping is adversely affected by the mite *Varroa jacobsoni* Oudemans (Griffiths and Bowman, 1982). The mite clings to adult bees and feeds by sucking their haemolymph. Reproduction of the mite takes place in capped honey bee brood cells, where the mite and its offspring feed on the pupa (Ifantidis, 1983). The resulting juvenile bee is often severely affected (De Jong *et al*, 1982). Heavily infected colonies will die. The effectiveness of some of the pesticides currently in use, *eg* Perizin® (Bayer AG) containing the active ingredient coumaphos and Apitol® (Ciba-Geigy) containing the active ingredient cymiazole hydrochloride are supposed to depend on the system of food transfer within a bee colony.

There are 3 ways by which the pesticide can be introduced into the trophallactic network of a colony; it can be supplied: a) in a sugar solution outside the hive, so

Abbreviations: MB = methylene blue.

that it can be collected by foragers; or b) *via* a feeder on top of the colony; c) or by sprinkling onto the bees, which subsequently lick it. Each of these methods will lead to a different distribution pattern. These differences might be crucial for effective control of the mite.

Nixon and Ribbands (1952), who trained foragers to feed on a radioactive sugar solution from a dish outside the hive found that after 27 h only 76% of the foragers and 43–60% of all bees were radioactive. We suppose that if the food is given through a feeder, a rapid and much more accelerated uptake will occur. Bayer AG recommends that Perizin be sprinkled without adding sugar. Ciba Geigy recommands the same for Apitol. The manufacturers assume that those bees that are not reached directly obtain the agent through trophallaxis. However, trophallaxis is not stimulated by the simultaneous introduction of sugar solution and Perizin.

Rösch (1925) and Lindauer (1953) found that most tasks within the colony are performed by bees of specific ages. The supply of nectar, its dehydration and storage are all activities in which age-dependent division of labour occurs. This could have implications for the distribution of a drug when mixed with a sugar solution.

Moritz (1982) found that the systemic effect is influenced by 2 factors: the volume applied and the time of the year. In this study we investigate possible differences in distribution patterns for 2 application methods, a feeding-jar and sprinking, in order to obtain more insight into the consequences of treatments with a systemic agent.

MATERIALS AND METHODS

Methylene blue (MB) was used as a tracer element to detect food distribution patterns at the colony level (*cf* Moritz *et al,* 1981; Moritz and Hallmen, 1986). MB was administered in 3 ways: a) in a sugar solution with Perizin, through a feeding-jar on top of the colony; b) *via* a feeding-jar in a sugar solution, but without the addition of Perizin; and c) MB in water, sprinkled over the bees resting on the combs.

Three experiments were carried out in summertime, using *Apis mellifera* L colonies accomodated in 6-frame hives. During 3 wk in each colony the newly emerged bees, characterized by their grey hairs, were colour-marked every day using a different colour every other day; ≈ 600 bees were marked in each age category. All bees in a colony descended from the same queen. Before the application of the MB solution the bees were shaken down from the combs and their total weight was measured. This enabled us to estimate the number of bees in the colony and the average volume of MB solution ingested by 1 bee. Additional experiments using small groups of bees kept in Liebefeld cages in an incubator were designed to provide information about whether the transfer of MB and that of Perizin among bees was comparable.

Experiment I: MB applied in a feeding-jar

A 50% sugar solution (water: sugar = 1:1, v/v) with MB (syrup: MB = 98 : 2, v/w), to which Perizin (0.5%, v/v) had been added was applied. We added Perizin to the solution to find out whether it would influence the uptake from the feeder. The colony was provided with ± 30 µl solution per bee. The effect of exposure time (3, 6, 12 or 24 h) on the distribution was determined, using 1 colony per time period. The colonies were kept in a closed hive without frames to prevent sugar solution from being stored in the comb. Half an hour before marked bees were collected the frames were put back into the hive, so that the bees could disperse over the comb. One hundred bees of each age category were sampled, killed and stored at –20 °C for further analysis. The amount of solution taken from the feeder was measured.

