Acute-paralysis infection of bee brood (*Apis mellifera* l.) from colonies treated with Tau-Fluvalinate

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ABSTRACT

Varroa mite control by the acaricide Apistan® (active substance Tau-Fluvalinate) acting as contact toxin leads to an increased mortality with APV infected bee pupae (*Apis mellifera*), during the first weeks after treatment. Approximately one month after the first treatment, the increased susceptibility diminishes. An improvement of the colonies' general constitution after removal of most *Varroa* mites can be expected if there were no secondary diseases before. Most probably, the different susceptibility of APV infected bee pupae treated with the acaricide and bee colonies not treated is caused by the synthetic pyrethroid. Genetically caused differences between the bee colonies seem to be irrelevant.

Key words: *Apis mellifera, Varroa destructor / acaricide / Tau-Fluvalinate / Acute Paralysis Virus / bee pupae mortality*

1. INTRODUCTION

Varroatosis is spread nearly world-wide and is very serious threat for bee colonies of *Apis mellifera*. The infestation and massive multiplication of the ecto-parasitic mite *Varroa destructor* leads to a weakening of the bee colony, brood care is neglected, and the colony mostly dies in autumn or winter (Ritter, 1981).

Early, examinations showed that the Acute Paralysis Virus (APV) was responsible for the mortality of adult bees and bee brood infested by the Varroa mite (Batuev,1979; Ball, 1985; Ball & Allen, 1988). Obviously Varroa mites can potentially transfer APV to the bee brood (vector function) when feeding on its haemolymph. On the other hand, inactive but prevalent APV can be activated by the mite during feeding (Ball, 1988; Ball & Allen, 1988).

Losses of colonies with *Apis mellifera* can only be restrained by yearly repeated control measures. To avoid the outbreak of varroatosis a chemical treatment can be neglected in most of the cases. For many years already, synthetic pyrethroids are used for mite control on a large scale, Apistan® (Vita Ltd.; active substance: Tau-Fluvalinate) with an effectiveness of ca. 99 % being regarded as especially efficient (Moosbeckhofer, 1990; Zoecon, 1993; Cabras et al., 1997).

Mite control by an acaricide acting as contact toxin becomes distributed throughout the bee hive including the unsealed brood. Up to now, the effect of yearly chemical treatments on the bees respectively their brood has not been carefully considered. Therefore, the effects of Tau-Fluvalinate on APV-infected bee pupae were examined in detail.

2. MATERIAL UND METHODS

2.1 PUPAE MATERIAL

The homogenous bee pupae material needed for the tests originated from 6 *Apis mellifera carnica* (Pollmann, 1979) colonies. The queens came from the queen-mating station of the "Landesverband Baden" (Badenia Federal Association). Before the examinations, no *Varroa* treatments were done.

For testing, large quantities of pupae of nearly same age were required. These quantities could not be found in every season in a comb under the same brood rearing conditions. Therefore, the queen was confined to an empty comb for a few days, depending on the intensity of laying eggs. Afterwards, the queen was released and the comb with eggs was marked and remained in the middle of the brood nest.

To maintain the egg laying and brood rearing during the months of September and October, in the absence of forage, small quantities of sugar dough (Apifonda®, Südzucker) were fed regularly.

Sealed brood combs with brood of the nearly same age were extracted from the colonies and stored for a few hours in an incubator (KT/L 3340, Fa. Ehret), at 34° C and 50 % relative air humidity, until they reached the necessary development stage.

2.2 BROOD TREATED WITH ACARICIDES

Bee colonies bound for brood removal, were divided into two groups (3 colonies each). One group was treated with Fluvalinate, according to the manufacturer's instructions. The control group received no treatment. After 14 days of application, brood of the late Pr-stage (eyes brown-red in colour) was removed from each colony to examine the survival rate when subjected to an artificially produced APV infection. According to the classification by Rembold et al. (1980), the stages showed a transition to beginning Pd-stage. Pupae with Varroa mites in their cells were not included in the experiment.

2.3 BEE BROOD IN RELATIONSHIP OF ORIGIN

To produce relationship conditions between bee colonies, one bee colony was divided, its old queen was removed and sister queens (origin: Lund) were introduced into both newly formed colonies. In dividing the original colony, honey, pollen, and brood combs were divided in equal parts to create identical starting-conditions. Both new colonies were afterwards moved to distant place to start the experiment as described under 2.2.

2.4 INFECTION BY APV

For infecting pupae, a dosage was needed to allow the survival of the infected brood. By using a dosage-mortality-curve for APV susceptibility, the injection of 10^{-7} ng viruses was chosen.

Immediately after comb removal, 0,2 µl of virus solution was injected per pupa, using a Hamilton syringe (Hamilton 1710, volumetric measuring device PB 600), and inserting the needle ventro-lateral between the 5^{th} and the 6^{th} abdominal segment.

