

## **Acephate Residues After Pre-Blossom Treatments: Effects on Small Colonies of Honey Bees**

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Treatments of crops with pesticides toxic to honey bees during the blooming period are not recommended or even prohibited in many countries to prevent hazard to honey bees. Pre-blossom treatments, however, are not restricted, though residues of various insecticides could be detected in nectar (Waller and Barker 1979, Fiedler and Drescher 1984, Barker et al. 1980). Because residues of acephate exceed those of other insecticides (Fiedler and Drescher 1984), we have continued analysing nectar of plants sprayed with acephate.

A feeding experiment similar to that used by Waller and Barker (1979) to determine the effects of dimethoate was carried out with various concentrations of acephate.

### **MATERIALS AND METHODS**

During the years 1982-1985 acephate ("Orthene", 50% a.i. WP) was applied at a rate of 750g a.i./ha as a pre-blossom spray to apples (malus sylvestris), sour cherries (prunus cerasus) and raspberries (rubus idaeus). During the flowering period nectar was collected several times with micropipettes; in most cases collection from apple and cherry was only possible after having covered the flowers with plastic bags for 24 h, which led to a production of more, but less concentrated nectar. The nectar was analyzed by GLC (Drescher and Fiedler 1983) for residues of acephate and its major toxic metabolite, methamidophos.

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To determine the effects of acephate by a feeding experiment 20 colonies of honey bees were established in miniature hives made of styrofoam ("Kirchhainer Kästen"). Each colony was supplied with a laying queen (half-sisters from artificial insemination) and 110 g (ca. 1100) workers. All bees had been shaken from brood combs of one strong and healthy colony. Two drawn combs (ca. 14 \* 10 cm) and two top bars were given to each colony. The bees were kept in a cool and dark room for 72 h to consolidate; during this time they were provided with pure sugar syrup. After that period they were set up near our laboratory for 14 days and provided with contaminated sugar syrup; the levels of treatment were: 0.00 (control), 0.25 , 0.50 and 1.00 ppm (5 colonies/group). The treatment started on August 13th; natural bee forage is limited at this time of the year. The sugar solution (50% w/w sugar; ca. 14% glucose, 14% fructose and 22% sucrose) was supplied in plastic bottles above the top bars (covered with an empty hive body to prevent robbing). It was replaced every 48 h by a freshly prepared solution. The amount was restricted to 200g/48 h; remaining food was weighed to determine consumption; remaining insecticide in the syrup was analyzed by GLC twice during the experiment (6th and 10th day) to determine the decomposition rate. Inspections were made at the 6th and 10th day of treatment to determine the presence of queen, eggs, larvae and pupae. After 14 days surviving bees were weighed, eggs, larvae and pupae were counted, and the presence of the queen was checked. The area of newly built comb was measured, stored food was removed, its weight was determined and the contamination by acephate and methamidophos was measured by GLC.

## RESULTS AND DISCUSSION

Results of residue analysis are given in Table 1. The results of inspections during the feeding experiment and of the final examination are summarized in Table 2.

While the control group developed normally and all stages of brood were present at the 10th day, all treatment groups were seriously damaged.

In the 0.25 ppm-group all queens continued laying eggs up to the end of the experiment; their number, however, was significantly reduced at the final examination. Only three colonies succeeded in rearing a few larvae,

**Table 1. Residues of acephate and methamidophos in nectar after pre-blossom treatments**

plant species; year	Treatment	days before flowering	----- residues in nectar --- days after beginning of flowering	ppm acephate/ methamidophos	
apple 1982	1		7	8.44/0.65	x
			14	0.73/0.08	x
apple 1982	7		7	2.93/0.33	x
			14	0.27 0.03	x
apple 1984	7		9	1.67/0.07	x
apple 1984	3		9	2.25/0.09	x
apple 1985	1		7	1.40/0.15	x
rasp- berry	1982	1	1	14.39/1.13	
			6	2.80/0.30	
			11	0.80/0.09	
			13	0.45/0.04	
rasp- berry	1984	20 and 5	3	2.15/0.20	
			6	0.45/0.04	
rasp- berry	1984	13	1	0.80/0.15	
			6	0.15/0.04	
rasp- berry	1985	2	5	1.99/0.33	
cherry 1982	3		7	1.95/0.18	x
			16	0.19/0.02	x
cherry 1984	9		0	0.85/0.08	x
			6	0.32/0.05	x
			11	0.21/0.03	x
			14	0.20/0.04	
cherry 1985	2		2	2.84/0.27	
			7	1.69/0.14	

