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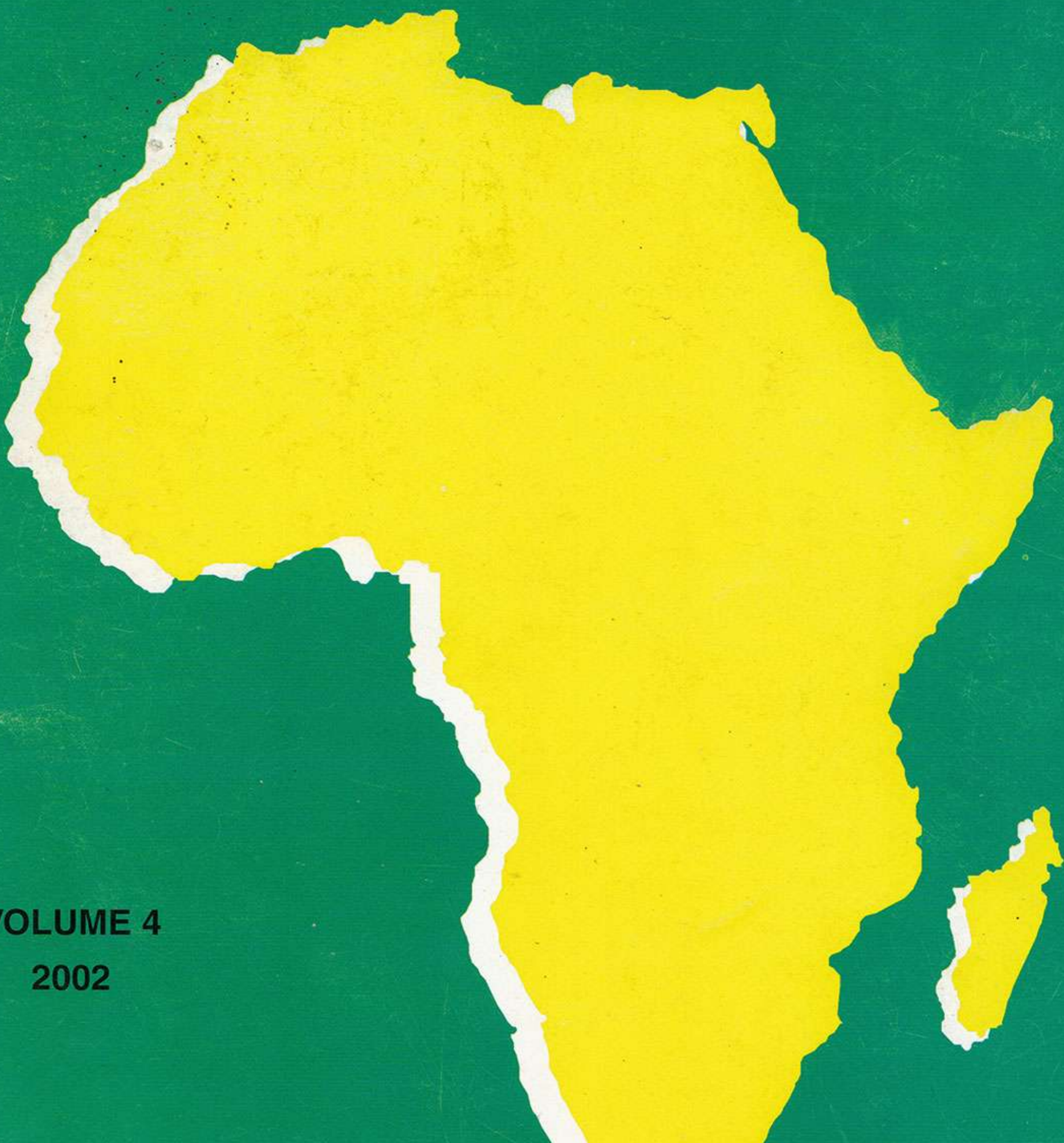
ENVIRONMENTAL SIDE – EFFECTS OF LOCUST AND GRASSHOPPER CONTROL



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ENVIRONMENTAL SIDE-EFFECTS OF LOCUST AND GRASSHOPPER CONTROL

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TABLE OF CONTENTS

PREFACE

As in the previous volumes, we present a number of field and laboratory tests with compounds used for locust and grasshopper control.

The field tests were all preceded by a full season of baseline observations : the temporary pond system and the savannah soil fauna, which are presented in this volume, and the millet agroecosystem, published in Volume 3 (Thiam & Van der Valk, 1998).

The temporary ponds show a typical trophic system : at the basis are highly suspended solid particles (soil and organic material) which are eaten or grazed upon by filter feeders (zooplankton) and detritus feeders (chironomids, tadpoles). The latter are predated by hemiptera and dytiscids. Top predators are birds and reptiles (turtles, snakes, toads, monitors).

