

TITLE PAGE

Side effects of Confidor SL 200 on bees following one application to  
Apple trees at the mouse-ear stage

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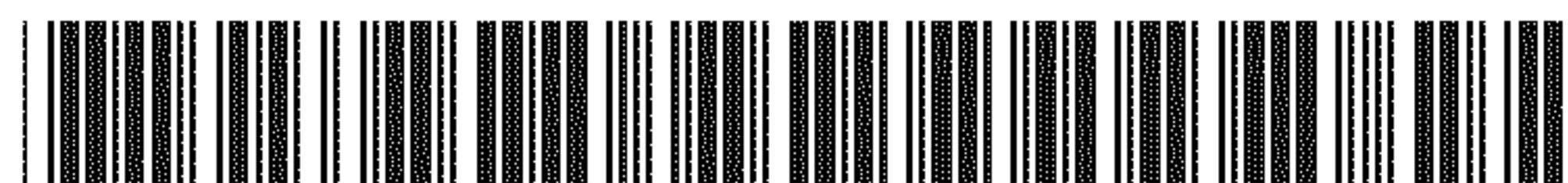
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## 1.0 INTRODUCTION

In 1998, a field trial on apple trees was performed to assess the risks to honey bees associated with use of Confidor 200 SL. Apple-growing represents a realistic worst-case scenario for such a risk assessment for the following reasons:

1. Apple trees blossom shortly after the point at which it is recommended that treatment takes place, i.e. the mouse-ear stage.
2. Apples are a crop that is usually pollinated by bees.
3. Bee colonies are in the early stages of development and are therefore more susceptible to interfering factors.
4. At the envisaged time of use, few other treatments are applied and so the risk of interference from other products is minimised.

## 2.0 MATERIAL AND METHODS

### 2.1 Investigational product

Test substance: Confidor SL 200 (& oliocin)  
 Active ingredients: imidacloprid (& white oil)  
 Chemical name of the active ingredients: 1-[6-CHLORO-3-PYRIDINYL]METHYL-N-NITRO-2-IMIDAZOLIDINIMINE  
 CAS no. of the active ingredients: 138261-41-3  
 Indication: spray  
 Tested application rate: 50 ml/hl Confidor SL 200 (& 500 ml/hl oliocin)

### 2.2 Reference product

No reference product is specified as yet for the tested dosage form (pre-blossom spraying).

### 2.3 Conduct of the study

The test site was treated with the investigational product on 24.03.1998.

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Study director:

Investigator:

Technician:

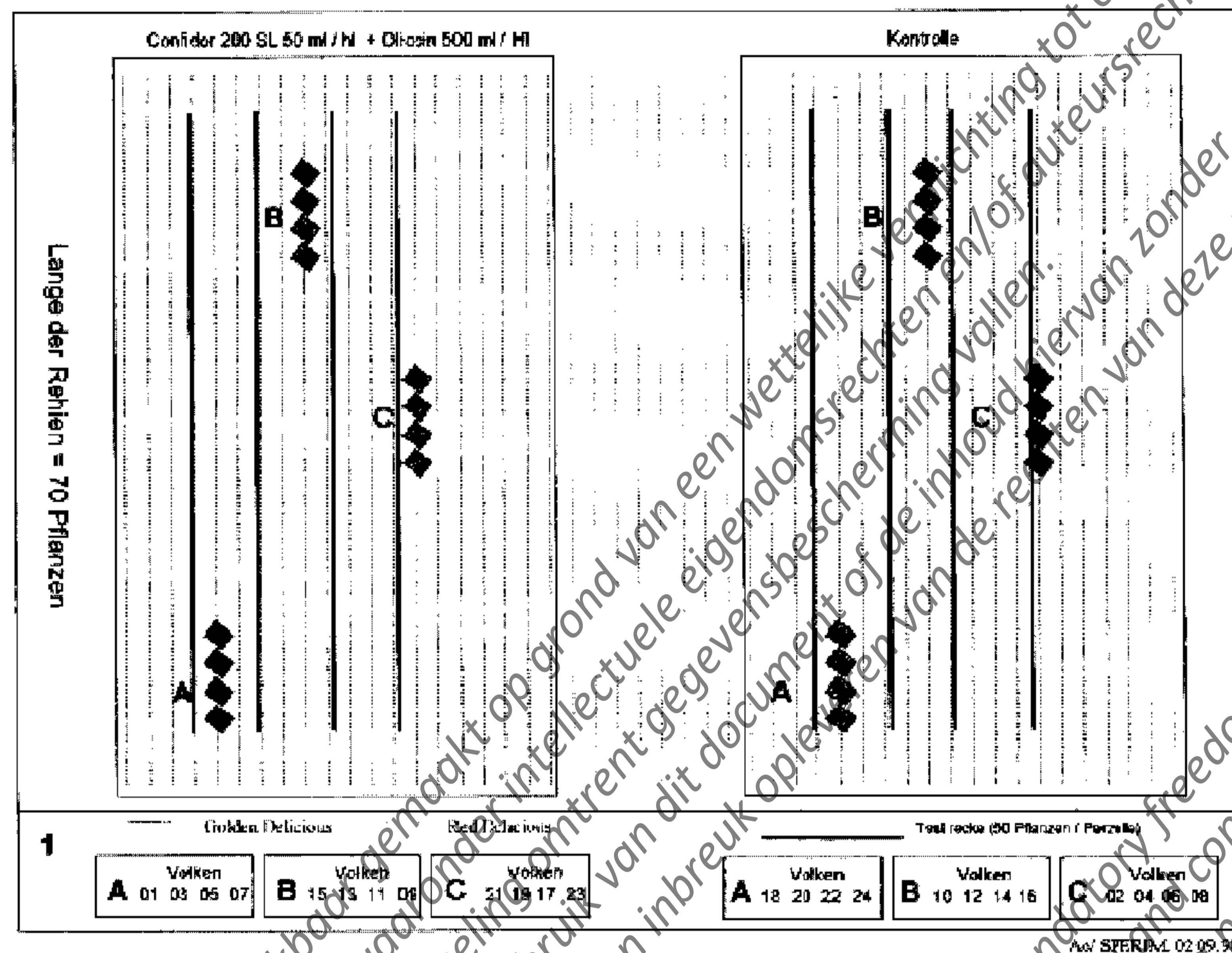
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## 2.4 Study location and establishment of test plots

