TRADE SECRET

Confidor SL 200: a multiple rate cage study to determine effects on honeybees, *Apis mellifera* L, when applied to flowering *Phacelia tanacetifolia*

Test Guidelines

OEPP/EPPO 1992: Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honeybees. Bulletin OEPP-EPPO Bulletin 22, 203-215

Study Report Date

6 December 2001

Study Sponsor

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Study/Reference Numbers

Test Substance: Confidor SL 200
MITOX Study Number: B074AMS

c.c.: [redacted] (Sponsor Representative)

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Table of contents

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Tables and figures

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I, the undersigned, hereby declare that the following persons have contributed to the study and that they were qualified to perform the tasks that they were assigned:

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Report Title:

Confidor SL 200: a multiple rate cage study to determine effects on honeybees, *Apis mellifera* L, when applied to flowering *Phacelia tanacetifolia*

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1 EXECUTIVE SUMMARY

1.1 Summary of the test

A multiple rate cage study with the insecticide Confidor SL 200 was performed in a fully replicated semi-field cage test design for honeybees, *Apis mellifera* L. Honeybees were exposed to flowering *Phacelia tanacetifolia* (fiddleneck) treated at several rates of the test product. The following nominal test application rates were used: 14 g a.i./ha, 9 g a.i./ha, 4 g a.i./ha, 2 g a.i./ha, 1.2 g a.i./ha and 0.6 g a.i./ha. The overall test design was in agreement with OEPP/EPPO-guidelines (EPPO, 1992) for cage studies with honeybees.

Small, standardised honeybee colonies were placed in meshed cages of 4 x 5 meter and 2 meter high. Each cage contained approximately 108 untreated flowering *Phacelia*-plants. Honeybees gained foraging experience for four days before exposure. During this period mortality was assessed after every period of honeybee flight. During the final two days before exposure, foraging activity was monitored on six moments during the day.

After this initial 4-day period, the exposure phase started by applying the test product to the *Phacelia* present inside the tents in the morning after the onset of the honeybee flight. All treatment groups were tested simultaneously and compared to a water treated control and a reference item (PennCap M, a 240 g/l CS formulation of methylparathion, at 1000 g a.i./ha). For each treatment there were four replicates. Foraging activity and mortality of the honeybees were assessed during 4 days after initiation of exposure.

Treatment effects were evaluated both by within-colony comparison of foraging activity and mortality before and after exposure (pre-post design) and by among-colony comparison of different treatment groups to the water treatment.

1.2 Summary of results

1.2.1 Foraging activity

Weather conditions were good for the trial. There was little rain and days were warm and light around the application day, which resulted in good flight activity. Before the exposure period, on average 5.0 to 14.1 bees were found foraging on the test plants at each observation moment. There were no statistically significant differences among treatment groups. The day of application was warm and sunny and mean foraging activity in the water control was 13.5 to 18.8 bees at each observation moment. This shows that a sufficient and constant number of bees were in principle exposed to the test product.

In-flight application of Confidor SL 200 at various rates led to statistically significant reductions in foraging activity. This finding applied to test rates of 14 g a.i./ha, 9 g a.i./ha, 4 g a.i./ha and 2 g a.i./ha (the latter depending on the in/exclusion of outliers); 0.6 g a.i./ha and 1.2 g a.i./ha of Confidor SL 200 had no effects on foraging activity. The reduction observed in the 14 g a.i./ha treatment persisted for two more days, but for the other test rates foraging activity returned to the level of the controls from 1 day after application onwards (Table 1.1).

Regression analysis showed a significant relationship between foraging activity and test concentration on the day of application and on the following day, but not

on the other days (Figure 6.1). Exposure to the toxic reference PennCap M caused highly significant reductions in foraging activity on all observation days.

Table 1.1. Average number (± SE, n=4) of foraging bees per day found before and after application of the test item Confidor SL 200.

Treatment	Pre-treatment		Post-treatment							
	11-Jun-01	12-Jun-01	13-Jun-01		14-Jun-01		15-Jun-01		16-Jun-01	
Deionised water	59.0 ± 6.7	74.3 ± 11.7	98.0 ± 19.3		87.3 ± 21.2		87.8 ± 22.0		74.0 ± 17.8	
Confidor 0.6	47.3 ± 3.6	47.0 ± 16.4	69.3 ± 12.2		77.5 ± 8.6		76.5 ± 16.1		61.8 ± 12.4	
Confidor 1.2	38.7 ± 16.8	54.0 ± 22.5	75.8 ± 10.0		85.0 ± 16.1		76.0 ± 11.4		67.8 ± 13.5	
Confidor 2.0	56.5 ± 9.5	64.3 ± 12.2	57.5 ± 3.0	*R1	89.5 ± 12.6		74.0 ± 7.7		74.0 ± 16.7	
Confidor 4.0	50.0 ± 9.2	65.5 ± 12.5	50.5 ± 9.4	*	58.5 ± 11.4		64.0 ± 16.8		48.5 ± 16.6	
Confidor 9.0	46.3 ± 6.6	72.0 ± 9.4	43.8 ± 6.0	*	58.5 ± 6.8		64.0 ± 5.5		51.3 ± 8.9	
Confidor 14	52.3 ± 6.3	61.0 ± 10.4	40.8 ± 3.0	*	48.0 ± 12.8	*	56.5 ± 12.5	*	49.5 ± 12.4	
PennCap	65.3 ± 17.4	84.3 ± 16.6	31.0 ± 3.1	*	2.5 ± 1.0	*	10.8 ± 4.2	*	11.0 ± 5.2	*

Numbers followed by an asterisk are statistically significantly different from the water control (P<0.05 ANCOVA followed by Fisher's LSD test).
R1: Exclusion of colonies with reduced foraging activity in the pre-exposure period, indentified as outliers in the statistical analysis, led to the conclusion that foraging activity in the Confidor 2 g a.i./ha treatment was also significantly affected.

1.2.2 Mortality

In the two days before treatment, mortality ranged on average from 0 to 28 dead bees per cage. There were no statistically significant differences among groups in the pre-exposure phase. In the post-exposure phase statistically significant increased mortality was observed for the reference item, but not for any of the other groups (see Table 1.2 for a summary of mortality data).

Table 1.2. Average number (± SE, n=4) of dead bees found before and after application of the test substance Confidor SL 200.

Treatment	Pre-treatment		Post-treatment							
	11-Jun-01	12-Jun-01	13-Jun-01		14-Jun-01		15-Jun-01		16-Jun-01	
Deionised water	14.3 ± 4.7	5.8 ± 1.7	12.3 ± 1.8		17.3 ± 5.0		20.3 ± 6.0		19.0 ± 2.7	
Confidor 0.6	8.0 ± 2.9	6.5 ± 3.4	13.8 ± 3.3		12.0 ± 0.0		13.5 ± 2.2		12.5 ± 1.9	
Confidor 1.2	13.3 ± 4.0	7.8 ± 2.1	14.0 ± 4.7		20.5 ± 7.4		13.8 ± 3.9		11.8 ± 3.1	*R1
Confidor 2.0	5.5 ± 1.4	4.8 ± 1.9	10.3 ± 3.1		8.5 ± 2.8		15.8 ± 5.6		14.8 ± 4.9	
Confidor 4.0	9.5 ± 4.9	4.0 ± 0.9	6.5 ± 3.6	*R1	10.5 ± 3.0		11.3 ± 1.8		17.3 ± 4.8	
Confidor 9.0	10.0 0.7	6.5 1.8	15.3 2.0		10.5 3.8		9.3 1.3		18.5 3.0	
Confidor 14.0	7.5 ± 2.4	7.8 ± 2.3	19.3 ± 3.8		10.3 ± 4.3		11.3 ± 1.7		17.8 ± 5.1	
PennCapp	12.0 ± 1.9	2.8 ± 1.4	217.3 ± 39.6	*	269.3 ± 52.1	*	169.8 ± 32.3	*	72.3 ± 16.9	*

Numbers followed by an asterisk are statistically significantly different from the water control (ANCOVA followed by Fisher's LSD test). Dates indicate the day mortality occurred. Counting was the next morning.
R1 Mortality in these groups was lower than in the control group.

1.2.3 Conclusions

When applied during bee flight, 0.6 g a.i./ha and 1.2 g a.i./ha of Confidor SL 200 had no effects on foraging activity and mortality of the honeybee *Apis mellifera*.
At a rate of 2.0 g a.i./ha, 4.0 g a.i./ha and 9.0 g a.i./ha foraging activity was reduced on the day of application, but no effects on mortality were observed.
At the highest test rate (14.0 g a.i./ha) statistically significant reduction in foraging was found during the first two days, but no effects on mortality were observed.
The reference item (PennCap M) was statistically significantly different for foraging and mortality during the whole period following application.

2 RATIONALE AND OUTLINE OF THE TEST

2.1 Study background

Honeybees play an important role as pollinators in a large number of crops. The purpose of the study was to determine effects of the insecticide Confidor SL 200, on honeybees, *Apis mellifera* L, in a fully replicated semi-field cage test design. Honeybees were exposed to flowering *Phacelia tanacetifolia* (fiddleneck) treated at six rates of the test item.
The overall test design was in agreement with OEPP/EPPO-guidelines (EPPO, 1992) for cage studies with honeybees, and performed in accordance with the study protocol, with the exception of the deviations listed in Appendix 7.

2.2 Principle of the trial

Honeybee colonies, standardised with respect to age structure and total honeybee weight, were individually enclosed in meshed cages. To enable a straightforward assessment of mortality, the colonies were manipulated such that no new adult honeybees would emerge during the trial period. To gain foraging experience each colony was confined to untreated flowering *Phacelia* plants inside the cage for a period of four days. In the morning following this period, after the start of honeybee flight, the plants were treated with different rates of the test product, with deionised water and with PennnCap M as reference items. This was the initiation of the exposure phase. The exposure lasted for a total of four days. Foraging activity and mortality were compared among treatments and before and after initiation of exposure.

2.3 Trial design

Treatments: The test was performed with Confidor SL 200 at the following nominal test application rates: 0.6 g a.i./ha, 1.2 g a.i./ha, 2.0 g a.i./ha, 4.0 g a.i./ha, 9.0 g a.i./ha and 14 g a.i./ha. Applications were performed in the morning after the onset of honeybee flight. All treatment groups were tested simultaneously and compared to a deionised water treated control and a toxic reference (PennCap M), a 240 g/l CS formulated methylparathion at 1000 g a.i./ha.