The APV for the experiments was isolated from infected bees according to the method by Bailey and Woods (1974). Using PCR, a virus genome analysis was made to verify the presence of APV. The virus concentration was calibrated by a photometric method according to Newman et al (1973) and Ball (1985).

In addition to the main control group, another control was included to measure the effect of the virus solvent potassium phosphate buffer (PPB) and the puncture. The main control group was not inoculated at all.

To determine the survival rate until emergence, all pupae were put into Eppendorf test tubes and closed by a cotton-wool ball. Until emergence all brood was kept in an incubator at 34° C and 50% relative air humidity. Those bees gnawing with their mandibules at the wad were regarded as being ready to emerge. Dead pupae could be identified from the melanisation at the end of their abdomen respectively at their tarsi. Additionally, they did not show any reaction with contact stimuli.

Some of the pupae were killed immediately after inoculation and examined for APV with the immune diffusion test, to make sure that a positive reaction was not due to the injection.

The bee pupae were controlled twice per day recording the stage of development and the period of development until the death.

The test was conducted twice. The test setup of 1998 was repeated to confirm the tests of 1997 and to determine any genetic effects.

The statistical calculations were produced with the software-program "Win-Stat“ (Version 3.1. R. Fitch-Software).

3. RESULTS

3.1 PUPAL MORTALITY FROM COLONIES OF DIFFERENT GENETIC ORIGIN

The pupal survival rates for Tau-Fluvalinate and PPB of the individual random samples are shown in Table 1.

There were no significant differences (χ^2, $P>0.05$) between treated and untreated bee brood, neither in the control group nor in the one inoculated with buffer solution (PPB), (Table 1). Only colony no. 8, in the group inoculated with buffer solution showed a greater difference to the adult rate of the control group.

More pronounced differences were found with pupae infected with APV. According to Table 1, in August 1997, pupae from untreated colonies showed a considerably higher survival rate compared to bee pupae from treated colonies (χ^2,.$P<0.001$).

Because of the great homogeneity of the control-group in August, it was only the PPB-group as the real control-group for APV-infection in the test in September included.

In the repeated test, one month later, the group inoculated with buffer solution (Table 2) only showed small differences between treated and untreated colonies. More serious losses in the PPB groups of the previous month of colony 5 and 8 were not observed anymore.

Pupae from colonies treated with acaricide and inoculated with APV showed a survival rate between 77.9 and 94.8 % (see Table 2). Similar values (V6 = 66.7 %, V8 = 94.9 %) were

observed from pupae of untreated colonies. Colony no. 9 (APV inoculated, untreated) with an adult rate of 28.1% differs greatly from the other results.

The pupae's development periods in the individual test groups showed that colony 9 an average value of 102.9 hours was a considerably lower average development time of 85 hours with colony no. 6 (table 3).

The serious loss of bee pupae inoculated with APV in colony no. 5 treated with Apistan®, in August 1997, is reflected in the short average development time of 42.6 h (χ^2, $P<0.001$), compared to the colonies no. 1 and 4. In September, the development period was adapted to the other colonies.

3.2 PUPAE MORTALITY OF COLONIES IN RELATIONSHIP OF ORIGIN

To factor out genetic differences on pupal survival sister queen colonies were produced

As it is shown in Table 4, the colony treated with Tau-Fluvalinate had only 30.6% of the pupae reached adult stage, versus 73.1% for the untreated colony (χ^2, $P<0.001$). The two control groups, control and KPP, did not show differences (χ^2, $P=0.15$).

Similarly differences regarding the average development period were observed from the APV infected groups with $APV_{treat..}$ = 70.1 h and APV-untreated = 91.9 h (see Table 5). The control and PPB groups, however, only showed small differences.

4. DISCUSSION

Experiments were conducted to test the effects of Tau-Fluvalinate and APV on pupal survival. Two weeks following the acaricide treatment pupae with brown to red eyes were removed from the colonies. These pupae were exposed as larva to Tau-Fluvalinate via contact with nurse bees (Ritter, 1996).

In unrelated colonies treated with the acaricide and their controls, different survival rates were not found (see Table 1). APV infected pupae, however, from colonies treated with the acaricide, showed a significantly lower survival rate compared to pupae from untreated colonies.

The acaricide did not cause obvious losses in healthy bee larvae. Only the virus infection produced increased mortality rates with pupae treated with Tau-Fluvalinate. The significant differences regarding the survival rate of pupae from colonies with mite treatment point at a different susceptibility concerning the acaricide in connection with a virus infection.