Experiment II: MB applied by sprinkling

In this experiment 25 ml of a 2% MB solution was sprinkled (applied in the same manner as

Perizin, according to the manufacturer's instructions). The hive was left open, and all combs were present. After periods of 3, 6, 12 and 24 h, marked bees, 100 bees per age category, were sampled, killed and stored in the manner mentioned above.

Experiment III : comparison between MB applied by a feeding-jar and by sprinkling

In this case no Perizin was added to the solution in the feeding-jar. The experimental time was 3 h and only one colony was used in each condition; each received 25 ml of the MB solution.

Recovery of MB

All bees were analysed individually. To remove and to determine potential body surface contamination we first washed a number of bees individually in 3 ml of 85% methanol. The amount ingested was determined by homogenizing the bee for 30 s in 3 ml of 96% ethanol. The homogenate was centrifuged at 4 000 rpm for 10 min. The amount of MB was measured in a spectrophotometer (MCP Vitatron) at 641 nm. The maximum light absorption found for a blank bee was taken as the background level, equivalent to 0.079 µg MB (SD = 0.06 µg, N = 139). The detection limit was 0.005 µg MB.

Experiment IV: distribution of coumaphos and MD

This experiment was designed to investigate whether Perizin and MB were distributed in the same way among the members of a bee colony. We used honey bees from colonies maintained in bee flight-rooms. They were kept in an incubator. One h before the experiment started we deprived the bees of food. One of the bees ≈ 7 d old, was fed 10 µl of a syrup solution (27%) containing 0.029 mg MB and 14.06 x 10^2 Bq of [^{14}C]-coumaphos (Bayer, spec act 21.9 x 10^5 Bq/mg). This donor bee was placed along with 7–11 acceptor bees for 1, 2, 4, 7 or 24 h. Four groups were used for each time period. At the end of the period all bees were killed and stored at –20 °C. The alimentary canal of each bee was isolated and homogenized in 0.9 ml ethanol (96%) for 30 s. The mixture was centrifuged and the amount of MB in the alimentary canal determined. The amount of coumaphos was analysed by solubilizing the sediment in 0.4 ml Lumasolve® (Lumac); this was added to the remainder of the supernatant. The total solution was kept for 3 d at 50 °C. Radioactivity was subsequently determined using a Packard scintillation counter (Type 4550). Detection limit for coumaphos was 0.001 µg.

Experiment V: recovery of MB over time

Each bee was fed a 10 µl MB sugar solution. Two concentrations were used: 0.3 and 0.03% MB solution in 50% sugar solution (water: sugar = 1:1, v/v). After feeding, the bees were kept apart. For both concentrations the amount of MB was determined in 10 bees after 0.5, 1, 2, 3, 4, 6, 10, 12, 16, 20 or 24 h.

Statistical analysis

The existence of normal distribution patterns in the amount of MB consumed was investigated by Shapiro–Wilk statistics (Shapiro and Wilk, 1965). In experiments I, II and III no normal distributions were found. Therefore, a logarithmic transformation was applied to the amount of MB to meet the criteria for linear regression and analysis of variance (*ie* normal distribution and equality of variance around the regression line cq among the groups). After this transformation these distributions became normal. Following the analysis of variance, the groups were summarized by their geometric mean (Sokal and Rohlf, 1981). A lower limit and an upper limit are given to illustrate the range of the amount of MB. These limits are asymmetric around the geometric mean as a logical consequence of the logarithmic transformation.

In experiment V the existence of a correlation between the amount of coumaphos and the amount of MB and differences in time were investigated by linear regression.

RESULTS

Experiment I: MB applied in a feeding-jar

In all colonies a quantity of the MB solution was left in the feeding-jar; therefore, the bees had been able to ingest food during the entire period. In the colony with an exposure time of 24 h the feeding-jar did not function properly; therefore the results of this colony were not taken into account.

The amount of solution consumed per colony was 15, 19 and 46 ml after 3, 6 and 12 h, respectively. When the weight of the colonies (compare table I) and the duration of the experiments were taken into account the amount consumed per 100 g bees per h was found to be 0.7, 0.6 and 0.7 ml during the 3, 6 and 12 h, respectively. The similarity of these values suggests that the amount of MB solution consumed was constant over time.