Application: The applications were performed using a hand-held compression sprayer fitted with a disposable reservoir containing 35 ml of test solution for each cage. Based on a carrier volume of 200 l/ha, this volume was the exact quantity of spray liquid for the surface area to be treated.

Test units: The test unit was a meshed cage of 20m^2 and 2m high containing about 108 flowering *Phacelia* plants. One standardised honeybee colony was placed in each tent. Colonies of honeybees were prepared on the day of initiation. Each colony was a small hive containing about 250 gram honeybees (approximately 2000 individuals) and included a queen and young brood.

Replicates: There were 4 replicates per treatment (in total 32 test units), arranged in random order over a single line of cages.

2.4 Endpoints of the test

Endpoints of the test were:

- Foraging activity as the number of honeybees observed in the crop daily at six moments during honeybee flight.
- The cumulative number of dead honeybees found daily inside the cages.
- Weight loss of the colony and evaluation of the development of the brood.

3 COMPOUNDS USED

The test product was stored at room temperature in the dark and all use was registered. Deionised water was the solvent for all treatment solutions and served as the harmless reference (control). PennCap M served as the toxic reference.

<u>Test item</u>

Product name:	Confidor SL 200
Active ingredient:	NTN 33893
CAS-number:	138261-41-3
Type of formulation:	soluble liquid
Formulation code:	SL
Nominal formulation concentration:	200 g/l
Analysis of sample	
• Purity [g/l.]:	194 g/l
• Date of analysis:	4 September 2000
• Expiration date:	4 September 2001
MITOX ID number:	20001121A/B
Test application volume:	equivalent to 200 l/ha
Test application rates (in g a.i./ha)	14, 9, 4, 2, 1.2 and 0.6 g a.i./ha
Solvent:	deionised water

<u>Reference item product</u>

Product name:	PennCap M
Active ingredient:	methyl parathion
CAS-number:	298-00-0
Type of formulation:	Micro capsules suspension
Formulation code:	CS
Nominal formulation concentration:	240 g/l
MITOX ID number:	19990819A
Test application volume:	equivalent to 200 l/ha
Test application rates (in g a.i./ha)	1000 g a.i./ha
Test concentration:	20.83 g product/l
Solvent:	deionised water

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4 DESCRIPTION OF THE METHOD

4.1 Test cages

Cages covered a surface of 4m x 5m and were approximately 2m high. The mesh size of the cover was 1mm, nominally. The floor of the cage was lined with white water permeable synthetic foil to facilitate recovery of dead honeybees. This foil was placed on top of a black water permeable cloth that was placed to suppress weed growth and as a support for the thin white foil. Honeybee hives were placed on poles at a height of approximately 1.5 meter. The high position of the hive made the orientation of the honeybees easier.
In each compartment 108 flowering plants were placed. The plants were contained in 36 pots divided over two plant trays with a total surface of 17550 cm^2. There were 3 plants per pot. Inside the cages the plants were connected to a drip irrigation system, so that watering of the plants could be done with minimal disturbance of the honeybees and without the risk of wetting the residue. To protect the residue from potential rainfall, a shelter of about 2 m^2 made of UV-transparent foil (Mevolux EVA) hang from the roof directly over the test plants.

4.2 Honeybee hives

Standardised colonies were obtained through a commercial supplier (INBUZZ, Imkersbedrijf Boot & Calis). As is common in The Netherlands, the bees used were not of a specific strain, but hybrids of existing genotypes.
Standardised mini-hives of 23.5 x 39 cm and 22.5 cm high (measured inside) were assembled on the day of initiation (8 June 2001). This was done by placing into each mini-hive: (1) an isolated queen, (2) a brood comb with brood up to 7 days old plus a comb with pollen and honey and (3) approximately 2000 young worker bees.

Ad (1) On 8 June 2001 preparation of the mini-hives started by collecting 32 queens from existing colonies. Each queen was put inside a small cage of 3.5 x 8.0 cm and 1.4 cm high together with two worker bees. The cage included a sugar plug (of 2.5 cm long and 3.5 x 1.4 cm) and was inserted into a mini-hive shortly after preparation. Release of the queen (by feeding of worker bees on the sugar plug) was expected to take at most 12 hours.

Ad (2) On 1 June 2001 empty combs were introduced into the centre of the brood nests of 40 healthy colonies. Seven days later (8 June 2001), 32 of these combs were removed and each comb, now containing eggs and larvae of up to 7 days old, was transferred to one mini-hive. In addition, each mini-hive received one comb with pollen and honey. This procedure ensured that new worker bees would not emerge during the trial, which facilitated the interpretation of the data.
Ad (3) To reduce background mortality due to ageing of the bees, the experiments were performed with predominantly young worker bees. To loose the older worker bees, 15 large colonies were moved about 25 meters away from their original site on 5 June 2001. Normally the foragers, generally old

honeybees, fly to the original site and drift into colonies neighbouring this original site, thus leaving the hive with young bees. Three days later, the young honeybees remaining in the colonies were introduced into the mini-hives.
To transfer the young worker bees to the mini-hives, the workers present in two to three colonies were collected together in a large container. From this container, approximately 250 grams of honeybees were transferred to each mini-hive. After 8 mini-hives were prepared the batch of bees in the large container was supplemented with the worker bees of 2 or 3 additional colonies. Hence, for the preparation of 32 mini-hives four batches, derived from a total of 10 colonies, were used. For each of the four replicates per treatment bees from a different source batch were used.
The total weight of the honeybees in the mini-hives was assessed by weighing the hives before and after entering of the honeybees. To relate the net weight of the mini-hive to the actual numbers of bees transferred, sub-samples of bees were collected, counted and weighed. For each of the four source batches one sample of between 77 and 104 honeybees was taken. From these data it could be shown that on average 2034 ± 91 (n=32) young worker bees were present in each mini-hive at the start of the experiment.

4.3 Test plants

Phacelia tanacetifolia was chosen as a test plant because it ensures a high foraging activity of the honeybees. Plants were obtained from MITOX nurseries, where they were grown prior to the experiment. The plants were grown the first 48 days in a tunnel greenhouse, after this period (BBCH scale 50) the trays were transferred to the test cages. The plants were potted in commercial pot soil contained in 64 plastic trays with approximate dimensions of 135x65 and 15cm high. These trays covered a total surface of 17550 cm^2.
In each tray 18 pots (Ø 12 cm) were moulded. In each of these pots 5 seeds of *Phacelia tanacetifolia* were put individually in prepared seed-holes. After germination, seedlings were removed such that 3 plants per pot remained. The pots were then connected to a drip irrigation system. Through this system the pots received water regularly. During their development period no treatments with plant protection products took place.
At least once a week, the phenological growth stage of the plants was registered, according to the BBCH-scale of oilseed rape (Enz and Dachler 1997). This was done each time for five randomly chosen plants. The trial was started when all plants were flowering (BBCH>65).

4.4 Spraying equipment and application

The test product was applied using a volume equivalent to 200 l/ha. The applications were performed using a hand-held compression sprayer fitted with a disposable reservoir containing 35 ml of test solution for each cage. Based on a carrier volume of 200 l/ha, this volume was the exact quantity of spray liquid for the surface area to be treated (17550 cm^2). The entire volume was homogeneously distributed over the test plants. Spray deposit distribution was documented with water-sensitive paper during application by placing 1 spray

card (manufacturer Novartis) just below the top flowers of one plant per tray in each of the 32 tents.
All applications were on 13 June 2001, 5 days after the introduction of the honeybees. Treatments were performed under windless conditions inside the cages from 10:25 to 11:45, *i.e.* after the start of the honeybee flight. The spraying order followed the order of the tent numbers.

4.5 Preparation of test solutions

All solutions were prepared with calibrated laboratory equipment on the day of application. In all cases a total of 200 ml was prepared. From this volume aliquots of 35 ml were transferred to each of 4 disposable containers to be fitted to a hand-held compression sprayer (one container for each replicate).

Preparation of Confidor SL 200 solutions

Preparing Stock solution:	ml product	Final volume (ml)
	2.00	200

Preparing final solutions

	g a.i./ha	Solution type:	ml solution:	Final volume (ml)
Solution 1	14.0	stock	7.2	200
Solution 2	9.0	stock	4.65	200
Solution 3	4.0	stock	2.05	200
Solution 4	2.0	stock	1.05	200
Solution 5	1.2	stock	0.620	200
Solution 6	0.6	stock	0.310	200

Preparation of the reference item
The test concentration of PennCap M was 5 g a.i./l or 20.83 g product/liter. On each occasion a total of 200 ml solution was prepared by dispersing 4.16 g product in 200 ml of deionised water.

4.6 Assessments

Throughout the experiment mortality was assessed on a daily basis. This was done by searching the test cages for dead bees. All dead bees found were collected and removed.
Foraging activity was determined six times per day, usually around 10:00, 11:30, 12:30, 13:30, 14:30 and 16:00 (see Appendix 3 for the precise timing of assessments). On these occasions the observer made a 18-24 seconds walk around the plants in each cage and recorded all the bees that were flying to or foraging on the test plants during this period. Other flight activities were not recorded.
At the end of the experiment the hives were closed and transported to the INBUZZ facilities in Wageningen. The condition of the mini-hives (brood development, and numbers of dead bees in hive) was evaluated two days after the last assessment. Hereto the hives were opened and the brood present on the combs was inspected and counted as eggs, larvae and pupae (capped brood) up to 50 individuals in each category. In case more than 50 individuals were counted brood development was considered normal and counting was stopped.

4.7 Test conditions

Throughout the experiment relative humidity and temperature were measured every 5 minutes by the internal sensors of a MiniMet meteostation (Skye instruments, model SDL2900) positioned outside one of the tents. Averages were logged every hour. Rainfall was measured with an external rain gauge connected to the MiniMet datalogger (Environmental Measurements, model ARG 100). Cumulative millimeters rainfall/hour were logged every hour. Light intensity, was measured every 5 minutes with an external pyranometer sensor (Skye Instruments, type SKS1110), measuring in the range of 350 to 1100 nm, also connected to the datalogger. Averages were logged every hour. Plants were shielded from potential rain by a UV-transparent rain cover of about 2 m^2.
In general, conditions were rather constant.