Two invertebrate species, the shrimp *Streptocephalus sudanicus* and the backswimmer *Anisops sardeus* represent 80% of the biofauna in these waters. Both are widely distributed over Africa. They have been selected for laboratory based toxicity tests (using Standard Operating Procedures), that can be validated in the field. The field tests have been published in Volume 1 and 2. A comparison of the data indicate a high predictivity of the tests for acute toxicity in the field .

Combining all data, including recovery in the field, and data from the literature on *Daphnia* allowed us to rank ten compounds used in locust control according to aquatic hazard. Ranking form least to most hazardous are : fipronil, bendiocarb, fenitrothion, malathion, diflubenzuron, triflumuron, teflubenzuron, chlorpyriphos, deltamethrin, lambdacyhalothrin.

The same species were used in bioassays for pesticide deposit studies. Here we describe how the method was used to determine the minimal width of buffer-zones for the protection of open water.

The terrestrial studies presented in this volume were mainly directed at social soil insects : termites and ants. The two groups are known often to play an ecological key role, specifically in arid soil ecosystems. Still, very little is known about their long-term response to pesticides.

Two field tests were carried out. In the first test, fenitrothion, bendiocarb and diflubenzuron were applied at " worst case dosages " (= 2x recommended dose for locust control). The termite *Psammotermes hybostoma* was strongly reduced by fenitrothion , but showed no effect the following year. Ants however, known from numerous other studies to be highly sensitive to fenitrothion, appear not to recover fully during the same season. Diflubenzuron on the other hand, also caused a reduction in termites one year later

A second test was carried out with chlorpyrifos and fipronil at recommended dosages (NB, the dose recommended for fipronil in barrier treatment was applied using the method for blanket spray, effectively resulting in an overdosage). The test started in 1996. *P. hybostoma* was strongly affected by fipronil and the population had not fully recovered before year 2000.

These and other studies show that social soil insects are extremely slow in rebuilding new populations after local depletion. Given their ecological importance, they should be given much more attention in risk assessments of pesticides used under similar conditions, than in the current practice.

The toxicity of fipronil to termites had been demonstrated previously in a laboratory test (Volume 3). Further tests with the tenebrionids *Pimelia senegalensis* and *Trachyderma hispida* which are natural enemies of desert locust and grasshoppers, showed a high toxicity to these species as well in comparison to bendiocarb, chlorpyriphos, deltamethrin, fenitrothion, lambda-cyhalothrin, and malathion.

A validation of these laboratory data in comparison with the field indicated that only for chlorpyrifos, laboratory tests tend to underestimate the real hazard.

Furthermore, chlorpyrifos induced a considerable increase in *Heliocheilus albipunctella*, (a major pest) when sprayed on millet. A treatment with deltamethrin did not show this effect.

We may conclude, that after 8 years of integrated field and laboratory research, a representative set of indicators has been selected and developed for ecotoxicological assessment of pesticides in the sahelian environment. The toxicity tests (all having standardized procedures) proved sufficiently predictive for the field, as well as to provide the necessary additional information over the " standard set " of globally required toxicity data for the lower tier screening of pesticides. We also demonstrated, however, that long-term field observations are indispensable for a balanced risk assessment, specifically in vulnerable and marginal ecosystems.

CHAPTER 1:

Seasonal changes in the physical and chemical environment and development of the invertebrate fauna in a temporary pond in central Senegal (West Africa)

Joost Lahr, Alpha Oumar Diallo, Khalifa Babacar Ndour & Aliou Badji

SUMMARY

Temporary ponds are surface waters that are formed during the rainy season in dry regions such as the Sahel. They contain an unique aquatic fauna that is adapted to the fluctuating environmental circumstances and the temporary nature of such an environment.

The present study was conducted in order to obtain background information on the hydrology, changes in physical and chemical parameters and on the succession (phenology) and ecological importance of species in sahelian temporary ponds. Similar pond had previously been used in ecotoxicological field experiments to assess the impact of locust control measures with different insecticides.

The hydrological dynamics and the faunal composition of a single temporary pond in central Senegal during a complete annual cycle, from the first rains until desiccation, were investigated. The biotic component of the study focused on the zooplankton and macro invertebrates. Samples of the fauna were taken once a day during the first month and twice a week during the remainder of the season. Physical and chemical parameters were measured on all these occasions. In addition meteorological data were obtained from other sources.

The pond was extant from late June 1994 until the end of January 1995 and contained a rich invertebrate fauna. Both the hydrological parameters and the biotic composition in the pond changed significantly during the season.

High air and water temperatures occurred during the rainy season from July until early November. Changes in water levels could be expressed as a function of precipitation and evaporation. Dissolved oxygen levels, pH and Secchi depths also fluctuated. Conductivity was related to the depth of the pond and increased considerably when most of the water in the pond had evaporated at the end of the wet period.