Two isolated large-sized plots, each consisting of approximately 1400 trees of the variety Golden Delicious, were established on a large fruit farm in the province of Cuneo (see diagram below). One of the two plots was treated on 21.03.98 (mouse-ear growth stage) with Confidor 200 SL (50 ml of product per hl) + oliocin (500 ml of product per hl). The other plot was left untreated.



**Kontrolle = Control**

**Länge der Reihen = 70 Pflanzen = length of rows = 70 plants**

**Test [illegible] (50 Pflanzen/Parzelle) = test [illegible] (50 plants/plot)**

**Völker = colonies**

The two plots were separated by 8 rows of Red Delicious that were likewise left untreated. To investigate the effects on bees, 12 hives were set up per plot, each of which contained 10 frames.

## 2.5 Crop treatments applied over the course of the study

The following treatments were applied to both plots during the study:

06.03.98	white oil	4 l/hl
21.03.98	Mancozeb	200 g/hl
25.03.98	Mancozeb	50 g/hl
30.03.98	bupirimate 256 EC	75 g/hl
04.04.98	kresoxym-methyl 50 WG	15 g/hl
15.04.98	kresoxym-methyl 50 WG	15 g/hl
28.04.98	kresoxym-methyl 50 WG	15 g/hl

## 2.6 Study parameters

The following study parameters were determined:

1. At the start and end of the study:

- colony size (% coverage of frames with bees)
- development of food reserves and brood (cells containing pollen, honey and brood together with empty cells as % of frame surface area)
- weight of the beehives



## 2. During the study:

- number of opened flowers
- number of flower clusters per shoot
- bee visits to flowers
- number of bees returning to the hive
- proportion of bees returning with pollen and type of pollen collected (apple or other plants).

## 2.7 Study procedure

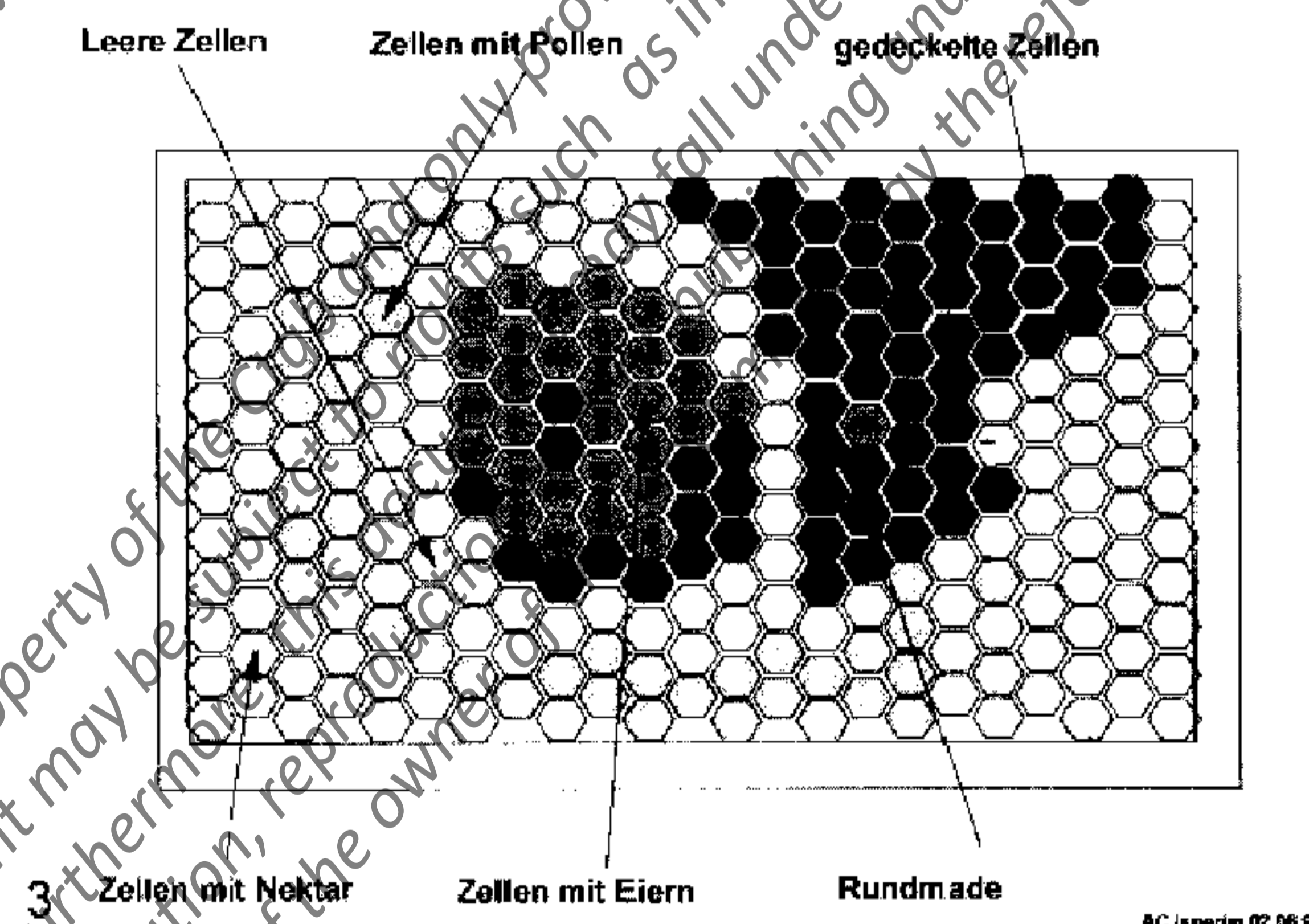
*Observations at the beginning and end of the study*