On average 10% of the bees in the colonies were analysed for the amount of MB ingested. The percentage of bees containing MB was 90.3, 96.3 and 99.8% after 3, 6 and 12 h, respectively. We therefore consider that after 12 h virtually all bees had received MB.

External contamination of the bees was very slight (± 0.12 μg) and seemed to be constant over time (table I).

No differences were found in the amount of MB ingested by bees of different ages, except for the period of 3 h. In this colony the amount ingested increased with age ($P = 2.0 \cdot 10^{-4}$) as did the amount of MB on the body surface ($P = 1.0 \cdot 10^{-4}$).

The total amount of MB per bee recovered from the outside and inside turned out to be 3.88, 20.36 and 71.52 μg after 3, 6 and 12 h, respectively. This is certainly not a linear increase, as found for the amounts taken from the feeding-jar. Theoretically the average amount of MB recovered per bee should be 42.86, 69.09 and 176.92 μg;

Table I. Distribution patterns of methylene blue (MB) on the outside of bees and in the alimentary tracts of bees, represented by the geometric mean values and their upper and lower limit (N = the number of bees analysed).

Treatment	*Duration (h)*	*External contamination (μg/bee)*				*Amount consumed (μg/bee)*				*Weight of colony (g)*
		Geometric mean	N	*Lower limit*	*Upper limit*	*Geometric mean*	N	*Lower limit*	*Upper limit*	
Feeding-jar (Exp I)	3	0.12	179	0.02	0.71	1.13	719	0.04	29.79	700
	6	0.09	180	0.01	0.63	5.87	667	0.12	281.18	550
	12	0.15	180	0.03	0.87	57.23	542	11.88	275.61	520
Sprinkling (Exp II)	3	0.25	180	0.05	1.36	12.76	526	1.50	108.42	700
	6	0.14	179	0.02	1.17	16.81	526	3.07	92.02	500
	12	0.07	180	0.01	0.36	12.03	503	2.16	67.02	750
	24	0.13	180	0.02	0.94	25.64	501	4.93	133.22	500

Colonies were either fed an MB–sugar–Perizin solution (Exp I) or received an MB solution by sprinkling (Exp II). Bees were sampled as indicated after 3, 6, 12 or 24 h after the start of the application.

this means that the recovery was 9.0, 29.5 and 40.4%, respectively.

Figure 1 shows the frequency distribution for the amount of MB (μg/bee); all ages have been lumped together. With time the distribution pattern changes, but after 12 h this pattern was still not normal (Shapiro–Wilk statistics, $P = 0.0$).

We investigated the possible differences between the uptake rate of MB solution and that of a mixture of MB and perizin. Eight groups each consisting of 10 bees of unknown age were maintained in Liebefeld cages. In 4 groups MB (0.03%) in syrup as available *ad libitum*, in 4 other groups the same solution containing perizin (0.02%) was available. The amount of solution consumed was determined every day for a period of 7 d. No significant differences were found between the 2 groups.

Experiment II: MB applied by sprinkling

On average 10% of the bees in the colony were analysed for the presence of MB. The percentage of bees containing MB was 100.0, 100.0, 99.8 and 100.0% after 3, 6, 12 and 24 h, respectively.

After 3, 6, 12 and 24 h the MB recovered per bee was 12.76, 16.81, 12.03 and 25.64 μg (geometric mean), respectively. The differences could be explained by variations in colony weight.

After all exposure times the amount of MB found was significantly higher when the bees were older (linear regression, $P = 1.0 .10^{-4}$, $y = 0.037x + 2.183$; $P = 1.0 .10^{-4}$, $y = 0.03x + 2.555$; $P = .0031$, $y = 0.019x + 2.293$; $P = 2.0 . 10^{-4}$, $y = 0.022x + 3.041$ after 3, 6, 12 and 24 h, respectively). The increase was ± 10% in bees aged from 2–20 d.