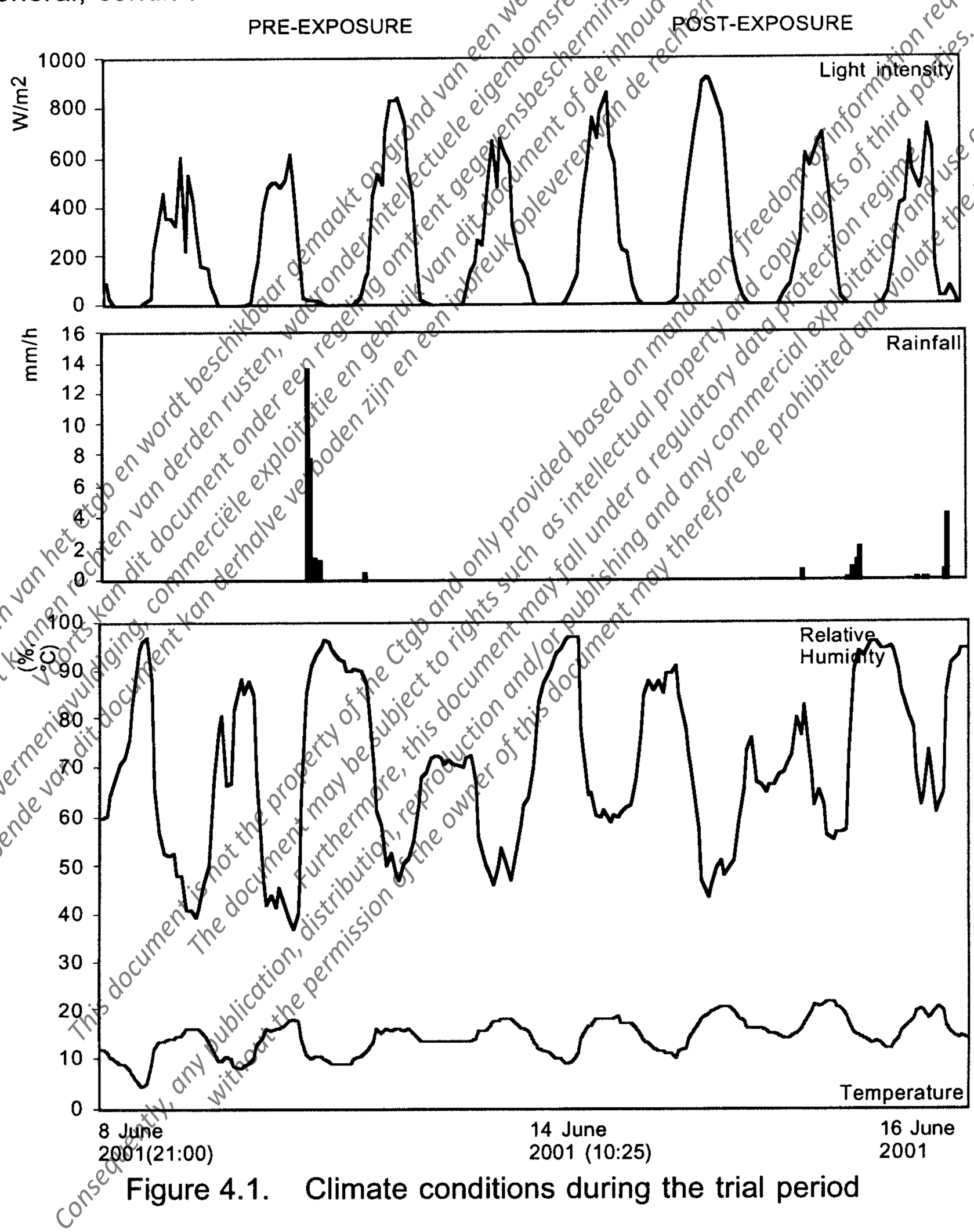

Figure 4.1. Climate conditions during the trial period

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5 DATA ANALYSIS

5.1 Foraging

As a first step cumulative foraging was calculated for the total pre- and for the total post-application periods. For both periods these data were analysed as a two-factor experiment, with the factors treatment (8 levels) and colony origin (4 levels corresponding to the source batch of bees). This was done using box plots and a 2-way ANOVA without interaction.
As a next step the data were analysed as a pre-post design, using a covariance alternative to repeated measures ANOVA. Hereto foraging, averaged over the last two-pre-application days, was taken as the covariate to predict post-treatment response levels for each day following application separately. The ANCOVA was followed by Fisher's LSD test to test for between-treatment differences. Foraging data was square root-transformed to improve ANOVA-conditions.
In addition, the logarithm of the ratio of post-/pre-treatment numbers of foraging bees was calculated for each replicate and each day. These data were analysed graphically (box plots and bar charts).
To investigate a possible relationship between the number of bees foraging and the dose rate applied, a regression analysis was performed on the logarithm of the test rates. These data were analysed graphically (fig. 6.1).

5.2 Mortality

Analysis of the mortality data was analogous to the foraging analysis described above, i.e. repeated measures ANOVA followed by ANCOVA and Fisher's LSD test. Mortality data were log-transformed to improve ANOVA-conditions. The dead bees that were observed during the first two days of the test were not included in the analysis, because mortality in this period was likely to reflect potential differences in manipulation during the preparation of the mini-hives

Null hypotheses were rejected if the probability of observing the test statistic (type I error level) fell below 5% (alpha = 0.05). Systat 5.2 for the Macintosh (Wilkinson, 1992) was used for all statistical analyses.

6 RESULTS

6.1 Foraging activity

Before the exposure period, on average 5.0 to 14.1 bees were found foraging on the test crop at any observation moment. There were no statistically significant differences between treatment groups in the pre-exposure period. However, outliers were observed. These were four colonies with exceptionally low foraging activity. There were no obvious reasons for this observation.
The day of application was warm and sunny and foraging activity in the water control was 13.5 to 18.1 bees on each observation moment on the day of

application. This shows a sufficient and constant number of bees were in principle exposed to the test product.
In-flight application of Confidor SL 200 at various rates led to statistically significant reductions in foraging activity. This finding applied to test rates of 14 g a.i./ha, 9 g a.i./ha, 4 g a.i./ha and 2 g a.i./ha (the latter depending on the in/exclusion of outliers). The reduction observed in the 14 g a.i./ha treatment persisted for two more days, whereas for the other test rates foraging activity returned to the same level as the controls from 1 day after application onwards (Table 6.1).

Table 6.1. Average number (± SE, n=4) of foraging bees per day found before and after application of the test substance Confidor SL 200.

Treatment	Pre-treatment		Post-treatment			
	11-Jun-01	12-Jun-01	13-Jun-01	14-Jun-01	15-Jun-01	16-Jun-01
Deionised water	59.0 ± 6.7	74.3 ± 11.7	98.0 ± 19.3	87.3 ± 21.2	87.8 ± 22.0	74.0 ± 17.8
Confidor 0.6	47.3 ± 3.6	47.0 ± 16.4	69.3 ± 12.2	77.5 ± 8.6	76.5 ± 16.1	61.8 ± 12.4
Confidor 1.2	38.7 ± 16.8	54.0 ± 22.5	75.8 ± 10.0	85.0 ± 16.1	76.0 ± 11.4	67.8 ± 13.5
Confidor 2.0	56.5 ± 9.5	64.3 ± 12.2	57.5 ± 3.0 *R1	89.5 ± 12.6	74.0 ± 7.7	74.0 ± 16.7
Confidor 4.0	50.0 ± 9.2	65.5 ± 12.5	50.5 ± 9.4 *	58.5 ± 11.4	64.0 ± 16.8	48.5 ± 16.6
Confidor 9.0	46.3 ± 6.6	72.0 ± 9.4	43.8 ± 6.0 *	58.5 ± 6.8	64.0 ± 5.5	51.3 ± 8.9
Confidor 14	52.3 ± 6.3	61.0 ± 10.4	40.8 ± 3.0 *	48.0 ± 12.8 *	56.5 ± 12.5 *	49.5 ± 12.4
PennCap	65.3 ± 17.4	84.3 ± 16.6	31.0 ± 3.1 *	2.5 ± 1.0 *	10.8 ± 4.2 *	11.0 ± 5.2 *

Numbers followed by an asterisk are statistically significantly different from the water control (ANCOVA followed by Fisher's LSD test).
R1: Exclusion of colonies with reduced foraging activity in the pre-exposure period, indentified as outliers in the statistical analysis, led to the conclusion that foraging activity in the Confidor 2 g a.i./ha treatment was also significantly affected.

Regression analysis showed a significant relationship between foraging activity and test concentration on the day of application and on the following day, but not on the other days (Figure 6.1). Exposure to the toxic reference PennCap M caused highly significant reductions in foraging activity on all observation days (Table 6.1).

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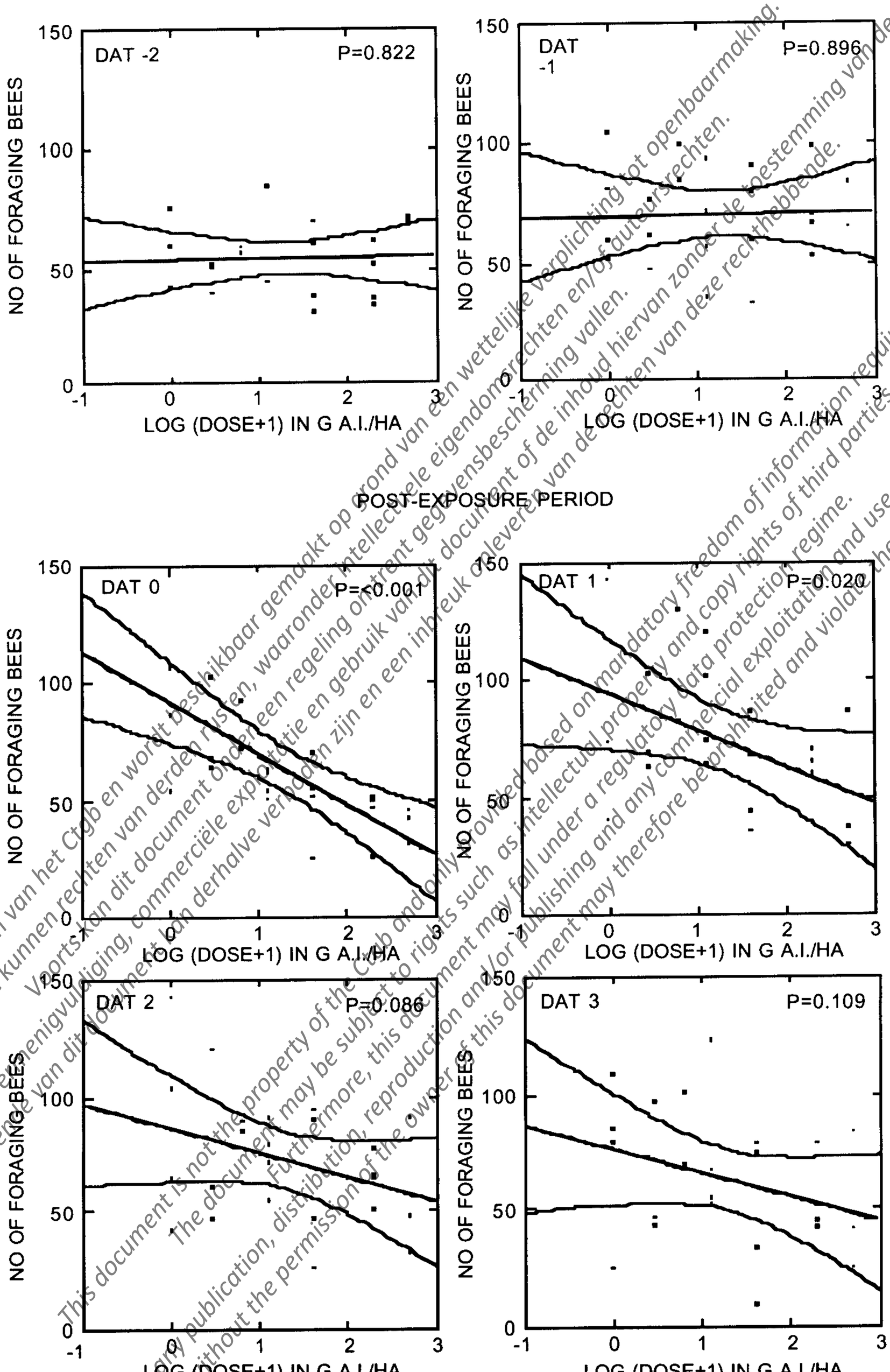