## 1. Assessment of colony size (% coverage of frames with bees):

Each frame of the hives was removed and both sides were assessed visually. Colony size was recorded in terms of the % of the frames covered with bees, i.e. 240 individual findings (12 hives x 10 frames x 2 sides) were recorded for each plot. These assessments were performed on 8.4.98, the day before the hives were transported to and set up at the study site. The assessment was repeated on the bees final day at the study site on 22.4.98 to see whether there were any differences in colony development between the control plot and the Confidor plot.

## 2. Assessment of development of food reserves and brood [in %]:

At the same time that colony size was assessed, the cells of both sides of the frames were examined to determine the extent (in %) to which they were filled with honey, pollen and brood. The proportion of empty cells was then calculated. In order to obtain worthwhile data, all the hives had to be inspected on the same day. For this reason, only half the beehives were assessed, i.e. 6 hives x 10 frames x 2 sides = 120 individual findings per plot.



- Leere Zellen = empty cells**  
**Zellen mit Pollen = cells containing pollen**  
**gedeckelte Zellen = capped cells**  
**Zellen mit Nektar = cells containing nectar**  
**Zellen mit Eiern = cells containing eggs**  
**Rundmade = larvae**

The remaining 6 hives per plot were merely evaluated qualitatively (i.e. presence or absence of pollen, nectar and brood) at the same time.



### 3. Recording of hive weights:

The hive weights were determined before the start of the study, i.e. before the hives were set up on the plot, and at the end of the study. One hive on the control plot was not included in this assessment, as the bees had swarmed shortly beforehand.

### *Observations during the study*

#### 1. Number of opened flowers

This parameter was recorded as a means of showing any differences between the plots in terms of the available amount of blossom. It is particularly important for evaluation of bee visits to the flowers. The number of opened flowers was recorded in 400 flower clusters per plot.

#### 2. Number of flower clusters per shoot

During the previous assessment re. (1), the number of flower clusters on 400 shoots on the inside of the plot was also determined.

#### 3. Bee visits to flowers

This parameter was assessed several times during the flowering period. 4 segments, each consisting of 50 trees in a row, were marked out per plot and the number of bees on the flowers or seeking nectar in these segments was recorded.

#### 4. Number of bees returning to the hive

A further parameter for recording the activity of the bees on the two plots is the number of bees returning to the hive. This was recorded per minute per hive.