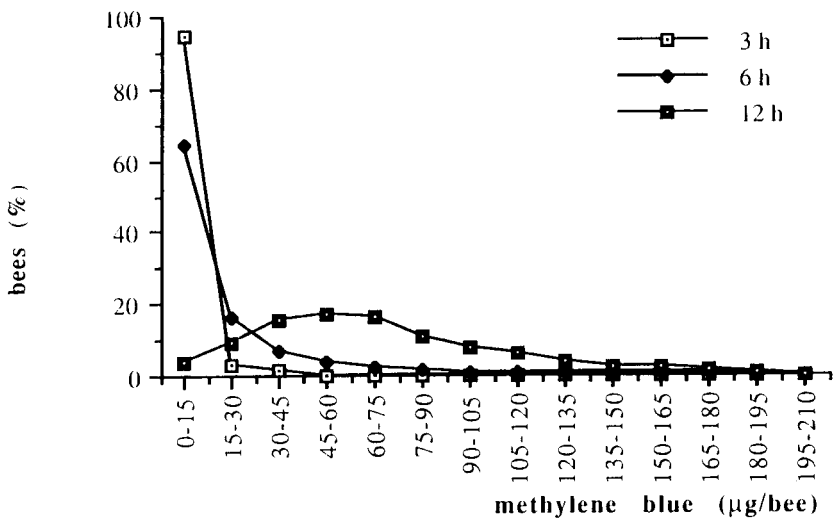

Fig 1. Frequency distribution of amounts of methylene blue (MB) (μg/bee) recovered from individual bees sampled from colonies that were exposed for 3, 6 or 12 h to a feeder containing a 2% MB–sugar–Perizin solution (Exp I).

Table II shows the total amount of MB recovered from the outside and inside of the bee. This means that 75, 78, 77 and 70% of the MB respectively has been lost. This could be caused by the fact that part of the solution remained on the top bars and combs in the sprinkled colony.

The small amount found on the outside of the bees (± 1% of the amount ingested) appears to be constant over time. Figure 2 shows the frequency distribution for the amount of MB (μg) in bees of all ages lumped together.

Experiment III: comparison between MB applied by a feeding-jar and by sprinkling

The fed colony consumed its 25 ml just within the 3 h of the experiment; it therefore received the same amount of MB as the other colony. The percentages of bees analysed were 19 and 17%, respectively.

Table III shows that the percentage of bees receiving MB did not differ between the 2 colonies. However, the amount of MB found outside and inside the bees (geometric

Table II. The total mount of methylene blue (MB) recovered from the outside and inside of the bee, the total amount applied per bee and the recovery of MB after 3, 6, 12 and 24 h after the start of the application (Exp II).

	Time-period (h)			
	3	*6*	*12*	*24*
Total amount of MB (μg/bee')	19.99	23.53	17.01	33.15
Àmount of MB applied (μg/bee)	78.6	110	73.3	110
Recovery (%)	25	22	23	30

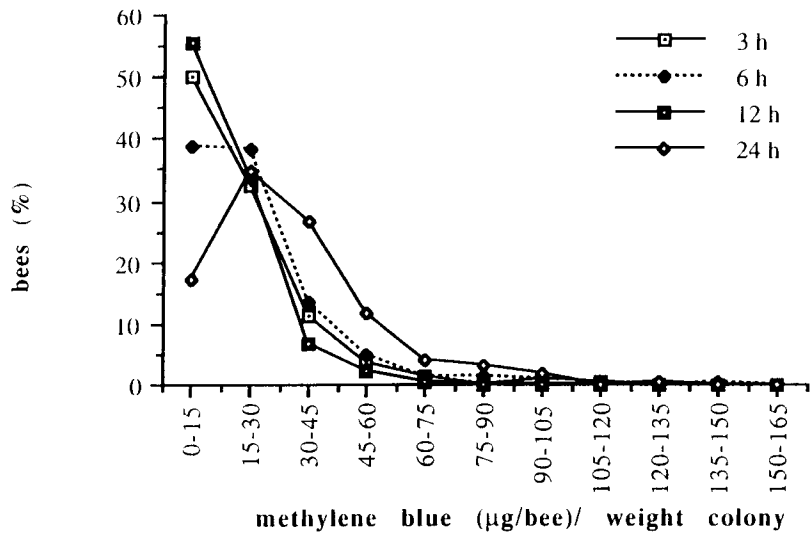

Fig 2. Frequency distribution of amounts of methylene blue (MB) (μg/bee) recovered from bees derived from colonies that were sprinkled with a 2% MB solution, 3, 6, 12 or 24 h, respectively, before the colony was sampled (Exp II).