x: flowers covered with plastic bags for 24 h

**Table 2. Results of inspections and final examination**

group (ppm) :	0.00	0.25	0.50	1.00
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1. Control (6th day), presence:				
Queen	+++++	+++++	+++++	+++++
Eggs	+++++	+++++	+++++	+++++
Larvae	+++++	+----	-----	-----
Pupae	+----	+----	-----	-----
2. Control (10th day), presence:				
Queen	+++++	+++++	+++++	+++++
Eggs	+++++	+++++	-++++	-++++
Larvae	+++++	-+---	-----	-----
Pupae	+++++	-+---	-----	-----
Final examination, presence:				
Queen	+++++	+++++	-++++	-+---
Eggs	+++++	+++++	-++++	-----
Larvae	+++++	+----	-+---	-----
Pupae	+++++	-+---	-----	-----
Final examination, counts + measurements:				
Surviv. bees (%)	89.5a	81.2a	72.5a	23.9b
Eggs	229.2a	42.2b	13.0b	0.0b
Larvae	196.0a	6.0b	3.4b	0.0b
Pupae	200.8a	0.6b	0.0b	0.0b
Food consumption (total, g)	1056.1a	423.5b	207.7c	231.0*
Insecticide consumption (mcg)	--	105.9a	103.9a	231.0*
Comb built (cm <sup>2</sup> )	219.4a	107.8b	56.3c	23.5d
Stored food (g)	298.3a	59.5b	11.4b	n.d.
Residues in stored food (ppm)	0.02	0.23	0.36	n.d.
Decomposition : 6th	-	n.d.	-38%	-32%
rate in food : 10th	-	-20%	-18%	-25%
after 48 h (%): day				

Duncan's multiple range test ( $P < 0.05$ ):

Values with the same letter are not significantly different.

\* : 1.00 ppm not included because of robbing.

n.d. = not determined (due to lack of remaining food-solution or stored food)

only two in rearing pupae; their average number was significantly reduced. The amount of newly built comb, food consumption and food storage was also reduced significantly, though the number of surviving adults was not different from that of the control. In the 0.50 ppm-group one queen stopped laying eggs about the 7th day and died between the 10th and 14th day; one queen stopped laying and died between the 10th and 14th day, and one stopped laying about the 11th day. The remaining two laid only a few eggs during the last days. Only one colony succeeded in rearing a few (11) larvae; pupae could not be observed in any colony of this group. The number of eggs, larvae and pupae and the amount of newly built comb, food consumption and food storage was significantly reduced; the number of surviving bees was reduced, but the difference was not significant. In the 1 ppm-group two queens stopped laying about the 7th day of the treatment. All colonies were robbed during the last 48 hours of the experiment; for this reason food storage (and residues in stored food) could not be determined. Results of the inspections show, that no larvae and pupae were reared at all. A decreasing number of adults could be observed too at the inspections; therefore we believe that the significantly reduced numbers of adults, eggs, larvae and pupae are (mainly) a result of intoxication and not of robbing. Food and insecticide consumption, however, was not included in the statistical test, because food bottles had also been robbed. Residues of acephate could be detected in the stored food of all treated colonies, but traces were also found in the stored food of the control; this was probably due to robbing of the 1.00 ppm-group (hives and food bottles). Decomposition rate of acephate in food solutions ranged from 38 to 18 % after 48 hours, which means that the real insecticide consumption is lower than the theoretical values given in Table 2. Methamidophos could neither be detected in stored honey nor in aged sugar solutions (the detection limit was 0.005 ppm). An abnormal behaviour of the bees could be observed for the 1.00 ppm-level at the first inspection and for all treatments (correlating with concentration) at the second inspection: Only a few or no bees started to fly when the hives were opened; the bees were moving slowly and activity in general seemed reduced. Also flight activity of the treatments was reduced and fewer bees collecting pollen could be observed. Flight activity of the 1.00 ppm-group was about zero after 10 days.