#### 5. Number of bees returning with pollen from apple trees or other plants

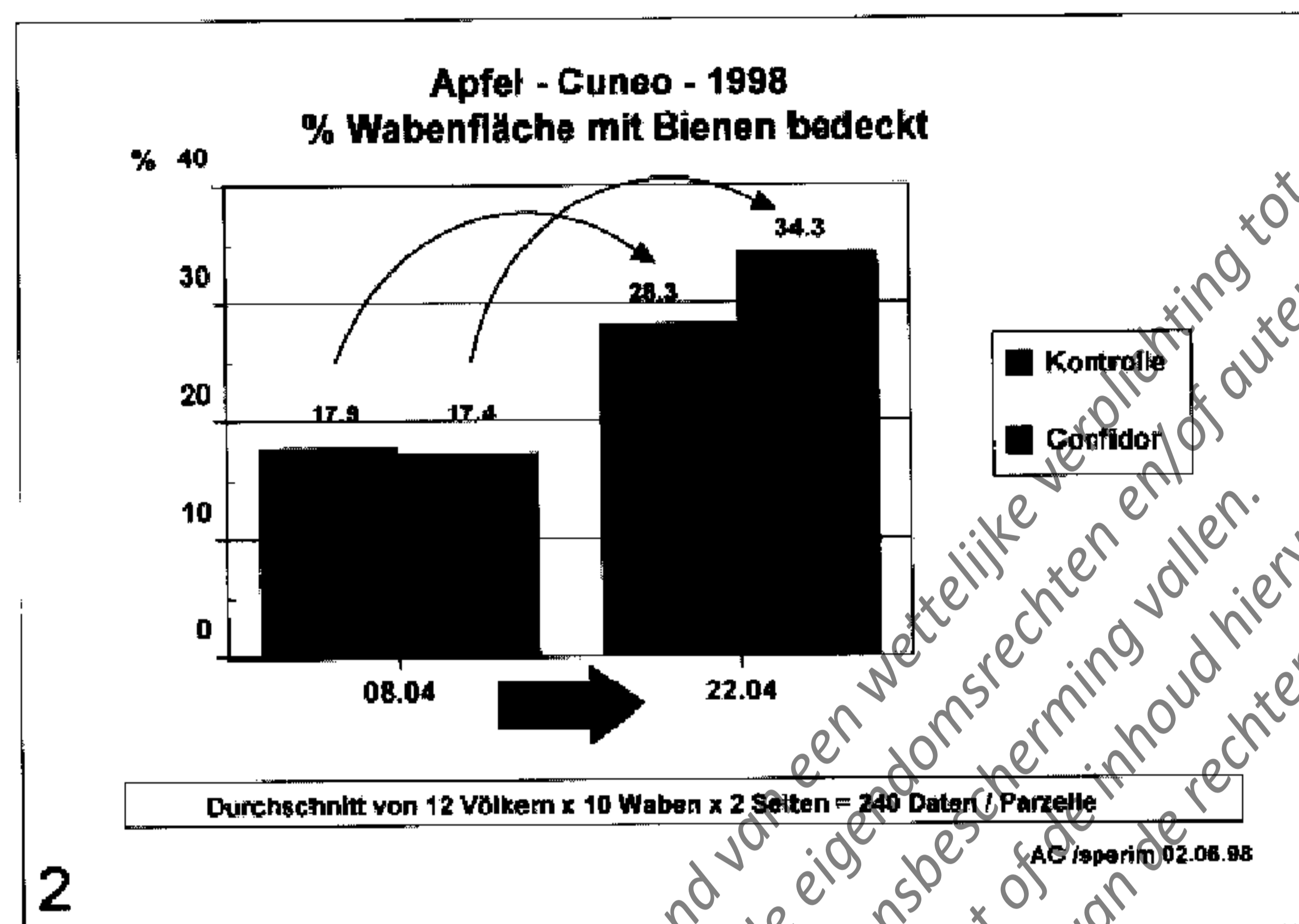
At the same time as recording the number of returning bees per minute per hive, bees carrying pollen were distinguished according to whether they had collected apple pollen or pollen from other plants, e.g. *Taraxacum officinale*.

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### 3.0 RESULTS

#### 3.1 Colony size progression over the course of the study

The results of assessment of colony size are summarised in the diagram below.