Table III. Comparison of the distribution patterns and recovery rates of methylene blue (MB) in 2 colonies that received MB either through sprinkling or through feeding. Exposure time was 3 h.

	Sprinkling	*Feeding*	P
% Bees containing MB	96.8	96.0	0.493 2
Geometric mean			
Amount of MB on outside (μg/bee)	0.07	0.08	0.009 9
Lower limit	0.01	0.01	
Upper lmit	0.36	0.53	
Amount of MB inside (μg/bee)	5.32	7.89	0.000 1
Lower limit	0.17	0.09	
Upper limit	163.69	665.81	
Arithmetic mean			
Total amount of MB recovered (μg/bee)	15.9	36.0	
Total amount of MB applied (μg/bee)	84.6	100.0	
% MB recovered	18.8	36.0	

The colonies contained 650 and 550 g of bees, respectively, and received 25 ml of a 2% MB solution (Exp III).

means) differed significantly. The amount of MB recovered in fed bees was twice as much as was found for the sprinkled ones. This difference could be caused by the fact that part of the solution remained at the top bars and combs in the sprinkled colony.

In the fed colony, there was a difference in the MB recovered from the outside as well as from the inside of the bee in relation to age (linear regression, $P = 0.0036$ and $P = 0.0014$, respectively); older bees were found to contain more MB than younger insects. In the sprinkled colony too, the older bees contained more MB than the younger ones (linear regression, $P = 1.10^{-4}$).

Experiment IV: distribution of coumaphos and MB

In this experiment no differences were found in the ratio of MB and coumaphos over time (linear regression, $P = 0.3245$); therefore the data were pooled. The average amount of coumaphos recovered was 62.6% (SD = 13.3; $N = 20$). However, in this experiment much of the MB was lost: 64.8% (SD = 38.9; $N = 182$). Using linear regression, the correlation between the amount of coumaphos and the amount of MB was found to be significant ($P = 1.10^{-4}$).

Experiment V: recovery of MB over time

Figure 3 shows the recovery after various time periods for 2 groups of bees fed different concentrations of MB. In both cases there is a decrease in the percentage of MB recovered. In the group of bees which was fed a 0.03% MB solution, the recovery is lower than in the group receiving a 0.3% MB solution.

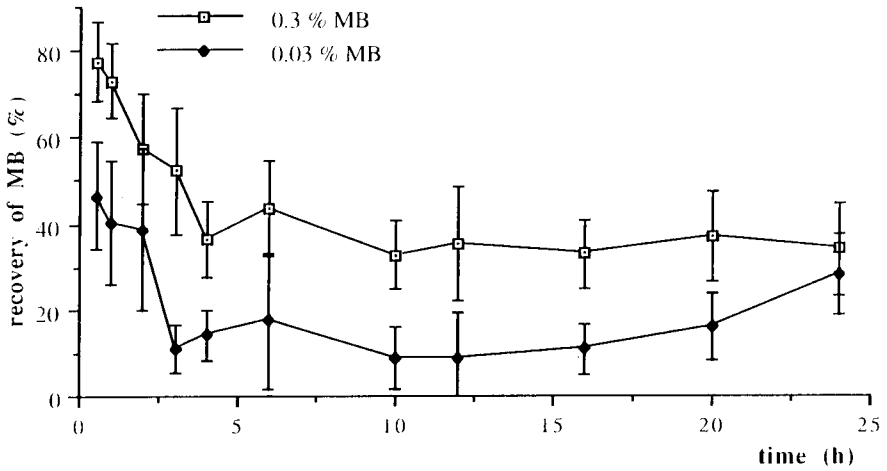

Fig 3. The recovery of methylene blue (MB) (%) in relation to time, when MB was fed either as a 0.3% or as a 0.03% MB–sugar solution (Exp V).