Zooplankton species showed distinct peak densities at different times for different species. Cladocera were the most important in numbers during the early season. The zooplankton was later dominated by different species of copepods. Ostracods seemed much less numerous, but this may be due to the fact that benthic zooplankton was not sampled separately.

The macro invertebrates consisted principally of phyllopods and aquatic insects. There was a clear trend in the succession of species. Anostraca and Notostraca dominated the early community of the pond. Aquatic insects became more important towards the end of the season: first the backswimmers of the genus *Anisops* (Notonectidae, Hemiptera) and at the last stage of the pond the diving beetles (Dytiscidae, Coleoptera). Four stages in the faunal succession in the pond were distinguished: colonization, dominance by crustaceans, a shift towards insects and desiccation.

In general micro- and macro crustaceans were succeeded by insects. The only exception was the clam shrimp *Cyclestheria hislopi* (Limnadiidae, Laevicaudata), that was present in large numbers from October until the pond dried out.

The growth rates of two fairy shrimps, *Branchinella chudeaui* and *Streptocephalus sudanicus* (Anostraca, Branchiopoda) were found to be constant, but very different for each species. *B. chudeaui* grew ten times as fast as *S. sudanicus*. Its longevity in the pond was also ten times shorter. Development of the *S.*

sudanicus population was studied as well. Sexual organs in the species became visible one month afetr hatching. Visible eggs in the ovisacs of the females were observed after 1-2

months. The ratio of males to females in the samples slowly declined from approximately 50% to 20% during the four months following adulthood.

Among the two most abundant back swimmer species (Notonectidae, Hemiptera) the percentage of males was 30% for *Anisops sardeus* and 50% for *A. debilis perplexus*. Changes in these ratios during the season were relatively small.

Possible trophic relationships between the different constituents of the biotic community in the pond are discussed. Input from organic matter and suspended particles may be highly important for the food chain in turbid temporary ponds such as the one in this study. Consumers consist mainly of particle and detritus feeders such as zooplankton, phyllopods, chironomid larvae and tadpoles. Many predatory insect species that were found may in turn feed on these groups. The most important top predators are probably water turtles and water birds.

Zooplankton (especially cladocerans), fairy shrimps of the genus *Streptocephalus* and backswimmers (*Anisops spp.*) are the most important representatives of the invertebrate fauna, in terms of numbers and biomass, in the pond. It is concluded that these may be important indicator species for anthropogenic disturbance studies such as side-effects of insecticides.

INTRODUCTION

Temporary ponds are small impoundments of water that occur on a seasonal basis in almost all arid and semi-arid parts of the world where total evaporation and infiltration exceed yearly precipitation. In West Africa these systems are found during the annual rainy season when the bulk of the rain falls, i.e. between May and November. They may occur in such different climate zones as the Sahara Desert, the arid Sahel zone and the somewhat more humid sudanic savannah. The typical temporary pond is a shallow, closed basin (Belk and Cole 1975). Its size, depth and duration depends on many factors such as the frequency and amount of rain, drainage area, temperature and morphometric/sediment characteristics (see for example Cole 1968).

Temporary ponds are well known for their special, adapted fauna in which aquatic invertebrates seem to play a more important role than in permanent aquatic ecosystems. Fish are usually absent. One of the most characteristic groups are the large Branchiopoda or phyllopods that consist of Anostraca (fairy shrimps), Notostraca (tadpole shrimps), Spinicaudata and Laevicaudata (clam shrimps). These organisms are rarely found in permanent waters where fast predators (fish) would quickly eliminate them. Other groups such as zooplankton, aquatic insects, amphibians and many more can also be found in these ephemeral systems. The species that represent these groups are adapted to survive the dry period. There are two main survival strategies: escape in time (dormancy) and escape in space (migration). Crustaceans for instance all apply dormancy, mostly as resting eggs. Aquatic insects mostly migrate to permanent waters if their adult stage is aquatic (hemipterans, coleopterans) or may survive as terrestrial imagos when only the larval stage is aquatic (mayflies, damselflies, dragonflies etc.). The adaptations of temporary pond dwellers are discussed in more detail by Hartland-Rowe (1972), Belk and Cole (1975), Wiggins *et al.* (1980), Williams (1985), Williams (1987), Brendonck and Persoone (1993) and Lahr (1997a).

Ephemeral aquatic habitats are of key importance to dry regions, both economically and ecologically. Rural people use them to supply drinking water for themselves and their livestock, or for agricultural and aquacultural purposes (Scoones 1991). They are important sites for natural production and serve as shelter and foraging areas to birds and game. In general much less is known of the biology and ecology of temporary waters than of permanent aquatic habitats.