% Wabenfläche mit Bienen bedeckt = % of comb surface area covered with bees

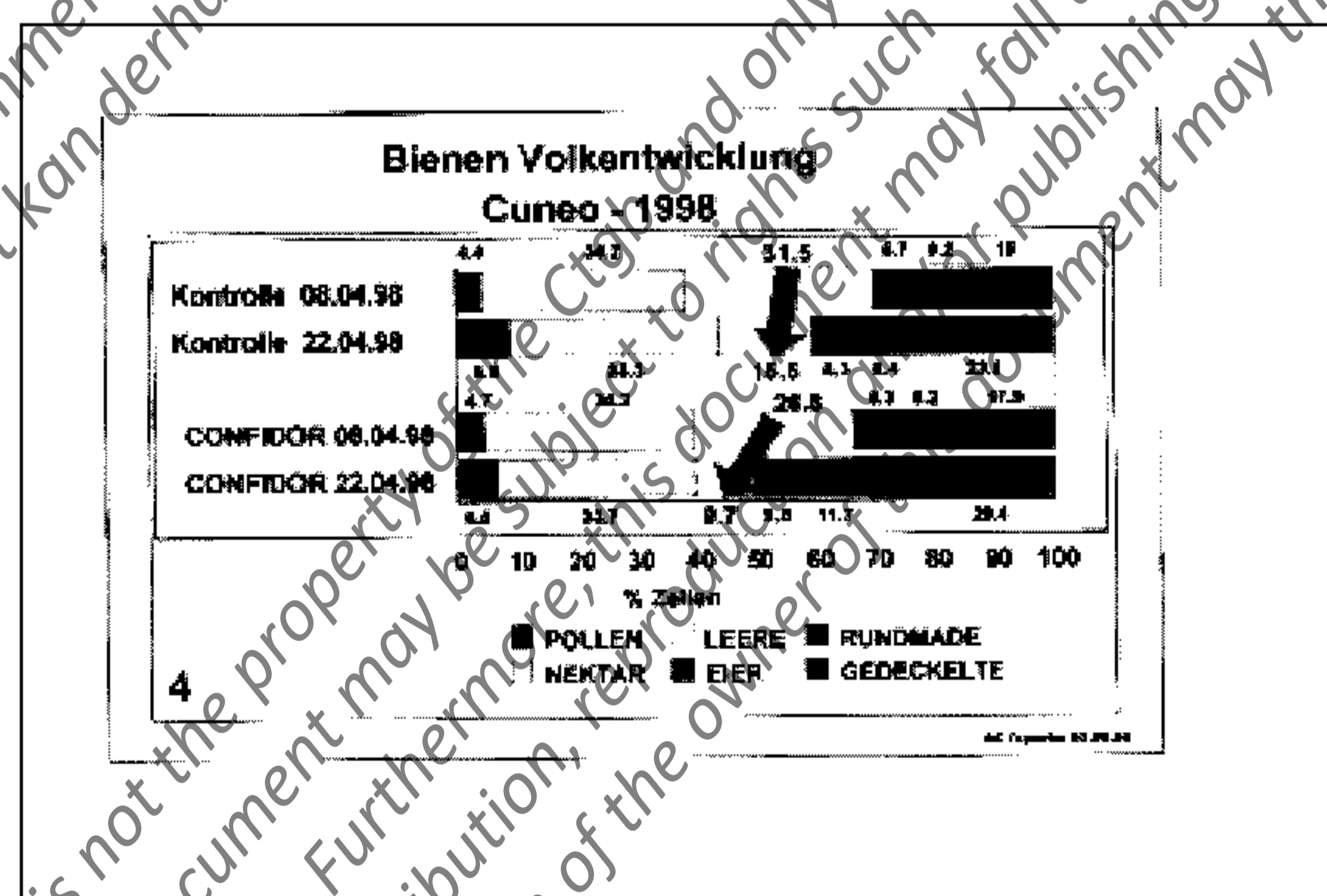
Kontrolle = control

Durchschnitt von 12 Völkern x 10 Waben x 2 Seiten = 240 Daten / Parzelle = mean of 12 colonies x 10 frames x 2 sides = 240 measurements / plot

Analysis of the data shows that the colonies in the beehives on the plot treated with Confidor increased in size to a greater extent than those on the control plot.

#### 3.2 Assessment of development of food reserves and brood (in %):

The diagram below shows the development of food reserves and brood over the course of the study in respect of the 6 hives that were quantitatively evaluated.



Bienen Volkentwicklung = bee colony development

Kontrolle = control

% Zellen = % of cells

Leere = empty

Rundmade = larvae

Nektar = nectar

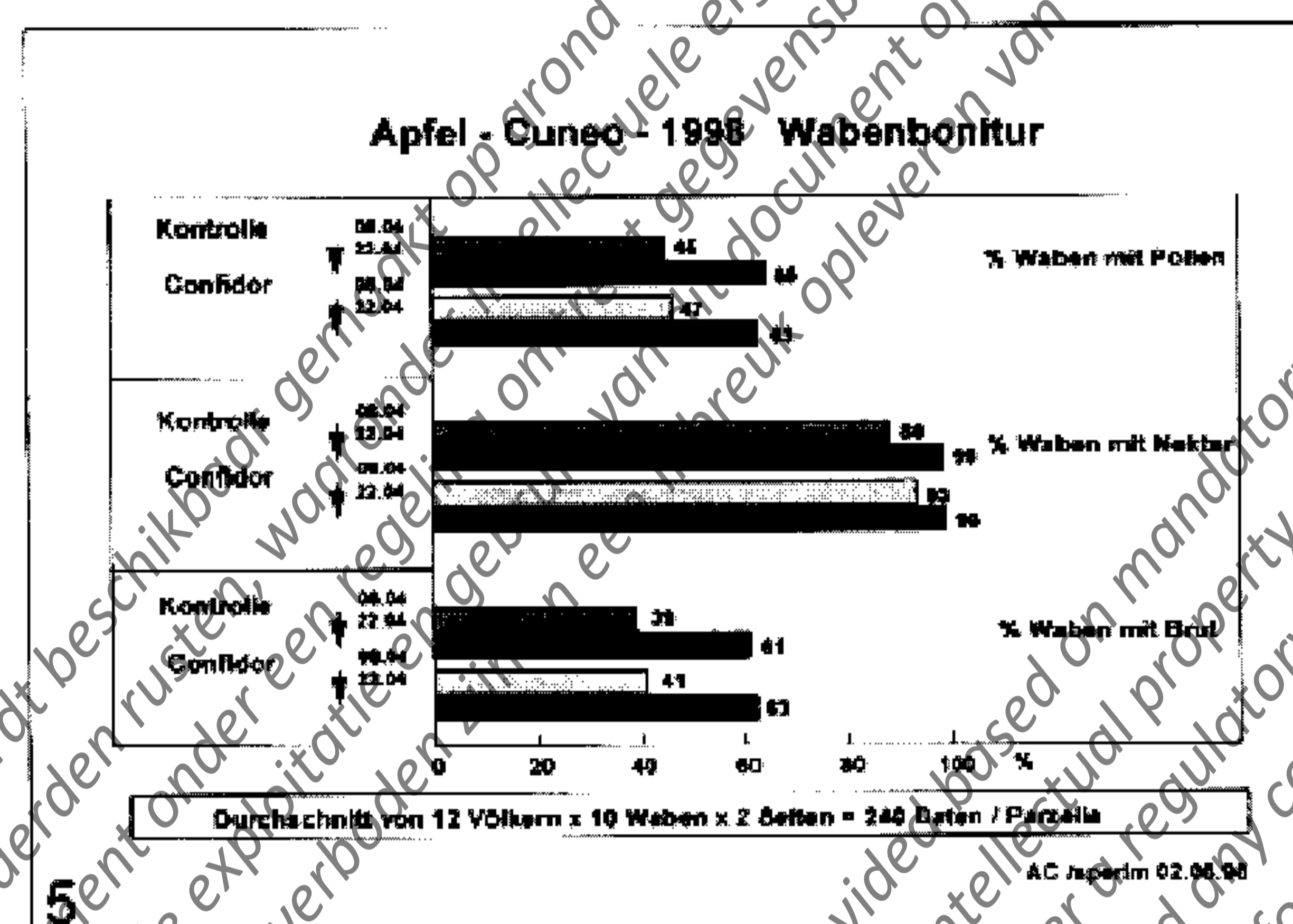
Eier = eggs

gedeckelte = capped



The population of the inspected hives grew at a normal rate over the course of the study. On both plots there was an increase in brood (control +34.8%, Confidor +50%) and amount of pollen collected (control +102.2%, Confidor +38.3%). The amount of honey on the frames remained unchanged or was slightly reduced at the end of the study (control +1.0%, Confidor -4.3%). This is attributable to enlargement of the brood nest, which causes the bees to store honey increasingly in the honey area above the frames where it would not have been included in this assessment. Comparison of hive weights (see 3.3) shows that the amount of honey in absolute terms increased substantially on both plots (control +4.8, Confidor +7.3). On both plots there was a considerable reduction in empty cells (control -50.8%; Confidor -63.8%).