DISCUSSION

In this study we have demonstrated that differences in the application methods of a systemic agent play a role in the distribution of the agent within a colony.

Table I reveals that sprinkling leads to a more even distribution than feeding: in all 4 time periods the geometric averages lie between 12 and 25 μg/bee; and the upper and lower limits are closer to the average than in the case of feeding.

It is not surprising that in our small colonies with sprinkling practically all bees are reached, whereas in the case of feeding it takes about 12 h to reach 100% of the bees. However, our data cannot support the general assumption that after sprinkling trophallactic activities lead to a considerable redistribution of Perizin; if this occurs, then it takes place within the first 3 h. None of the distribution patterns tends to approximate a normal distribution curve and the skewed distributions do not differ significantly between the tested time periods for sprinkling. Figure 2 shows that the smaller the colony, the smaller the percentage of bees receiving the lowest dose category.

The data in experiments I and II, confirmed by the data in table II, demonstrate that probably half of the MB is left on the frames. If this result is extrapolated to a conventional Perizin treatment (sprinkling the bees), it implies a considerable contamination.

Although in the feeding experiments the intake of MB solution was linear over time, recovery is not. A considerable amount of MB was lost, and this cannot be explained by purely technical inaccuracies. These losses could be due to several factors. As figure 3 shows, bees have a means of neutralizing MB. Especially during the first few

h after feeding, recovery is negatively correlated with time. The losses are more marked with lower dosages, and may reach levels of 80% when 0.03% MB is fed. Each bee received 10 µl of this solution, about one-third of the capacity of its crop. If we extrapolate to lower amounts of MB solution, we conclude that in such cases probably almost all MB remains undetected. The time-relatedness of these losses indicates a biological origin, such as absorption into tissue and metabolic degradation. Whatever the mechanism, its capacity to conceal MB is limited, for recovery rates improve when the colony has received larger amounts. Moritz and Hallmen (1986) reported a recovery of 95% when much higher doses were used.

In several cases we found a relation between the age of the bees and the average amount of MB recovered, both from the outside and the inside of the bee. Body surface contamination was low in all cases. There are probably 2 reasons for this relation between age and amount of MB recovered. In the first place older bees might be more inclined to ingest from the feeder or to store food in their crop, and therefore some of them have large amounts which influences the average. When MB was sprinkled, we assume that all age-categories received equal amounts. The differences can be due to the fact that younger bees might be more efficient in neutralizing MB.

On average the recovery of [^{14}C]-coumaphos is about twice as high as the recovery of MB; 64% and about 30% respectively. Although there are important fluctuations in the recovered coumaphos: MB ratio, we consider MB to be a suitable tracer element, since it permits a safer and more rapid detection of the distributional pattern than would be possible by a radiographic or a chromatographic technique. However, MB is not a good tracer if fine details of a trophallactic network are to be discovered.

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Résumé — L'efficacité des produits systémiques utilisés contre *Varroa jacobsoni* dans les colonies d'abeilles *(Apis mellifera)* dépend du mode de distribution de la nourriture. Les produits systémiques sont largement utilisés dans la lutte contre l'acarien *Varroa jacobsoni*. L'efficacité de ces acaricides dépend de leur distribution au sein de la colonie d'abeilles. Il est donc important d'avoir des informations sur les voies de distribution dans la colonie. Moritz a déjà montré en 1982 que la distribution du produit était influencée par la quantité d'acaricide et la saison à laquelle a lieu le traitement. Nous avons étudié les différences possibles dans les voies de distribution en fonction de 2 modes d'application (méthode du nourrisseur et méthode de l'aspersion) pour déterminer le meilleur mode d'application. Le bleu de méthylène a été utilisé comme marqueur pour suivre les circuits de distribution. Les expériences ont montré que ce colorant pouvait être utilisé comme modèle pour étudier la distribution de l'acaricide systémique Périzin. Trois expériences ont été faites en fonction du mode d'application du bleu de méthylène: 1) dans un nourrisseur; 2) par aspersion; 3) simultanément dans un nourrisseur et par aspersion. Pour les 3 types d'expériences, on a utilisé des colonies d'*Apis mellifera* dont les abeilles étaient marquées par