The study described here deals with some of the hydrological and ecological features of a single temporary pond in an area in central Senegal. The study was set up to provide ecological background information on the kind of ponds in which previous ecotoxicological experiments with insecticides had taken place and to support the choice of indicator-species for side-effects (Lahr and Diallo 1993, Lahr *et al.* 1995). The work focused on the occurrence, succession and relative importance of the most numerous zooplankton, pelagic and epibenthic macro invertebrate groups and tadpoles during an entire hydrological cycle. The groups and organisms that had previously been used as indicators for side-effects of insecticides were fairy shrimps, Notonectidae (backswimmers) and cladocerans.

METHODS AND MATERIALS

Study area

The temporary pond monitored in this study is situated 13°50N. 15°56W, approximately one hour driving north from the departmental capital Nioro du Rip and one kilometre east from the village Guènte Khaye. The region is extensively farmed during the rainy season, groundnuts, millet and maize being the principal crops. The area is slightly sloping. Higher grounds alternate with depressions, thus creating large drainage areas for temporary ponds. The soil is sandy and sometimes rocky on the plateaus, but in the lower parts it is comprised of fine clay. When water is present in the ponds these can become very muddy and opaque. Most of the ponds in the area are surrounded by a natural ring of trees and scrubs.

The pond, known in the local language as "Passoula", had been used as a control during our previous ecotoxicological experiments. It had therefore never received an insecticide treatment. It is a typical example of the temporary ponds in the region. Its maximum water surface is approximately one hectare, but during most of the rainy season it is much smaller (Fig 1). People were often found in the area when it was sampled, enjoying a short bath or doing the laundry. At the end of the season, when smaller ponds elsewhere had already dried out, it became frequently visited by shepherds and their cattle. Although surrounded by natural vegetation, the pond itself did not develop any significant aquatic vegetation.

Experimental design, meteorology and sampling

The study was started in June 1994 and included meteorological measurements, water parameters and biological samples.

Regional meteorological data were obtained from the Meteorological Station of the Senegalese Ministry of Agriculture in Nioro du Rip (13°45N, 15°46W). These included air temperature, relative air humidity, and daily precipitation and evaporation.

All measurements and samples at the site were taken in the morning between 8:30 and 10:00. During its first month of existence Passoula was sampled on a daily basis (June 22 to July 20). This period was judged of particular importance to study colonization and development of populations of fast growing species. From the end of July until the end of January 1995 sampling took place twice a week.

In deepest part of the pond, close to its centre (Fig 1), a pole was placed. Each time the pond was sampled, the depth was measured at this spot. Chemical and physical water parameters were measured at the same site using portable devices. The measurements included water temperature, pH, dissolved oxygen (DO, as % saturation), conductivity and Secchi depth.

Biological samples were taken in three different directions from the centre of the pond (Fig 1). Zooplankton was captured just underneath the surface with a zooplankton net (diameter 28cm, mesh width 200µm). Pelagic macro invertebrates were sampled similarly using a circular scoop net with a diameter of 35cm and a mesh width of 1mm. Benthic macro invertebrates were captured with a square scoop net (32x32cm, mesh width 1mm) which was dragged, partly submerged, through the 5 cm top layer of the sediment. Catches were quantified by multiplying the length of the samples with the surface of the net openings. Sample length could be varied according to the amount of water and the number of organisms present in the pond (macro invertebrate samples only). The average catches from the three sampling stations were expressed as the number per cubic metre for swimming organisms and as the number per square metre for sediment dwellers. At the beginning and at the end of the rainy season, when water levels were lower than the diameter of the nets, both macro invertebrate samples were taken with the square net. The amounts per cubic metre for these samples were calculated from the sample lengths and the measured depth of the pond. When the water level dropped beyond 28 cm 10L zooplankton samples were taken with a 2L volumetric cylinder and filtered through the zooplankton net.

Macro invertebrates were stored in bottles containing a 5% formaldehyde solution. Prior to identification

they were transferred into 70% ethanol. All zooplankton samples were preserved in 5% buffered formaldehyde.

Macro invertebrate weights and estimation of biomass

Macro invertebrate specimens for weight measurement were collected from different ponds in the area around Nioro du Rip during 1995 and 1996. They were taken to the laboratory, rinsed with clean water and frozen at -15°C prior to analysis. To establish individual weights ten or more individuals of each species were put into a small crucible. All weights were measured using an analytical balance (precision 0.001 g). Wet weights were calculated from the difference in weight between the empty and the full crucible divided by the number of specimens. Dry weights (DW) were measured after drying the organisms for 2 hours in an oven at 105°C.

For the fairy shrimps *Branchinella chudeaui* (Branchinectidae) and *Streptocephalus sudanicus* (Streptocephalidae), which grew continuously, different size classes of individuals were analyzed to establish length-weight relationships. For other macro invertebrate species only the weights of the adult, nymphal or larval life stages were measured.

The results of the individual weight measurements were used to estimate the total biomass of different groups of species by multiplying them with the densities. The total biomass of larger taxonomic groups and categories was calculated by adding the biomass estimations of their constituents.