Figure 6.1 Regression of foraging bees on the logarithm of test rate. Water was included as 0 g a.i./ha and 1 was added to the dose to avoid zero values. DAT= day after treatment, P-values indicate the significance of the regression coefficient.

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6.2 Mortality

In the two days before treatment, mortality ranged on average from 0 to 28 dead bees per cage per treatment group. There were no statistically significant differences among treatment groups, and no statistically significant differences among colony.

Table 6.2. Average number (± SE, n=4 in all cases) of dead bees found before and after application of the test substance Confidor SL 200.

Treatment	Pre-treatment		Post-treatment			
	11-Jun-01	12-Jun-01	13-Jun-01	14-Jun-01	15-Jun-01	16-Jun-01
Deionised water	14.3 ± 4.7	5.8 ± 1.7	12.3 ± 1.8	17.3 ± 5.0	20.3 ± 6.0	19.0 ± 2.7
Confidor 0.6	8.0 ± 2.9	6.5 ± 3.4	13.8 ± 3.3	12.0 ± 0.0	13.5 ± 2.2	12.5 ± 1.9
Confidor 1.2	13.3 ± 4.0	7.8 ± 2.1	14.0 ± 4.7	20.5 ± 7.4	13.8 ± 3.9	11.8 ± 3.1 *R1
Confidor 2.0	5.5 ± 1.4	4.8 ± 1.9	10.3 ± 3.1	8.5 ± 2.8	15.8 ± 5.6	14.8 ± 4.9
Confidor 4.0	9.5 ± 4.9	4.0 ± 0.9	6.5 ± 3.6 *R1	10.5 ± 3.0	11.3 ± 1.8	17.3 ± 4.8
Confidor 9.0	10.0 0.7	6.5 1.8	15.3 2.0	10.5 3.8	9.3 1.3	18.5 3.0
Confidor 14.0	7.5 ± 2.4	7.8 ± 2.3	19.3 ± 3.8	10.3 ± 4.3	11.3 ± 1.7	17.8 ± 5.1
PennCapp	12.0 ± 1.9	2.8 ± 1.4	217.3 ± 39.6 *	269.3 ± 52.1 *	169.8 ± 32.3 *	72.3 ± 16.9 *

Numbers followed by an asterisk are statistically significantly different from the water control (ANCOVA followed by Fisher's LSD test). Dates indicate the day mortality occurred. Counting was the next morning.
R1: Mortality in these groups was lower than in the control group

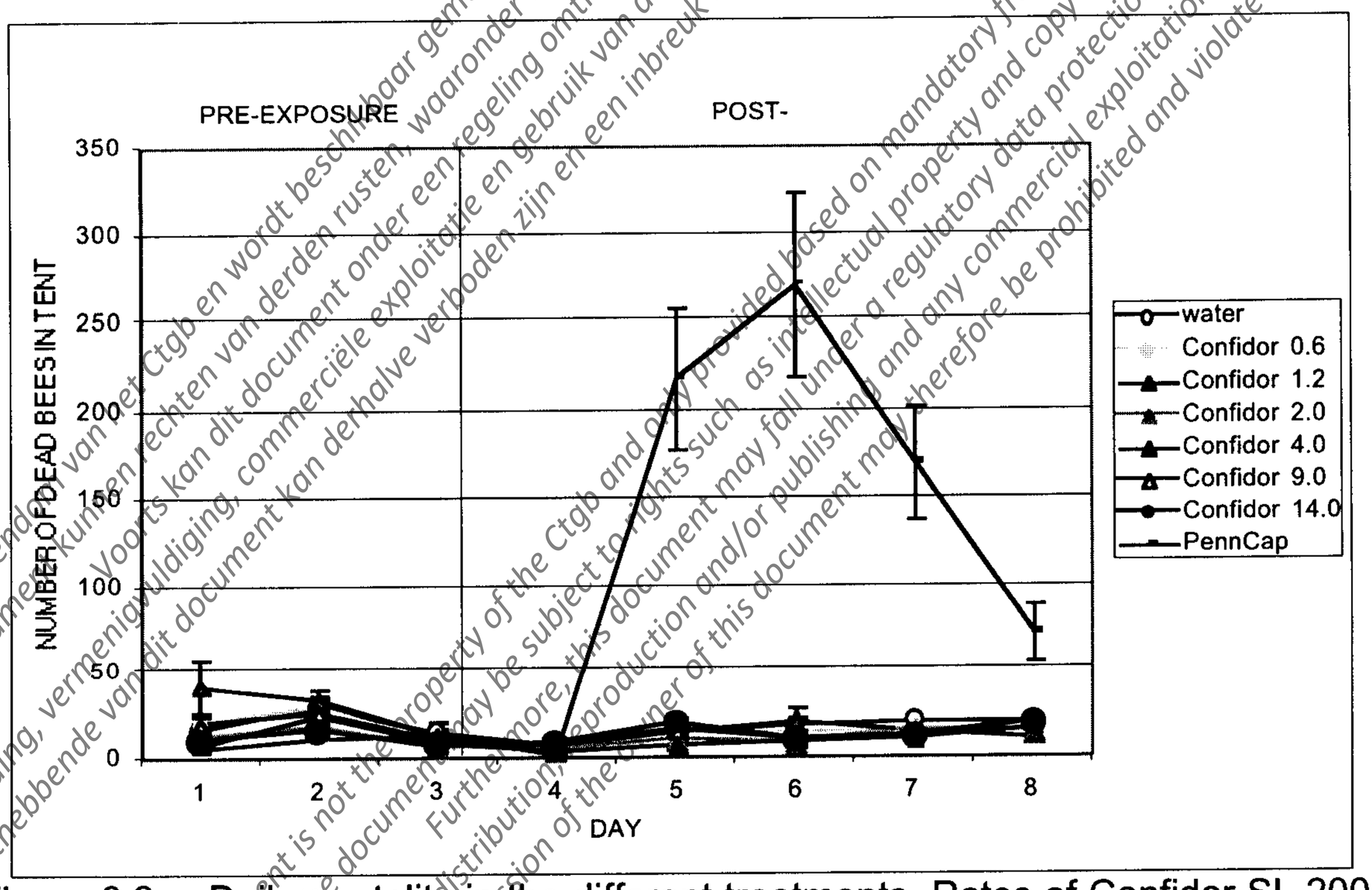

Figure 6.2 Daily mortality in the different treatments. Rates of Confidor SL 200 are expressed in g a.i./ha.

The number of dead bees found during the entire post-treatment period was not statistically significantly different from the water control for any of the Confidor SL 200 test rates. The toxic reference was statistically significantly different from the water control. The raw data are shown in Appendix 5 and summaries are in Table 6.2 and Figure 6.2.

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6.3 Colony evaluation

Seven colonies had an abnormal development of the brood. In one of these the queen was absent, no eggs and no larvae were observed. In the second colony the queen was found damaged in the test cage, in this hive 11 eggs and 12 larvae were present, but pupae were plentiful. In the third and fourth mini-hive no larvae were found, but eggs and pupae were plentiful. In the fifth mini-hive eggs and pupae were present, but only 32 larvae were present. Finally in the sixth and seventh mini-hive eggs and larvae were present, but only 29 and 40 pupae were present. See Appendix 2 for details on colony development. These seven colonies were not identified as outliers in the statistical analysis and their foraging and mortality levels were not deviant. Effects and observations were not related to test compounds or to concentrations of the test compounds.

7 CONCLUSIONS

The test was designed as a multiple rate cage study to determine effects on honeybees, *Apis mellifera* L, starting during beeflight, when Confidor SL 200 is applied to flowering *Phacelia tanacetifolia*. The trial was valid for the purposes to which it was designed. High foraging activity and low mortality in the deionised water indicates that the test animals were in good condition. Low foraging and high mortality in the toxic reference treatment showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.
Confidor SL 200 applied during bee flight, 0.6 g a.i./ha and 1.2 g a.i./ha had no effects on foraging activity and mortality of the honeybee *Apis mellifera*.
At a rate of 2.0 g a.i./ha, 4.0 g a.i./ha and 9.0 g a.i./ha foraging activity was reduced on the day of application, but no effects on mortality were observed.
At the highest test rates (14.0 g a.i./ha) statistically significant reduction in foraging was found during the first two days, but no effects on mortality were observed.
The reference item (PennCap M) was statistically significantly different for foraging and mortality the entire period following application.
Seven colonies had an abnormal development of the brood. These seven colonies had normal foraging and mortality. Effects and observations were not related to test compounds or to concentrations of the test compounds.

8 STUDY LOCATIONS

The study was carried out at MITOX Stichting Bevordering Duurzame Plaagbestrijding, Kruislaan 320, 1098 SM Amsterdam, The Netherlands. MITOX laboratories are GLP compliant (see Appendix XI) and are located on the premises of the Faculty of Science, University of Amsterdam.
Preparation and evaluation of the mini-hives took place at INBUZZ Imkersbedrijf Boot & Calis, Wageningen, The Netherlands. Both employees of INBUZZ had GLP training at MITOX.