For the sake of greater clarity, the individual parameters are shown qualitatively in the following diagram in a further comparison between control colonies and treated colonies for all assessed hives (12 hives x 10 frames x 2 sides). This again shows that there are no significant differences between the Confidor plot and the control plot in terms of either pollen and honey yield or brood development.



**Wabenbonitur = frame evaluation**

**Kontrolle = control**

**% Waben mit Pollen = % of frames containing pollen**

**% Waben mit Nektar = % of frames containing nectar**

**% Waben mit Brut = % of frames containing brood**

**Durchschnitt von 12 Völkern x 10 Waben x 2 Seiten = 240 Daten / Parzelle = mean of 12 colonies x 10 frames x 2 sides = 240 measurements / plot**

### 3.3 Hive weight progression over the course of the study

Hive weight progression over the course of the study is shown in the following table. This likewise revealed no evidence of any differences between treated and untreated plots.

	Mean hive weights (kg)		Difference
	08.04.98	22.04.98	
Untreated	34.53	36.20	+ 4.8
Confidor	38.39	41.18	+ 7.3



### 3.4 Number of opened flowers

The number of opened flowers in 400 flower clusters per plot was recorded. The results are shown in the following table. Significant differences between the untreated plot and the Confidor plot were observed throughout the entire flowering period. The Confidor plot situated on the northern boundary of the farm exhibited a slower rate of growth compared with the control plot.

	Number of opened flowers [%]		
	09.04	16.04	21.04
Untreated	27.8	79.6	91.0
Confidor	18.3	60.9	83.3

### 3.5 Number of flower clusters per shoot

Significant differences between the untreated plot and the Confidor plot emerged likewise in terms of the number of flowers per shoot (see table below). More than twice as many flowers were counted on the better-protected untreated plot than on the treated plot.

	Mean number of flower clusters per shoot
Untreated	3.96
Confidor	1.70

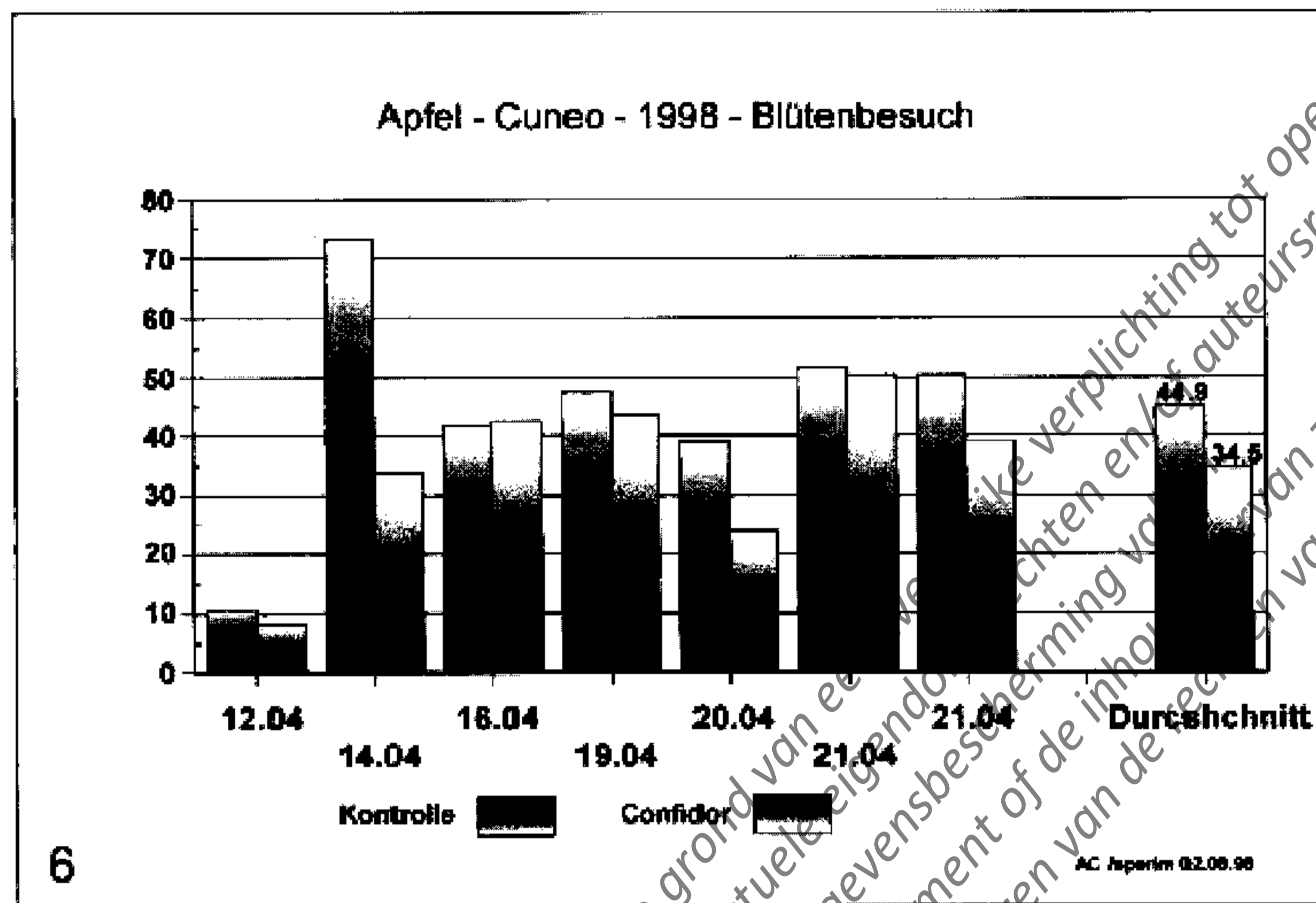
If the number of flower clusters per shoot on 21.04 is multiplied by the mean number of opened flowers per flower cluster on the same date, the results are as follows:

Evaluation on 21.04	Mean no. of flower clusters per shoot	Mean no. of opened flowers per flower cluster	Mean no. of opened flowers per shoot
Untreated	3.96	4.40	17.42
Confidor	1.70	3.85	6.55

The results show that the amount of available blossom on the untreated plot was roughly three times that on the Confidor plot.

### 3.6 Bee visits to flowers

Bee visits to flowers are shown in the following diagram.

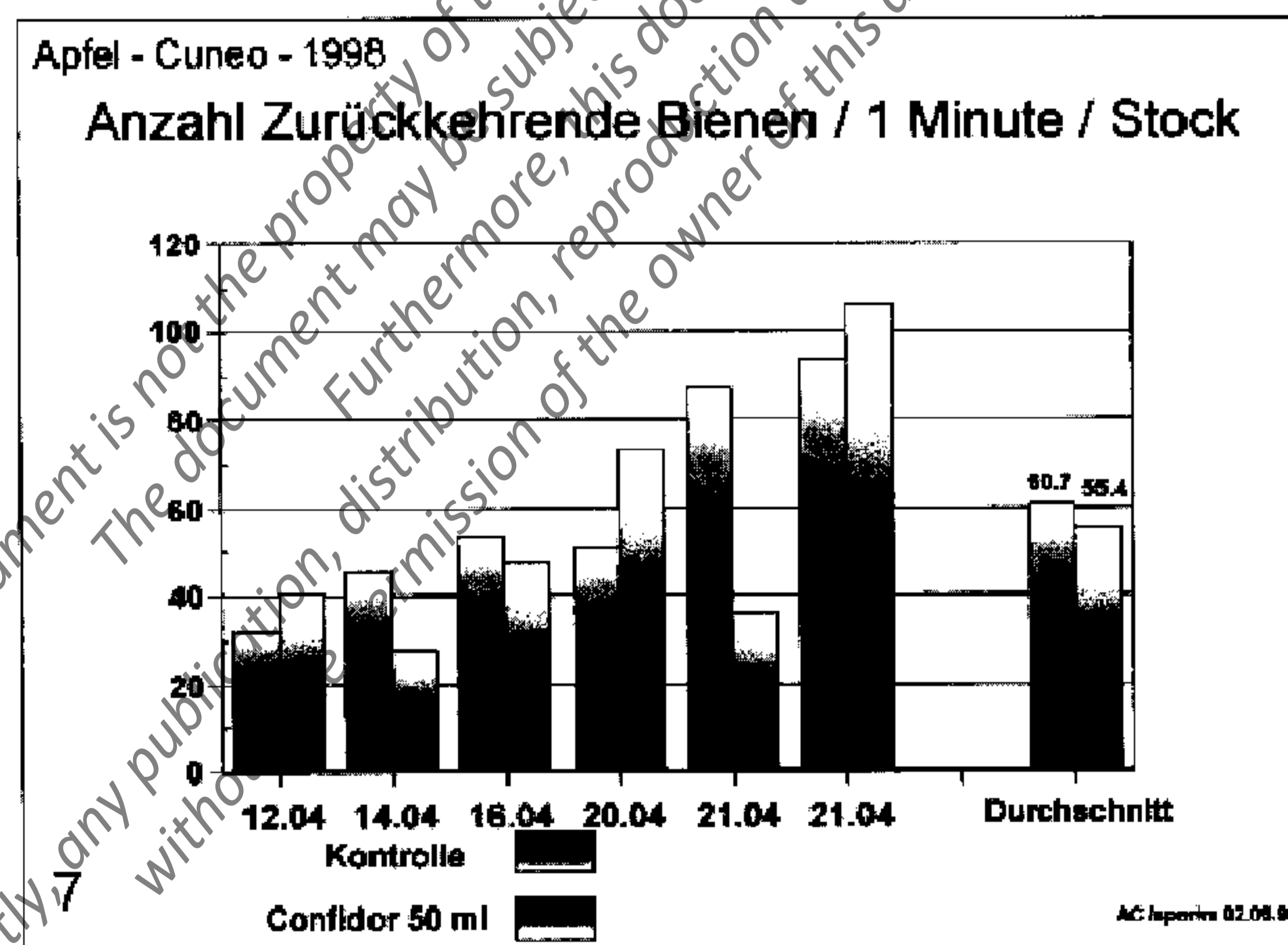


*Blütenbesuch* = bee visits to flowers  
*Durchschnitt* = mean  
*Kontrolle* = control

The mean number of visits to the flowers was lower for the plot treated with Confidor. It must be borne in mind, however, that this parameter was evaluated per tree and that the amount of available blossom was three times higher on the untreated plot than on the treated plot. Taking into account the results for all the other parameters studied, the larger amount of blossom available on the control plot is a plausible explanation for the observed difference in terms of bee visits to the flowers.