Statistical analyses

Relationships between several parameters were analyzed using least-squares linear or log-linear regression using the module available in the spreadsheet computer programme Quattro-Pro v6.01 for Windows 3.x (Novell Applications Group, Orem, Utah): the logarithm of the depth of the pond versus the net water balance (cumulative precipitation minus cumulative evaporation), the logarithm of the conductivity versus the depth of the pond, the ratio between the logarithm of the dry weight of two fairy shrimps and their length, and the individual dry weight of these two species versus time. Significance of the correlation coefficients R was determined with the table provided by Steel and Torrie (1981) (level of acceptance $\alpha<0.05$).

RESULTS

Meteorology and physical and chemical parameters

Although some initial rain fell in the region during the first half of June, Passoula did not become filled with water until the first big rainstorm on June 20 (18.3mm). Larger and smaller showers continued falling until early November. The last two downpours occurred on October 23 (23.0mm) and November 1 (49.8mm). Both of these took place rather late in the season. This explains why Passoula did not desiccate until the end of January 1995, which was unusually late. Temporary ponds more often dry out by the end of November or early December (Lahr and Diallo 1993, Lahr *et al.* 1995). The total precipitation during the rainy season of 1994 measured in Nioro du Rip was 795mm. The bulk of this amount fell during August, September and October.

Figure 2 shows the results of the meteorological measurements and the depth of the pond. The start of the dry period can clearly be observed in the graphs for air temperature and relative air humidity. During the wet period median daily temperature fluctuated around 30°C with minima and maxima of approximately 25 and 35°C. The dry season began in the first half of November. This is illustrated by a drop in relative air humidity. At the same time minimum daily temperature decreased considerably and maximum temperature became slightly higher.

The average daily evaporation during the study was approximately 5.3 mm/day. As can be seen in Fig 2, the rate became somewhat higher when the air humidity dropped at the end of the season. The fluctuations of the net water balance (the difference between the cumulative precipitation and evaporation) closely resembled the measured water depth of Passoula (Fig 2). Linear regression of the logarithm of the depth against this net balance revealed a significant positive correlation between these parameters (Fig 3).

The temperature of the water in the pond (Fig 4) strongly reflected the daily minimum air temperatures measured during the study (Fig 2). This is not surprising given that the water temperature was measured early in the morning when the pond was sampled and that the pond was rather shallow.

The water in the pond was slightly acid with pH values fluctuating around 6.3 (Fig 4). DO could not be measured from mid-October until mid-December due to malfunction of the measuring device. Most values were between 50 and 100%. When the pond starts to dry out in December, DO levels dropped to zero.

The Secchi depth was almost zero at the beginning and at the end of the wet season (Fig 4). Turbidity only decreased when the depth at the centre of the pond exceeded 60cm (Fig 2). During this period, from August to until mid November, the water was clearer.

Conductivity was around 50 µS/cm during most of the season, but values increased considerably when the pond started to desiccate (Fig 4). A value of almost 500 µS/cm was reached the day before Passoula became completely dry. It is assumed that this was caused by concentration of dissolved ions when water evaporates and the level drops (see Fig 5). A high correlation was found between the logarithm of the conductivity and the water depth.

Biota

Table 1 shows the most abundant species or groups of species of invertebrates captured during the season, the type of sample from which average densities for each group were calculated. Many organisms could be identified at the species level, but some could only be distinguished at higher taxonomic levels. The group called Dytiscidae spp. is probably a mixture of two species, *Hydroglyphus sp.* and *Bidessus sp.* The tadpole shrimp *Triops sp.* is most likely *Triops cancriformis mauritanicus*. *Alona spp.* is a mixture of *A. diaphana* and *A. karua* and *Micronecta spp.* may consist of *M. scutellaris* and other species such as *M. eupompe*.

The species mentioned in the table and in this report include only those of which a total of more than 20 individuals was obtained when the average densities of all 83 sample dates during the study were added.

These relative measures of yearly abundance are shown in Fig 6. The total number of zooplankton captured was ten times as high as the total number of macro invertebrate individuals.

Measured individual macro invertebrate dry weights are also given in Table 1. Three species, *Triops sp.*, *Sigara sexlineata* and *Hydrovatus sp.*, were not found in sufficient numbers during our searches in 1995 and 1996. These species are therefore not included in the total estimated biomass, but it is assumed that this is of minor importance because the three of them were only present in very low numbers throughout the season (Fig 6). The length-weight relationships established for *B. chudeaui* and *S. sudanicus* are shown in Fig 7. For *B. chudeaui* only three measures were obtained, but correlation was nonetheless significant. The data for *S. sudanicus* show a clear significant linear relationship between the logarithm of the dry weight and the animals' length.

Zooplankton

The average numbers of the abundant cladocerans are shown in Fig 8. All four species occur in peaks. *M. micrura* is very numerous shortly after the first rains with densities up to almost 30 000 individuals/m^3. It is followed by lower peak densities of *D. senegal* in August, *C. quadrangula* during early September and *Alona spp.* in November.