The results of feeding acephate to small colonies correspond to those Waller and Barker (1979) found for dimethoate. They are in contrast with the results of Stoner, Wilson and Harvey (1985), who fed acephate to large colonies for 8 weeks; they observed no effect on adults after consumption of 1.00 ppm and a reduction of sealed brood which was about 20% at 0.1 ppm and 16% at 1.00 ppm. The differences are probably caused by two factors:

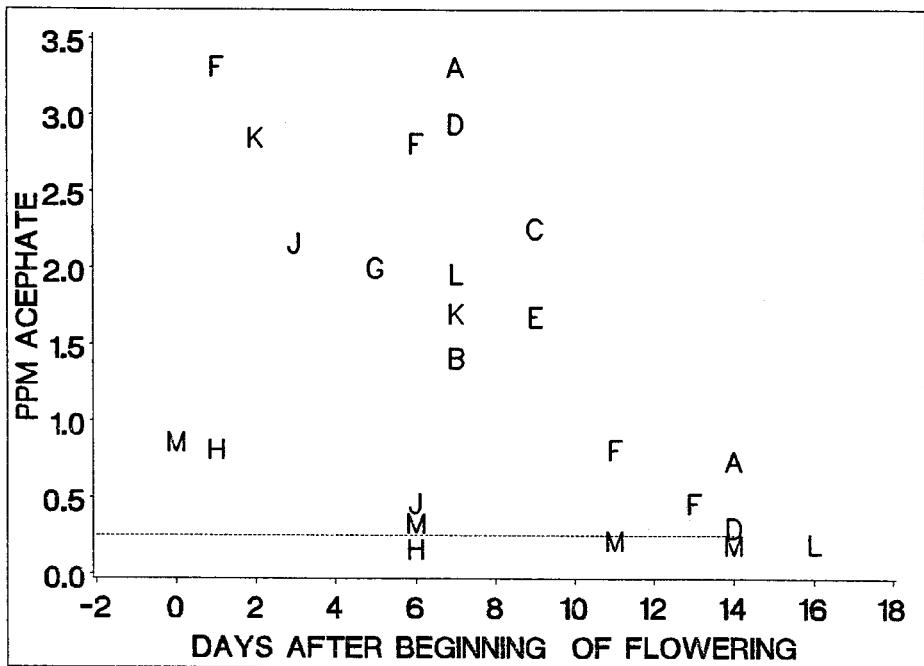
a) dilution effect:

In the experiment of Stoner et al. the contaminated sugar syrup was "diluted" to a high extent by the collection of non-contaminated nectar from natural sources: At the 1 ppm-level the average food-consumption/colony was ca. 9 kg in 8 weeks while the weight gain/colony was 28.8 kg after 8 weeks (Control 7.2 kg and 26.6 kg resp.). It must be considered that the weight gain is: total consumption less metabolized sugar less evaporated water. In our experiment the food-consumption of the colonies was limited nearly totally to the contaminated sugar syrup: The consumption of caged colonies (controls of a similar experiment in 1982) was computed and compared to the values obtained from 1985's controls; the daily consumption of sugar syrup/bee was ca. 75 mg in both cases. The relations (g/g) of food-consumption / stored food in our experiment were 3.54 (control), 7.12 (0.25 ppm) and 18.22 (0.50 ppm). If we assume a ratio of 3:1, the colonies of Stoner et al. would have had to consume ca. 90 kg of sugar solution (instead of 9 kg) for a weight gain of 28.8 kg.

b) decomposition of acephate in the sugar syrup:

Sugar syrup was exchanged weekly in the experiment of Stoner et al.; the authors state, that in a syrup contaminated with 10 ppm acephate the insecticide was totally decomposed (0.00 ppm) after one week.

A dilution of contaminated food by nectar from non-contaminated sources may happen to some extent; it should be considered, however, that under conditions of agriculture, where bees are used as pollinators or to increase yield (e.g. fruit orchards, rape) the dilution effect will unfortunately not be very important. It may be assumed that a concentration of 0.25 ppm acephate in nectar results in damages to honey bee colonies; first effects on brood could be observed after ca. 7 days,



Legend:

(Symbol: plant species, year / treatment days before beginning of flowering)

A: apple 1982 / 1	G: raspb. 1985 / 2
B: apple 1985 / 1	H: raspb. 1984 / 13
C: apple 1984 / 3	J: raspb. 1984 / 20+5
D: apple 1982 / 7	K: cherry 1985 / 2
E: apple 1984 / 7	L: cherry 1982 / 3
F: raspb. 1982 / 2	M: cherry 1984 / 9

----- : 0.25 ppm

**Figure 1. Residues of acephate in nectar after pre-blossom treatments**

significant effects were recorded after 14 days. If we compare the residues of acephate in nectar after pre-blossom sprays to that critical concentration of 0.25 ppm, residues clearly exceed this limit (Fig.1). (Additional effects of methamidophos are even not considered here.) For this reason pre-blossom sprays with acephate should be avoided, if bees are to visit the flowers.

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