9 ARCHIVING

All raw data and records are kept in a file labelled with the study number. They are on loose sheets and forms, each bearing the study number, date and initials of the originator. The report and study records were assigned the study number B074AMS.
For the periods given by the proper authorities, study documents and materials will be stored in the archives of MITOX Stichting Bevordering Duurzame Plaagbestrijding, including:

- study protocol
- any protocol and/or report amendments or addenda or SOP deviations
- all raw data as described above
- comments of the sponsor on the draft report
- one original signed copy of the final report
- all documentation generated by the Quality Assurance Unit (to be archived by the MITOX Quality Assurance Unit, separate from study records)
- laboratory-specific or site-specific raw data such as personnel files, instrument, equipment,
- refrigerator, and/or freezer raw data
- (samples of) the test items used in this study

At the conclusion of the testing any unused portions of the experimental materials will disposed of after their expiry, or returned to Sponsor as the Sponsor so directs with written notice. MITOX reserves the rights to use data obtained in this study on reference treatments to set up reference databases.

10 REFERENCES

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Appendix 1. Certificate of Analysis for Confidor SL 200

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Appendix 2. Colony Characteristics.

Table A2.1. Net weight of colonies before the trial. From these weights and from the weight of individual bees that were sampled during transfer, the numbers of bees introduced were calculated. Also indicated is the source batch code of the mini-hive (A, B, C or D) and the test cage number.

identity					colony weight		sample weight		individual bee	no. bees
hive number	source number	tent	treatment	code	initial weight hive (kg)		g. bees (net)	net g.	no. bees	weight before test (g)	introduced
B6	A	8	CONFIDOR 0.6	2	3.378	3.64	257	12	90	0.13	1958
D14	B	9	CONFIDOR 0.6	2	4.274	4.53	253	13	104	0.13	1985
F22	C	15	CONFIDOR 0.6	2	4.358	4.61	251	12	101	0.12	2061
H30	D	19	CONFIDOR 0.6	2	3.289	3.55	259	10	77	0.13	1929
B8	A	12	CONFIDOR 1.2	3	3.704	3.96	257	12	90	0.13	1958
D16	B	16	CONFIDOR 1.2	3	4.221	4.49	273	13	104	0.13	2142
F24	C	17	CONFIDOR 1.2	3	3.264	3.54	271	12	101	0.12	2225
H32	D	28	CONFIDOR 1.2	3	3.695	3.96	264	10	77	0.13	1966
A4	A	4	CONFIDOR 14	7	3.516	3.77	251	12	90	0.13	1913
C12	B	21	CONFIDOR 14	7	3.068	3.33	257	13	104	0.13	2016
E20	C	23	CONFIDOR 14	7	3.576	3.85	269	12	101	0.12	2208
G28	D	24	CONFIDOR 14	7	3.51	3.76	254	10	77	0.13	1891
A2	A	2	CONFIDOR 2	4	4.727	5	268	12	90	0.13	2042
C10	B	26	CONFIDOR 2	4	2.932	3.19	256	13	104	0.13	2009
E18	C	31	CONFIDOR 2	4	3.514	3.77	258	12	101	0.12	2118
G26	D	32	CONFIDOR 2	4	4.033	4.31	272	10	77	0.13	2025
B5	A	5	CONFIDOR 4	5	3.372	3.63	261	12	90	0.13	1989
D13	B	6	CONFIDOR 4	5	4.104	4.36	253	13	104	0.13	1985
F21	C	13	CONFIDOR 4	5	3.599	3.85	250	12	101	0.12	2052
H29	D	27	CONFIDOR 4	5	3.203	3.46	256	10	77	0.13	1906
B7	A	11	CONFIDOR 9	6	3.847	4.1	255	12	90	0.13	1943
D15	B	18	CONFIDOR 9	6	3.249	3.51	263	13	104	0.13	2064
F23	C	22	CONFIDOR 9	6	3.639	3.89	255	12	101	0.12	2093
H31	D	29	CONFIDOR 9	6	3.27	3.53	260	10	77	0.13	1936
A1	A	1	PennCap M	8	3.562	3.83	263	12	90	0.13	2004
C9	B	10	PennCap M	8	4.202	4.47	263	13	104	0.13	2064
E17	C	14	PennCap M	8	3.219	3.49	268	12	101	0.12	2200
G25	D	30	PennCap M	8	3.831	4.11	278	10	77	0.13	2070
A3	A	3	water	1	5.042	5.32	275	12	90	0.13	2096
C11	B	7	water	1	2.928	3.2	273	13	104	0.13	2142
E19	C	20	water	1	4.839	5.1	261	12	101	0.12	2143
G27	D	25	water	1	3.878	4.14	264	10	77	0.13	1966

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Table A2.2 Results of hive evaluation at conclusion of the testing. Shown are brood development, status of the queen and status of food.

identity					queen	brood			food	
hive number	source number	tent	treatment	code	present	eggs	larvae	pupae	pollen	sugar
B6	A	8	Confidor 0.6	2	Yes	>50	>50	>50	Yes	Yes
D14	B	9	Confidor 0.6	2	No, R1	0	0	>50	Yes	Yes
F22, R3	C	15	Confidor 0.6	2	Yes	>50	>50	>50	Yes	Yes
H30	D	19	Confidor 0.6	2	Yes	>50	>50	29	Yes	Yes
B8	A	12	Confidor 1.2	3	Yes	>50	>50	>50	Yes	Yes
D16, R3	B	16	Confidor 1.2	3	Yes	>50	>50	>50	Yes	Yes
F24	C	17	Confidor 1.2	3	Yes	>50	>50	>50	Yes	Yes
H32	D	28	Confidor 1.2	3	Yes	>50	>50	2	Yes	Yes
A4	A	4	Confidor 14	7	Yes	>50	>50	>50	Yes	Yes
C12	B	21	Confidor 14	7	Yes	>50	>50	>50	Yes	Yes
E20, R3	C	23	Confidor 14	7	Yes	>50	>50	>50	Yes	Yes
G28	D	24	Confidor 14	7	Yes	>50	>50	>50	Yes	Yes
A2	A	2	Confidor 2	4	Yes	>50	>50	>50	Yes	Yes
C10	B	26	Confidor 2	4	Yes	>50	0	>50	No	No
E18	C	31	Confidor 2	4	Yes	>50	>50	>50	Yes	Yes
G26	D	32	Confidor 2	4	Yes	>50	>50	>50	Yes	Yes
B5	A	5	Confidor 4	5	Yes	>50	>50	>50	Yes	Yes
D13	B	6	Confidor 4	5	Yes	>50	>50	>50	Yes	Yes
F21	C	13	Confidor 4	5	Yes	>50	>50	>50	Yes	Yes
H29	D	27	Confidor 4	5	Yes	>50	>50	>50	Yes	Yes
B7	A	11	Confidor 9	6	Yes	>50	>50	>50	Yes	Yes
D15	B	18	Confidor 9	6	Yes	>50	>50	>50	Yes	Yes
F23	C	22	Confidor 9	6	Yes, R2	11	12	>50	No	Yes
H31	D	29	Confidor 9	6	Yes	>50	>50	>50	Yes	Yes
A1	A	1	PennCap M	8	Yes	>50	>50	>50	Yes	Yes
C9	B	10	PennCap M	8	Yes	>50	>50	>50	Yes	Yes
E17	C	14	PennCap M	8	Yes	>50	>50	>50	Yes	Yes
G25	D	30	PennCap M	8	Yes	>50	32	>50	Yes	Yes
A3	A	3	water	1	Yes	>50	>50	>50	Yes	Yes
C11	B	7	water	1	Yes	>50	0	>50	No	Yes
E19	C	20	water	1	Yes	>50	>50	40	Yes	Yes
G27	D	25	water	1	Yes	>50	>50	>50	Yes	Yes

R1: queen absent in hive (D14) on 18/6/01
R2: queen damaged (one leg missing) in hive (F23) on 18/6/01
R3: hives F22, D16 and E20 pre-exposure outliers in statistically analysis

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Appendix 3. Time schedule of the test

PREPARATORY ACTIVITIES AT MITOX

Growing of *Phacelia*

Installing test cages and drip irrigation

PREPARATORY ACTIVITIES AT INBUZZ (Wageningen)

D-12	1-Jun			Preparation of 40 broodcombs for the mini-hives
D-7	5-Jun			Relocation of 15 standard hives to shed older foragers
D-5	8-Jun	12:30		Weighing of bee samples for determine initial bee weight
				Preparation of 32 queen cages
				Preparation and weight determination of mini-hive
		20:30	21:50	Placement of mini-hives in test cages
D3	16-Jun			Transport of hives to Wageningen
D5	18-Jun	10:25	11:45	Evaluation of the mini-hives

APPLICATION *PHACELIA* PLANTS

D0	13-Jun	10:25	11:45	Application of test substances, Penncap M and water,

ASSESSMENTS MORTALITY AND FORAGING ACTIVITY

		mortality		foraging activity											
				I		II		III		IV		V		VI	
		from	to	from	to	from	to	from	to	from	to	from	to	from	to
D-3	10-Jun	07:00	08:40												
D-2	11-Jun	07:30	09:00	10:00	10:20	11:30	11:45	12:30	12:50	13:30	13:45	14:30	14:45	16:05	16:20
D-1	12-Jun	07:00	08:30	10:00	10:15	11:30	11:45	12:30	12:45	13:30	13:40	14:35	14:50	16:00	16:10
D0	13-Jun	07:00	08:00	12:05	12:15	13:10	13:25	14:00	14:15	14:50	15:05	15:40	15:52	16:30	16:40
D1	14-Jun	07:15	08:30	10:05	10:20	11:30	11:45	12:.35	12:50	13:35	13:50	14:30	14:45	16:00	16:15
D2	15-Jun	07:00	09:00	10:00	10:25	11:30	11:45	12:45	13:00	13:55	14:10	15:00	15:15	16:00	16:15
D3	16-Jun	07:00	09:00	10:00	10:15	11:15	11:30	12:00	12:15	13:00	13:15	14:00	14:15	15:00	15:15
D5	18-Jun	12:45	13:45	(note cages were removed 16/6/00 around 22:00) hives examined at Inbuzz											

Note: all dates are in 2001.

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Appendix 4. Foraging activity

Table A4.1 Raw data foraging activity.
Given are the actual numbers of bees observed to fly towards the test plants on each of the six observation moments per assessment date.
A graphical summary of the data is given in Figure A4.1.