Copepods also display short peak densities (Fig 9). All three species peak in July, a few weeks after the rains started. The cyclopoids *M. kieferi* and *T. decipiens* were more numerous than the calanoid *P. rex*. Only *T. decipiens* seemed to last longer than a few weeks. It was found until January and was also very abundant during the second half of November.

Ostracods were less abundant than the cladocerans and copepods. The four species were mostly found during November and December (Fig 10). The only exception was *H. symmetrica*, which also peaked several times during July and August.

The total number of zooplankton, and the percentage that each of the three main groups attributed to this during the season, are shown in Fig. 11. Zooplankton was predominant during the first month of the existence of the pond. Cladocera constitute the bulk of the numbers until mid July and during August. During the rest of the period copepods dominated the zooplankton. The contribution of ostracods to the total number was marginal.

Table 1: Most abundant invertebrate species captured in a temporary pond in central Senegal during the rainy season of 1994-1995. Z, P and S refer to the type of samples from which the densities of the organisms were calculated: zooplankton, pelagic or sediment macro invertebrate samples respectively (see text). The individual weights for most macro invertebrate species or categories are given as well.

Taxon	Sampling method	Dry weight (mg/ind.)
Branchiopoda		
Ceriodaphnia quadrangula (Cladocera, Daphnidae)	Z	-
Moina micrura (Cladocera, Moinidae)	Z	-
Alona spp. (Cladocera, Chydoridae)	Z	-
Diaphanosoma senegal (Cladocera, Sididae)	Z	-
Branchinella chudeaui (Anostraca, Branchinectidae)	P	see Fig 7
Streptocephalus sudanicus (Anostraca, Streptocephalidae)	P	see Fig 7
Triops sp. (Notostraca, Triopsidae)	P	-
Cyclestheria hislopi (Laevicaudata, Lymnadiidae)	S	0.25
Copepoda		
Mesocyclops kieferi (Cyclopoida, Cyclopidae)	Z	-
Thermocyclops decipiens (Cyclopoida, Cyclopidae)	Z	-
Peradiaptomus rex (Calanoida, Diaptomidae)	Z	-
Ostracoda		
Heterocypris symmetrica (Cypridae)	Z	-
Cypris latissima (Cypridae)	Z	-
Cyprette murati (Cypridae)	Z	-
Oncocypris muelleri (Cypridae)	Z	-
Hemiptera		
Anisops sardeus (Notonectidae)	P	4.90
Anisops varius (Notonectidae)	P	8.23
Anisops debilis perplexus (Notonectidae)	P	3.03
Anisops jaczewskii (Notonectidae)	P	2.4
Anisops spp. nymphs (Notonectidae)	P	2.16
Agraptacorixa senegalensis (Corixidae)	S	9.10
A. senegalensis nymphs (Corixidae)	S	2.80
Micronecta spp. (Corixidae)	S	0.43
Sigara sexlineata (Corixidae)	S	-
Coleoptera		
Eretes sticticus (Dytiscidae)	P	55.4
E. Sticticus larvae (Dytiscidae)	S	10.9
Dytiscidae spp.	S	0.3
Yola enigmatica (Dytiscidae)	S	0.7
Hydrovatus sp. (Dytiscidae)	S	-
Berosus sp. (Hydrophilidae)	S	0.7

Macro invertebrates and tadpoles

Densities of four phyllopods are shown in Fig 12. *Triops sp.* and *B. chudeaui* are typically fast growing organisms. They appeared only days after the pond became filled in June and disappeared in two or three weeks. Their densities never became very high. In contrast, *S. sudanicus* was also found in a matter of days after the first showers, but the species remained present until mid November. *C. hislopi* was the most numerous phyllopod. This small clam shrimp species was found in large numbers during October and November, but it peaked shortly before the pond dried out.

The difference in growth rate between the fairy shrimps *B. chudeaui* and *S. sudanicus* is illustrated in Fig. 13. Average growth rates for both species derived from these graphs using linear regression of individual dry weight or length against time were 166 and 17 µg DW/day (or 1.63 and 0.18 mm/day) respectively. *B. chudeaui* therefore grew ten times as fast as *S. sudanicus*. The dip in the average dry weight for *S. sudanicus* observed at the end of July may be due to heavy rainfall which inundated new parts of the pond. Younger juveniles that hatched from these newly inundated areas got mixed with the older and slightly longer ones already present. The ratio between the length of the periods during which both fairy shrimps were present (two weeks for *B. chudeaui* and approximately 150 days for *S. sudanicus*, Fig 12) was similar to the ratio between the growth rates (approximately 1:10).