### 3.7 Number of bees returning to the hive

The number of bees returning to the hive is shown in the following diagram.



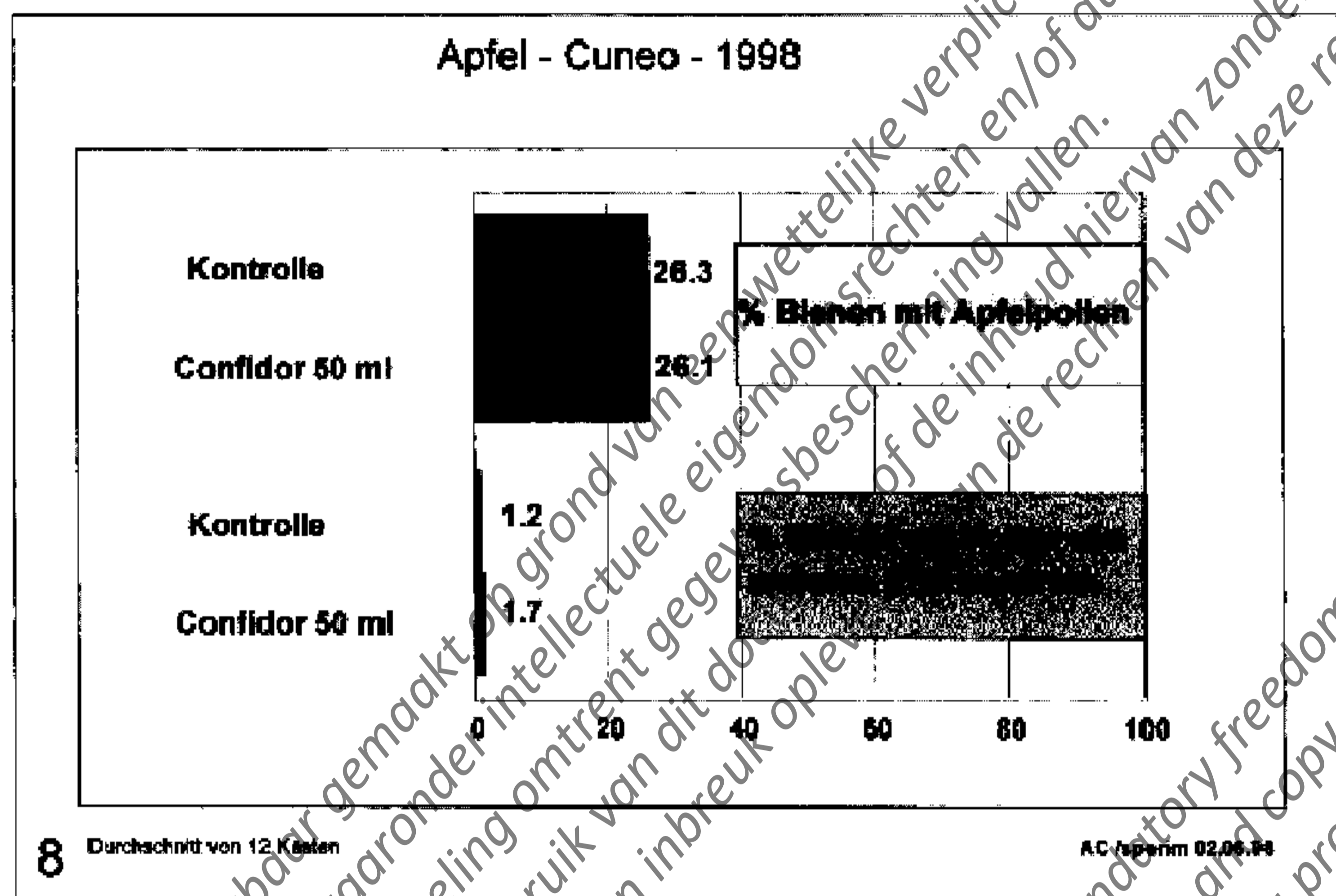
*Anzahl zurückkehrende Bienen / 1 Minute / Stock* = number of returning bees / minute / hive  
*Durchschnitt* = mean  
*Kontrolle* = control



From the means for all the hives, it is evident that this parameter likewise revealed no significant differences between the Confidor plot and the control plot.

### 3.8 Number of bees returning to the hive with pollen

The results for this parameter are shown in the diagram below. No differences were observed between the beehives on treated and untreated plots in terms of pollen collection.



**Kontrolle = control**

**% Bienen mit Apfelpollen = % of bees with apple pollen**

**% Bienen mit Pollen von anderen Pflanzen = % of bees with pollen from other plants**

**Durchschnitt von 12 Kolonien = mean of 12 colonies**

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## 4.0 CONCLUSIONS

The extensive observations in this study permit the conclusion that use of 50 ml/hl Confidor SL 200 on apples at the mouse-ear stage has no adverse effects on bee colonies and does not interfere with pollination on treated land.

The data on brood development over the course of the study are of particular significance. The substantial reduction in the number of empty cells in the hives on the plot treated with Confidor shows that, if used correctly, the product has no effect on bee colony development. This conclusion is reinforced by the observed progression of hive weights over the course of the study.

Observation of the hives was not fully complete when the study came to an end. They were repeatedly inspected at later dates. These inspections revealed no changes or abnormalities in the development of the bee colonies.

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