PRE-TREATMENT

TENT	TREAT	June 11, 2001						June 12, 2001					
		10:00	11:30	12:30	13:30	14:30	16:05	10:00	11:30	12:30	13:30	14:35	16:00
3	water	0	6	10	9	8	9	8	10	8	11	8	7
7	water	6	15	12	4	12	10	4	16	10	8	5	17
20	water	11	11	20	11	13	9	18	18	25	13	17	13
25	water	11	14	7	8	11	9	10	21	10	11	17	12
8	Confidor 0.6	7	7	13	8	9	8	6	11	13	5	12	15
9	Confidor 0.6	4	3	7	6	10	9	3	8	7	10	5	15
15	Confidor 0.6	0	0	0	0	0	0	0	0	0	0	0	1
19	Confidor 0.6	2	8	9	13	12	7	12	14	10	12	12	17
12	Confidor 1.2	0	0	0	0	0	0	0	0	0	0	0	4
16	Confidor 1.2	0	0	0	0	0	0	0	0	1	12	8	8
17	Confidor 1.2	5	8	10	11	11	14	6	16	20	19	15	23
28	Confidor 1.2	3	12	12	6	17	7	12	17	18	7	18	12
2	Confidor 2.0	3	5	15	12	12	7	6	5	14	6	14	12
26	Confidor 2.0	6	8	7	7	8	8	6	8	10	9	28	11
31	Confidor 2.0	6	22	11	14	12	19	12	22	17	12	12	18
32	Confidor 2.0	2	14	8	6	6	8	4	7	6	5	5	8
5	Confidor 4.0	3	6	6	6	7	3	2	5	5	6	7	8
6	Confidor 4.0	9	7	20	13	17	4	9	7	9	14	8	13
13	Confidor 4.0	5	0	6	6	10	11	5	9	12	15	10	27
27	Confidor 4.0	4	12	8	10	15	12	18	14	17	13	15	13
11	Confidor 9.0	4	6	6	5	9	4	7	12	14	14	2	18
18	Confidor 9.0	1	3	9	4	6	14	1	9	12	11	9	11
22	Confidor 9.0	8	7	19	5	9	14	9	13	15	21	12	28
29	Confidor 9.0	2	12	11	8	10	9	7	13	10	11	17	12
4	Confidor 14.0	9	9	12	13	15	10	8	14	12	10	12	9
21	Confidor 14.0	7	11	9	13	14	15	7	19	14	15	11	17
23	Confidor 14.0	0	0	0	0	0	0	0	0	0	0	2	11
24	Confidor 14.0	9	7	19	10	18	9	9	13	17	10	23	11
1	PennCapp	9	8	13	14	20	16	9	20	18	17	16	30
10	PennCapp	8	5	10	4	15	9	7	17	16	10	9	12
14	PennCapp	7	3	18	5	14	13	10	21	15	14	11	21
30	PennCapp	9	10	15	4	25	7	7	7	7	14	13	16

POST TREATMENT

TENT	TREAT	June 13, 2001						June 14, 2001					
		12:05	13:10	14:00	14:50	15:40	16:30	10:05	11:30	12:35	13:35	14:30	16:00
3	water	8	8	8	12	8	10	7	7	11	6	4	6
7	water	11	17	18	11	16	13	7	9	9	19	15	14
20	water	23	25	22	28	19	29	18	24	25	28	24	23
25	water	12	13	17	22	19	23	9	12	13	23	12	24
8	Confidor 0.6	11	8	14	13	10	12	4	8	11	13	16	11
9	Confidor 0.6	8	8	15	18	3	12	10	11	8	17	12	11
15	Confidor 0.6	8	3	12	7	7	6	9	9	12	14	16	16
19	Confidor 0.6	14	16	19	19	21	13	7	18	19	17	24	17
12	Confidor 1.2	15	4	7	9	7	7	6	4	13	13	7	9
16	Confidor 1.2	9	16	17	16	13	19	7	8	18	18	12	14
17	Confidor 1.2	16	11	9	12	11	13	7	11	15	19	16	14
28	Confidor 1.2	16	11	17	14	16	18	14	22	19	23	23	28
2	Confidor 2.0	8	9	12	11	6	5	11	11	13	12	15	12
26	Confidor 2.0	9	7	13	11	9	13	15	18	10	16	19	23
31	Confidor 2.0	13	9	9	10	11	11	15	11	13	29	21	30
32	Confidor 2.0	8	4	9	14	11	8	9	13	8	12	8	14
5	Confidor 4.0	7	1	5	4	3	5	4	4	5	5	10	8
6	Confidor 4.0	3	11	11	16	4	7	10	6	10	7	6	5
13	Confidor 4.0	5	7	14	10	9	10	7	9	9	16	14	13
27	Confidor 4.0	9	8	14	18	13	8	8	6	15	20	18	19
11	Confidor 9.0	6	2	4	2	3	9	13	15	5	20	6	11
18	Confidor 9.0	7	4	8	13	6	9	2	6	7	7	8	9
22	Confidor 9.0	10	6	6	11	13	4	7	10	12	7	17	7
29	Confidor 9.0	10	6	12	8	9	7	6	12	9	12	10	16
4	Confidor 14.0	7	5	5	4	5	6	5	5	6	8	9	5
21	Confidor 14.0	10	11	7	6	3	6	3	4	1	3	6	13
23	Confidor 14.0	6	6	6	4	9	11	3	6	7	8	8	6
24	Confidor 14.0	6	5	10	11	7	7	14	10	17	16	15	14
1	PennCapp	11	2	1	8	4	3	0	1	0	0	1	2
10	PennCapp	1	1	2	4	7	9	0	0	0	0	1	3
14	PennCapp	4	6	7	7	4	4	0	0	0	0	0	0
30	PennCapp	14	1	1	4	12	7	1	1	0	0	0	0

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POST TREATMENT

TENT	TREAT	June 15, 2001 10:00	11:30	12:30	13:30	14:30	16:00	June 16, 2001 10:00	11:30	12:30	13:30	14:30	16:00
3	water	5	9	11	9	5	3	5	3	6	4	3	3
7	water	1	9	11	20	8	15	6	8	14	19	15	17
20	water	2	28	22	28	31	31	22	15	22	16	21	12
25	water	1	15	22	18	24	23	14	13	14	11	13	20
8	Confidor 0.6	1	6	11	13	8	8	10	5	3	10	10	5
9	Confidor 0.6	3	10	12	11	9	15	8	10	9	7	9	3
15	Confidor 0.6	1	15	15	13	14	20	10	7	16	6	13	9
19	Confidor 0.6	1	19	17	25	31	28	16	12	17	19	14	19
12	Confidor 1.2	0	6	9	9	5	13	3	3	8	11	7	3
16	Confidor 1.2	4	17	18	19	14	16	9	7	14	11	13	12
17	Confidor 1.2	1	12	18	16	15	23	11	10	15	10	12	11
28	Confidor 1.2	0	15	17	18	16	23	14	14	15	14	21	23
2	Confidor 2.0	6	13	9	13	13	18	16	10	6	7	8	5
26	Confidor 2.0	4	12	12	8	19	24	4	13	13	12	13	12
31	Confidor 2.0	1	14	14	12	23	27	18	28	21	14	17	25
32	Confidor 2.0	1	11	9	9	11	13	5	10	13	12	5	9
5	Confidor 4.0	0	5	7	2	6	5	1	1	3	0	3	1
6	Confidor 4.0	0	7	11	7	13	9	8	3	9	6	6	1
13	Confidor 4.0	2	16	24	17	13	22	12	12	9	10	17	14
27	Confidor 4.0	0	11	18	25	26	10	17	12	12	10	11	16
11	Confidor 9.0	4	10	14	14	10	13	7	5	11	5	10	6
18	Confidor 9.0	0	6	8	10	14	12	4	9	8	6	8	6
22	Confidor 9.0	0	11	9	12	17	15	9	11	10	15	13	20
29	Confidor 9.0	1	17	6	21	16	16	8	8	8	9	7	2
4	Confidor 14.0	1	7	12	9	5	14	14	4	6	4	11	2
21	Confidor 14.0	1	6	5	5	9	6	2	2	3	7	5	5
23	Confidor 14.0	0	7	11	12	11	14	4	9	7	9	7	14
24	Confidor 14.0	0	17	18	18	23	15	16	14	16	16	16	5
1	PennCapp	0	1	0	1	0	7	0	0	0	2	2	2
10	PennCapp	0	0	0	0	1	1	0	0	0	8	4	2
14	PennCapp	0	0	0	0	6	4	0	0	0	0	0	0
30	PennCapp	0	1	6	4	7	4	3	4	4	4	6	3

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Figure A4.1. Mean foraging activity and the ratio of post- to pre-application activity.