The development of the population of *S. sudanicus* is shown in more detail in Fig 14. Until the end of July the sex of the organisms could not be determined, i.e. they were still juvenile. Adulthood therefore was reached after one and a half month at the earliest. Females with visible eggs in their ovisacs did not appear until August. Time to first reproduction in the species therefore may be almost two months. An other interesting phenomenon is that the sex ratio changed to the advantage of the females during the season. From September until the end of October the percentage of males gradually dropped from 50 to around 20%.

Anisops species were the predominant hemipterans in the pond (Fig 15). For *A. sardeus* and *A. varius* a small peak can be observed approximately 12 days after the pond was newly filled with rain. For the other two species these peaks do also exist, but due to the scale of the graphs the few individuals involved cannot be distinguished. The initial peaks are followed by an almost complete absence of adults until the end of September. During this time the number of nymphs that are observed reached its maximum. Numbers of adults of the four species strongly increased from October onwards. The species disappeared two weeks before the desiccation of the pond. The corixids *A. senegalensis* and *Micronecta spp.* were mostly found from September until December. *S. sexlineata* on the contrary seems only to occur at the beginning and the end of the rainy season when the pond is very shallow. Its numbers were very low.

The sex ratios of adult *A. sardeus* and *A. debilis perplexus* populations in the pond are shown in Fig 16. Males of *A. sardeus* were less numerous than females and showed a slight tendency to decrease during the season. For *A. debilis perplexus* a relatively stable sex ratio of 50/50% was observed during the whole study.

Most coleopterans (Fig 17) were not found in large numbers until the end of the wet cycle of the pond. Only *E. sticticus* whose larvae were observed early in the season was regularly found from September onwards. All species of beetles peaked shortly before the pond dried out.

Chironomid larvae were mostly found at the end of the season (Fig 18). The species have not been identified.

The estimated total macro invertebrate mass in the pond is shown in Fig 19. It increased from September onwards and peaked during early November and during the last weeks of the pond. Fairy shrimps (Anostraca) contributed significantly to the total mass from June until October. During the later stages *C. hislopi* and *Anisops spp.* became more important. The contribution of coleopterans to the total mass was not very high except for a short period in early July and during the last two months when most other groups had virtually disappeared. Together, Anostraca and *Anisops spp.* made up more than 50% of the total macro invertebrate biomass during the whole period from June until January.

Tadpoles (Fig 20) were mostly found at the beginning of the season. They developed rapidly and disappeared after reaching adulthood.

DISCUSSION

Physical and chemical features

The data in Figs 2 and 4 clearly showed the onset of the dry season by the end of November 1994. The last significant rains fell in the first week of November, but the intertropical rain front did not disappear until four weeks later. This change was marked by a strong decrease in relative air humidity and median temperatures. Likewise, the water temperatures (only measured in the morning) dropped from between 25 and 30°C to approximately 17°C. The temperature data in Fig 1 also show that although the median daily temperature is reduced, the difference between the maximum and minimum temperatures becomes much larger (maximum temperatures even increase somewhat). Many authors which studied diurnal temperature cycles in temporary ponds report strong fluctuations between day and night temperatures (e.g. Yaron 1964; Barclay 1966; Weir 1969; Moore 1970; Morton and Bayly 1977). It is therefore assumed that the temperature in the water of Passoula must also have fluctuated on a daily basis, although this was not investigated. When ponds are turbid penetration of solar radiation is greatly reduced (Kirk 1985) and micro stratification of the water may occur, even though temporary ponds are usually shallow (Eriksen 1966). On many occasions during sampling we noticed a clear temperature difference between the surface and the deeper parts of the pond.

Water temperature is not the only parameter known to alternate strongly on a daily basis in temporary ponds. DO, pH and alkalinity may also vary considerably from hour to hour in shallow waters (Yaron 1964; Barclay 1966; Cole 1968; Hartland-Rowe 1972; Hamer and Appleton 1991a). DO and pH in Passoula varied during the season. These parameters are related to the metabolism of the biotic community in the ponds. There was no clear pattern, although DO seemed to be somewhat higher when the water was relatively clear from August until November. This could have been an indication for autotrophic activity, but unfortunately most DO-measures during this period are lacking. The water of the pond was more transparent when water levels were relatively high, probably because the agitation of the water surface at this stage was not sufficient to affect the deeper sediments below.

When the dry season started, daily evaporation slightly increased (Fig 2) and this process and the lack of rain finally caused dessication of the pond. This process caused several simultaneous phenomena. Turbidity increased rapidly and the Secchi depth became less than a few centimetres (Fig 2). Dissolved oxygen levels dropped to zero shortly before the pond dried out. The effect on the conductivity was also clearly demonstrated (Fig 4). This parameter increased steeply when dissolved ions in the water of the basin became more concentrated. These effects on DO and conductivity are well known from other studies in temporary waters and sometimes occur simultaneously with increased hardness (DeLépiney 1961; Sublette and Sublette 1967; Dabom and Clifford 1974; Daborn 1976; Morton and Bayly 1977).