classes d'âge. L'application par aspersion conduit à une distribution plus rapide et plus uniforme du produit entre les membres de la colonie que l'administration par nourrisseur. Néanmoins, l'efficacité de l'aspersion est plus faible et la contamination des rayons et des barettes supérieures est bien supérieure, comparée à l'administration par nourrisseur. La saison et la quantité ne sont donc pas les seuls facteurs qui déterminent la façon dont le produit est distribué. Il faut utiliser avec précaution le bleu de méthylène comme marqueur des échanges trophallactiques car, lorsque les quantités sont faibles, il est difficile de le retrouver, probablement parce qu'il adhère aux tissus et qu'il est modifié par le métabolisme. On conclut que la trophallaxic est de faible importance pour obtenir une distribution uniforme d'un produit systémique dans une colonie d'abeilles.

***Varroa jacobsoni* / acaricide / Perizin / trophallaxie / marqueur biologique**

Zusammenfassung — Die Wirksamkeit von systemischen Mitteln zur Behandlung der Milbe *Varroa jacobsoni* in den Völkern der Honigbiene, *Apis mellifera*, hängt von der Art der Futterverteilung ab. Systemische Mittel finden im Kampf gegen die Varroa-Milbe weite Anwendung. Die Wirksamkeit dieser Akarizide hängt von ihrer Verteilung im Bienenvolk ab. Es ist deshalb von entscheidender Bedeutung, Informationen über das Verteilungsmuster zu besitzen. Moritz (1982) hatte schon früher gefunden, daß die Menge des benutzten Akarizids und die Jahreszeit die Verteilung des Mittels beeinflussen.

Wir haben die möglichen Unterschiede zwischen zwei Anwendungsmethoden untersucht (Fütterung mittels eines Futterglases und Sprühen), um die beste Weise zur Anwendung von systemischen Mitteln herauszufinden. Wir benutzten den Farbstoff Methylenblau als Markierungsmittel, um das Verteilungsmuster zu untersuchen. Vorversuche ergaben, daß dieser Farbstoff als Modell für die Verteilung des systemischen Akarizids Perizin benutzt werden kann. Es wurden drei Experimente durchgeführt: I) Methylenblau wurde in einem Fütterungsglas dargeboten, II) Methylenblau wurde durch Sprühen ausgebracht, und III) Methylenblau wurde gleichzeitig mittels Fütterungsglas und Sprühen dargeboten. Bei allen drei Versuchen wurden Völker von Apis mellifera mit nach Altersstufen markierten Bienen benutzt.

Es zeigte sich, daß durch Sprühen eine schnellere und gleichmäßigere Verteilung des Mittels über die Bienen des Volkes erreicht wurde als durch Ausbringung in einem Fütterungsglas (Abb 1, 2 Tabelle I). Die Wirksamkeit des Sprühens ist jedoch geringer und die Verunreinigung von Waben und Rähmchenträgern ist viel höher im Vergleich zur Fütterung mit dem Glas. Wir schließen daraus, daß für die Verteilung eines systemischen Mittels nicht nur Jahreszeit und ausgebrachte Menge eine Rolle spielen (Moritz, 1982), sondern auch die Ausbringungsmethode. Ferner stellten wir fest, daß bei Verwendung von Methylenblau als Markierungsfarbe für den trophallaktischen Futteraustausch Vorsicht geboten ist; denn in geringen Mengen kann es nur schwer wiedergewonnen werden, wahrscheinlich weil es an der Körperoberfläche hängen bleibt oder weil es durch den Stoffwechsel verändert wird. Man zicht den Schluß, daß der Futteraustausch für die gleichmüßige Verteilung eines systemischen Mittels im Bienenvolk nur eine geringe Bedeutung hat.

***Varroa jacobsoni* / Akarizid / Perizin / Futteraustausch / Markierungsmittel**

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