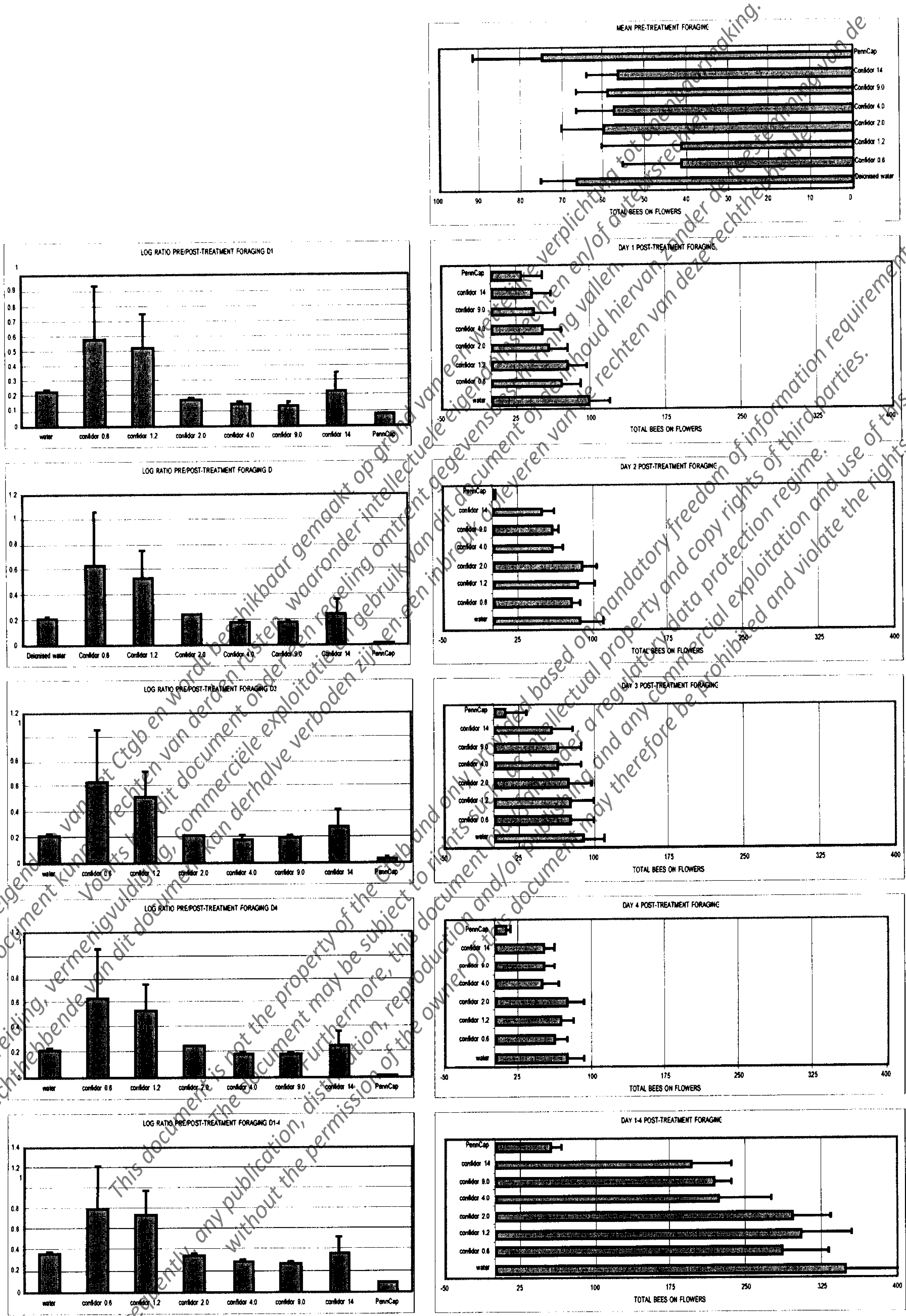

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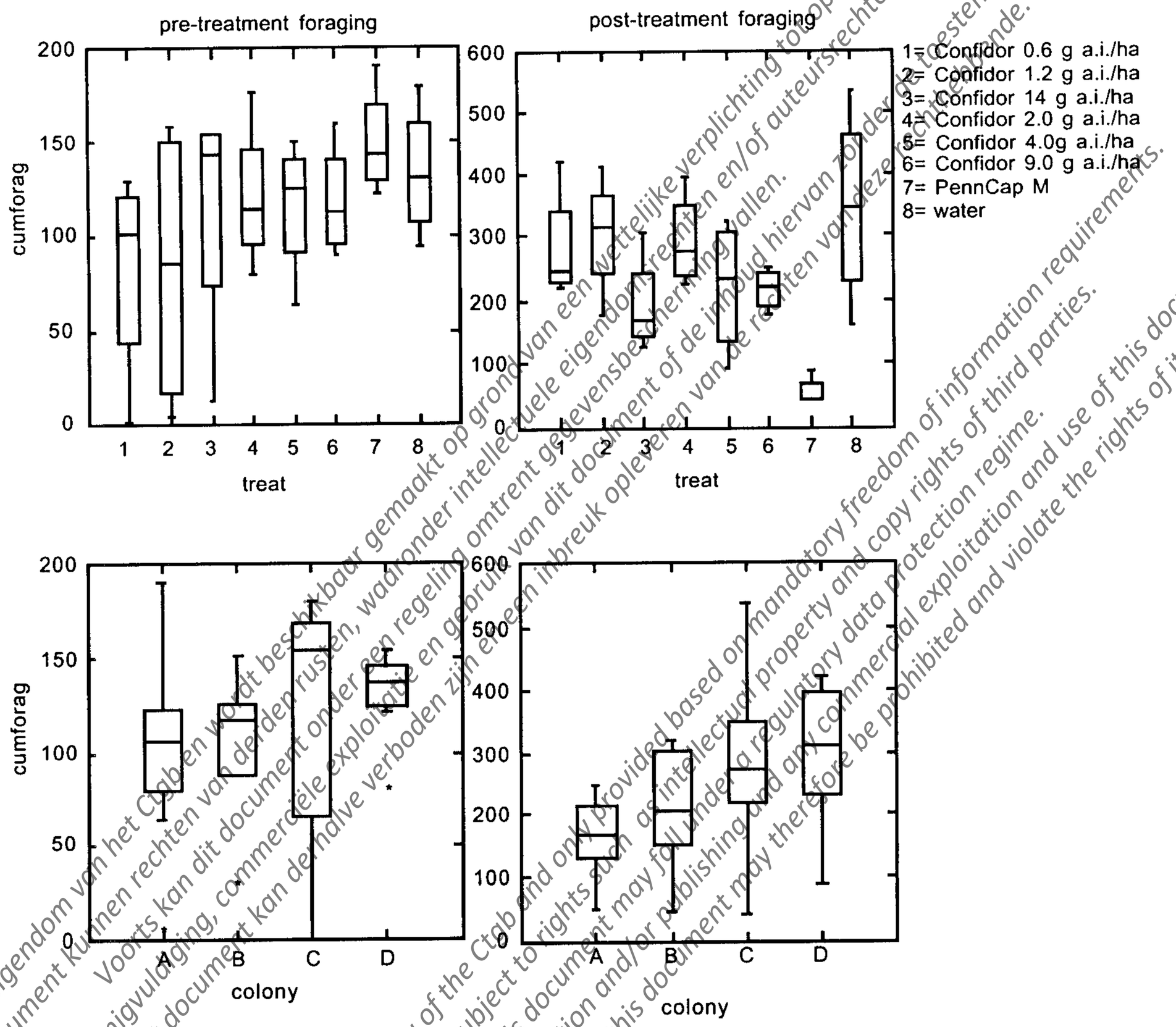
Figure A4.2 Box plots of the cumulative number of foragers pre- and post-treatment in relation to (A) Treatment (B) Colony. Note: in the pre-exposure period, no significant differences among groups were observed, but in the post exposure period both the effect of treatment and the effect of source batch were significant factors.



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APPENDIX 5. Mortality

Table A5.1 Raw data mortality.
Given are the actual numbers of dead bees found on each of the four assessment dates. The box "log pre-post ratio plus 1" gives the logarithm of the cumulative number of bees post-application over the total number pre-application. One was added to this ratio to avoid negative values.
A graphical summary of the data is given in Figure A5.1.

PRE-TREATMENT

TENT	TREAT	10-Jun worker	11-Jun worker	12-Jun worker	13-Jun worker	SUM
3	water	20	44	28	10	102
7	water	16	33	13	2	64
20	water	17	26	8	5	56
25	water	7	7	8	6	28
8	Confidor 0.6	25	37	5	4	71
9	Confidor 0.6	23	50	18	18	109
15	Confidor 0.6	13	14	3	0	30
19	Confidor 0.6	10	12	6	4	32
12	Confidor 1.2	11	18	7	5	41
16	Confidor 1.2	60	48	18	7	133
17	Confidor 1.2	16	27	6	5	54
28	Confidor 1.2	76	37	22	14	149
2	Confidor 2.0	18	22	6	5	51
26	Confidor 2.0	13	19	5	1	38
31	Confidor 2.0	2	9	2	10	23
32	Confidor 2.0	12	18	9	3	42
5	Confidor 4.0	9	39	24	5	77
6	Confidor 4.0	7	29	7	3	46
13	Confidor 4.0	7	9	3	2	21
27	Confidor 4.0	10	21	4	6	41
11	Confidor 9.0	32	42	10	8	92
18	Confidor 9.0	10	18	8	4	40
22	Confidor 9.0	15	16	11	3	45
29	Confidor 9.0	17	28	11	11	67
4	Confidor 14.0	12	24	11	4	51
21	Confidor 14.0	6	17	7	5	35
23	Confidor 14.0	14	7	1	8	30
24	Confidor 14.0	9	16	11	14	50
1	PennCapp	7	11	15	1	34
10	PennCapp	7	13	11	2	33
14	PennCapp	4	11	7	7	29
30	PennCapp	6	10	15	1	32

LOG PRE-POST RAT PLUS 1

D1	D2	D3	D4	D1-2	D1-3	D1-4
0.052	0.06	0.115	0.071	0.105	0.198	0.244
0.063	0.097	0.243	0.08	0.148	0.202	0.255
0.065	0.051	0.196	0.127	0.109	0.155	0.247
0.206	0.324	0.723	0.285	0.434	0.578	0.673
0.098	0.068	0.144	0.052	0.153	0.182	0.217
0.063	0.045	0.19	0.042	0.102	0.147	0.177
0.186	0.146	0.514	0.146	0.286	0.398	0.462
0.051	0.138	0.459	0.194	0.176	0.294	0.403
0.103	0.059	0.173	0.04	0.151	0.187	0.213
0.041	0.058	0.114	0.046	0.094	0.119	0.155
0.038	0.113	0.354	0.074	0.143	0.231	0.276
0.072	0.106	0.209	0.05	0.163	0.207	0.238
0.056	0.071	0.535	0.15	0.119	0.284	0.368
0.101	0.064	0.374	0.092	0.153	0.246	0.301
0.085	0.053	0.229	0.07	0.13	0.182	0.229
0.162	0.14	0.385	0.203	0.263	0.341	0.445
0.027	0.087	0.224	0.139	0.109	0.163	0.263
0.018	0.045	0.346	0.15	0.062	0.163	0.272
0.040	0.109	0.331	0.109	0.14	0.222	0.291
0.151	0.128	0.337	0.135	0.245	0.311	0.383
0.053	0.057	0.157	0.104	0.104	0.14	0.218
0.176	0.061	0.204	0.122	0.217	0.255	0.327
0.139	0.028	0.255	0.118	0.16	0.216	0.291
0.072	0.113	0.235	0.123	0.17	0.219	0.298
0.190	0.048	0.353	0.212	0.222	0.297	0.416
0.155	0.012	0.282	0.089	0.164	0.227	0.282
0.136	0.156	0.392	0.195	0.255	0.336	0.437
0.164	0.152	0.255	0.107	0.274	0.318	0.373
0.823	0.898	1.383	0.393	1.132	1.287	1.319
0.933	1.071	1.16	0.327	1.287	1.357	1.377
1.063	1.115	1.547	0.665	1.373	1.507	1.553
0.680	0.723	1.214	0.605	0.957	1.111	1.202