The same experiment was repeated around four weeks later. In August, there were severe losses of APV inoculated bee pupae from colonies treated with APV, but the results in September were similar to those of the untreated colonies. Whereas, in treated colonies, 80.3% on an average reached the adult stage, in untreated colonies only colony no. 6 and 8 showed similar values (see Table 2). The APV infected pupae from colony 9 was much loss than other colonies. Its average development period of 102.9 h (Table 3) indicates a relatively late average pupal loss. An examination of the dead pupae did not reveal any additionally diseases. The high mortality could be a late effect of the APV infection.

The control groups did not show larger differences in the survival rate between treated and untreated colonies. A negative effect of the acaricide on virus infected pupae was not observed.

To exclude genetic effects on pupal survival, two sister queen colonies were produced and one exposed to the acaricide.

It is unlikely that the difference in the survival rates between treated and untreated virus infected pupae is due to genetic effects since in that respect the colonies are similar. But rather the differences may be due to the acaricide and APV.

Even the colony's constitution probably did not play an important role in this respect, because the original colony had been divided into two nearly equally colonies with the same number, type and content of combs. A totally different development of the colonies can be excluded as a reason, because between the dividing of the original colony and the extraction of pupae there was only an interval of 3 weeks. Moreover, a *Varroa* mite population relevant for influencing susceptibility could not have developed due to a lack of brood. Thus it seems the enhanced susceptibility was initiated by the synthetic pyrethroid.

The *Varroa* mite is thought to be a vector and initiator of the APV (Bailey & Ball, 1991). According to Ball (1996) the APV was a world-wide distribution, at least in an inactive state. Activation of APV by *Varroa destructor* in combination with Tau-Fluvalinate could, for a short time, provoke an increased death of pupae. In temperate climate zones, an acaricide treatment is generally done after the last honey harvest, and this could lead to a reduction of the winter bees developing at that time.

Ritter et al. (1984) measured colony and Varroa populations and observed that increasing *Varroa* populations was correlated with an increase in colony susceptibility.

At the same time the bees were examined on APV, a steady increase in subordination to the infestation of the mites was the result (Ball & Allen, 1988).

In the present examination, it was easily possible that the weakening of the general constitution of colonies without *Varroa* treatment was due to the increasing mite population and the correspondingly increasing rate of damages.

Without additional infection the control groups of colony no. 9 did not show remarkable differences compared to the other colonies. This indicates that at least a latent weakening of the defence mechanism occurred, the manifestation of which, however, only appeared in case of an additional infection. In this respect, the infestation rate by the mite *Varroa destructor* seems to be of essential importance but not seasonal influences.

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TABLES

Table 1: Pupal survival for exposure to Tau-Fluvalinate and PPB without APV

	colony	Control N	Control %	PPB N	PPB %	APV N	APV %
treated	1	33	100,0	40	97,5	78	53,8
	4	30	100,0	50	92,0	101	41,5
	5	33	97,0	41	80,5	78	17,9
untreated	6	33	100,0	41	92,7	78	87,2
	8	25	92,5	40	67,5	78	80,8
	9	33	93,1	38	100,0	78	64,1

ΣN = 928

Table 2: Survival rate of treated and untreated bee pupae

	colony	PPB N	PPB %	APV N	APV %
treated	1	40	100,0	78	94,8
	4	41	97,6	76	68,4
	5	40	100,0	77	77,9
untreated	6	41	97,6	78	66,7
	8	40	92,5	78	94,8
	9	30	96,7	56	28,6

The colonies no. 1, 4 and 5 were treated with Tau-Fluvalinate (September 1997), (N=675).

Table 3: Average development periods of the bee pupae

development time (h)		treated			untreated		
colonies (V)		V1	V4	V5	V6	V8	V9
August 97	control	114.0	114.0	113.8	114.0	113.5	113.6
	PPB	111.8	113.6	112.8	113.5	112.0	114.0
	APV	74.8	65.5	42.6	102.8	97.2	86.9
Sept. 97	control	114.0	113.8	114.0	112.5	113.7	114.0
	PPB	114.0	113.8	114.0	113.8	113.5	113.8
	APV	113.6	112.1	107.4	85.0	113.6	102.9

Bee pupae originating from untreated colonies and those treated with Tau-Fluvalinate (August and September 1997).

Table 4: Pupal survival from sister queen colonies with treatments Tau-Fluvalinate and APV

	Control		**PPB**		**APV**	
	N	%	**N**	**%**	**N**	**%**
treated	12	100.0	25	100.0	49	30.6
untreated	14	100.0	27	92.6	52	73.1

August 1998 ($N_{treated}$ = 89; $N_{untreated}$ = 93).

Table 5: Pupal development times of untreated bee pupae and those treated with Tau-Fluvalinate in August 1998

Development time (h)	**Control**	**PPB**	**APV**
treated	114.0	113.5	70.1
untreated	114.0	114.0	91.9

August 1998 ($N_{treat.}$ = 89; $N_{untreat.}$ = 93).