POST TREATMENT

TENT	TREAT	14-Jun worker	15-Jun worker	16-Jun worker	17-Jun worker	SUM
3	water	13	15	31	18	77
7	water	10	16	12	13	51
20	water	9	7	8	19	43
25	water	17	31	30	26	104
8	Confidor 0.6	18	12	7	9	46
9	Confidor 0.6	17	12	15	11	55
15	Confidor 0.6	16	12	17	12	57
19	Confidor 0.6	4	12	15	18	49
12	Confidor 1.2	11	6	5	4	26
16	Confidor 1.2	13	19	10	15	57
17	Confidor 1.2	5	16	17	10	48
28	Confidor 1.2	27	41	23	18	109
2	Confidor 2.0	7	9	31	21	68
26	Confidor 2.0	10	6	13	9	38
31	Confidor 2.0	5	3	4	4	16
32	Confidor 2.0	19	16	15	25	75
5	Confidor 4.0	5	17	13	29	64
6	Confidor 4.0	2	5	14	19	40
13	Confidor 4.0	2	6	6	6	20
27	Confidor 4.0	17	14	12	15	58
11	Confidor 9.0	12	13	10	25	60
18	Confidor 9.0	20	6	6	13	45
22	Confidor 9.0	17	3	9	14	43
29	Confidor 9.0	12	20	12	22	66
4	Confidor 14.0	28	6	16	32	82
21	Confidor 14.0	15	1	8	8	32
23	Confidor 14.0	11	13	11	17	52
24	Confidor 14.0	23	21	10	14	68
1	PennCapp	192	235	197	50	674
10	PennCapp	250	356	111	37	754
14	PennCapp	306	349	248	105	1008
30	PennCapp	121	137	123	97	478

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Figure A5.1. Mean numbers of dead bees and the ratio of post- to pre-application mortality.

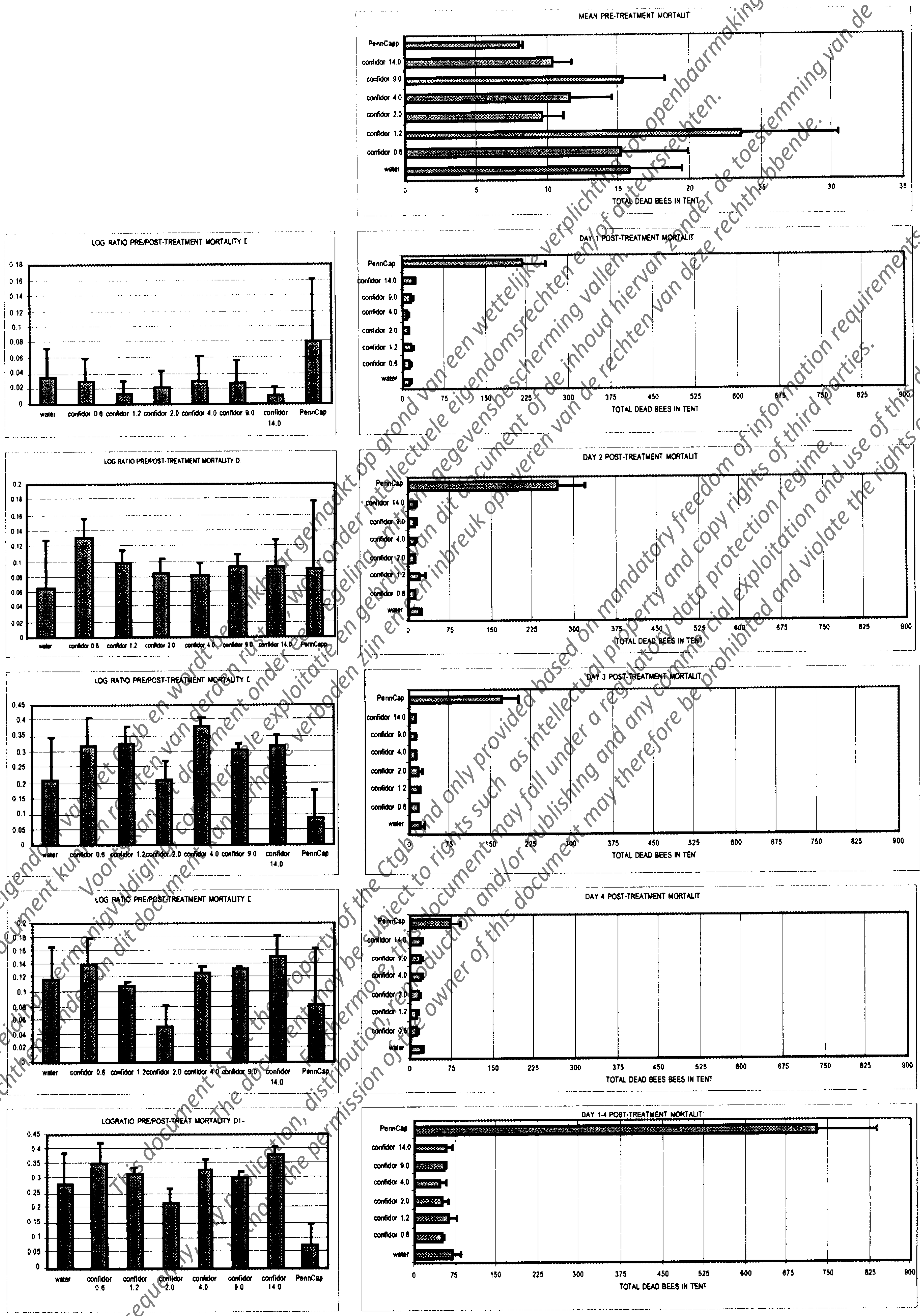

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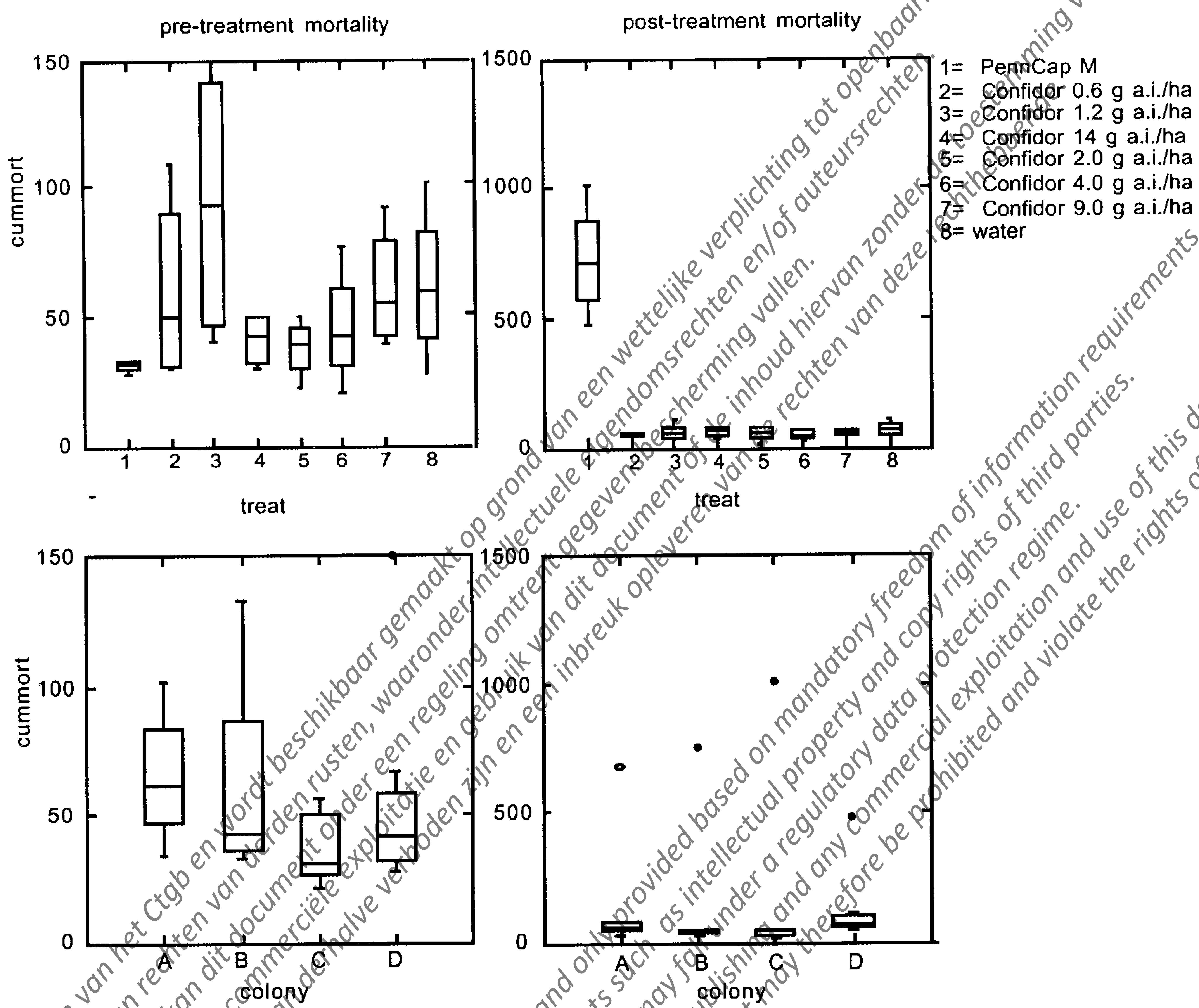
Figure A5.2 Box plots of the cumulative number of dead bees pre- and post-treatment in relation to (A) treatment and (B) colony origin . Note there is no effect of origin and treatment (except for PennCap M).



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Appendix 6: ENDORSEMENT OF COMPLIANCE

[redacted]

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Appendix 7: Protocol deviations and errata

Protocol	Contamination of the tents to be avoided by placing a screen around the test plants during application.
Deviation	No screen was placed.
Rationale	Practical reasons.
Implication	Probably none as there was no wind and no spray drift was observed.
Date	13 June 2001
Protocol	Suggestions for the preparation of test solutions
Deviation	Solutions were prepared in 200 ml instead of 150 ml
Rationale	Availability of volumetric glassware
Implication	None concentration was the same
Date	13 June 2001
Protocol	§ 3.5 during a 10 seconds observation period.
Deviation	All bees present on the flowers were counted, irrespective of the observation period needed. The actual observation periods ranged from 18 sec. to 24 sec. In this period, bees flying towards and away from the plants were not counted.
Rationale	10 seconds was too short to count all foraging bees.
Implication	Counting of the bees in the test cages was more accurate because usually more than 10 seconds were needed. However, flying bees were not included in the assessments. Because their number was generally small, this is expected to have a negligible impact on the outcome.
Date	11 June 2001 until 16 June 2001

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