The difference between the cumulative precipitation and the cumulative evaporation is called the net water balance (Fig1). If the pond would only receive water from direct precipitation on its surface the changes of this parameter would have been similar to the fluctuations of the water level in the pond. But since ponds such as Passoula receive most of their input from runoff, the fluctuations in depth are relatively higher. This was corrected by plotting the logarithm of depth against the net water balance. These parameters were indeed strongly correlated (Fig 3). The regression coefficient of this relationship, e.g. the slope of the regression line, will depend on the drainage area and infiltration rate of any particular pond.

When dissolved ions are concentrated or diluted, the conductivity of the water must be inversely correlated with the volume of the pond. The volume, in turn, is a function of the third power of the depth. This relationship was clearly shown by the almost straight line obtained by plotting the logarithm of the conductivity against depth (Fig 5).

Faunal composition

Temporary astatic and isolated waters are usually characterized by the large branchiopods commonly named phyllopods or euphyllopods. Assemblages of species of these groups are found all over the world in hot arid and semi-arid regions: in southern Africa (Seaman and Kok 1987; Hamer and Appleton 1991a, 1991b), East Africa (Rzòska 1961; Hildrew 1985), North-West Africa (Gauthier 1951; DeLépiney 1961; Monod 1969a, 1969b; Boutin *et al.* 1982, Thiéry 1986; Roux and Thiéry 1988; Beladjal *et al.* 1995), the Middle East (Yaron 1964), India (Belk and Esparza 1995), Australia (Morton and Bayly 1977; Bayly 1982;

Williams 1985), the southwestern United States (Sublette and Sublette 1967; Simovich and Fugate 1992), Mexico (Maeda-Martinez 1991; Campoy-Favela and Mascareñas 1993), and even on the Galapagos Islands (Brendonck *et al.* 1990). The phyllopods that were found in Passoula were *Triops cf. cancriformis mauretanicus* (Notostraca), *Branchinella chudeaui* and *Streptocephalus sudanicus* (both Anostraca) and *Cyclestheria hislopi* (Laevicaudata). *T. cancriformis mauretanicus* also occurs north of the Sahara (Thiéry 1986). The two fairy shrimp species are typical of northwestern Africa south of the Sahara (Hamer *et al.* 1994, Belk and Brtek 1995). *C. hislopi* is a circumtropical species occurring on all continents except Antarctica (Roessler 1995). The latter species is the only phyllopod regularly found in perennial waters.

The zooplankton found in Passoula contains species that are cosmopolitan and species with a more limited range. Zooplankton assemblages with many similar species as in our study were reported from several West African locations, Yemen and southern Africa (Dumont and Van der Velde 1977; Dumont 1981; Dumont and Verheye 1984; Dumont 1986; Dumont *et al.* 1986).

Many insects found during this study have a wide distribution. Most species of aquatic Hemiptera encountered in Passoula, even the rarer ones that have not been shown in the figures, were also found by Weir (1966) in Zimbabwean temporary ponds. *Anisops sardeus* (Notonectidae) for instance is found in Africa, Europe and Asia, but always in the proximity of seas and oceans (Poisson 1926). It is even adapted to life at sea (Brooks 1951). Among the Corixidae *Agraptacorixa senegalensis* may be endemic, but *Micronecta scutellaris* for instance plays an important role in the food chain of Lake Naivasha in Kenya (Clark 1992) and different *Sigara* species are reported from temporary ponds all over the world (e.g. Barclay 1966; Weir 1966, 1969; Sublette and Sublette 1967; Morton and Bayly 1977; Bayly 1982). Among the Dytiscidae (Coleoptera) *Eretes sticticus* has a worldwide distribution in temporary waters with little vegetation, *Yola enigmatica* occurs in a belt across Africa, and *Rhantaticus congestus* (not mentioned in this chapter but abundant in other parts of our study area) is widespread from Africa to Australia (Nilsson and Persson 1993; Nilsson *et al.* 1995). Other insects that are often found in temporary ponds are Lestidae larvae (Odonata). These were not very abundant in Passoula during 1994, but were regularly found in the region (Lahr and Diallo 1993).

Colonization, succession and disappearance

The results of this study once more show how dynamic the community of a temporary pond can be. Most species densities showed peaks during distinct periods of the annual cycle. Succession and development of the invertebrate populations could roughly be divided into four separate stages:

Stage 1- colonization (June-July)

The pond is instantly colonized by crustaceans that survive the dry period as resting eggs or diapausing immature stages. Most of these rapidly complete their life-cycle and then disappear before there are too many predators (the cladoceran *M. micrura*, two species of copepods *M. kieferi* and *P. rex*, the phyllopods *Triops sp.* and *B. chudeaui*). Other crustacean species that are observed during this period, but are present for longer periods of time, were the anostracan *S. sudanicus*, the cyclopoid *T. decipiens* and the ostracod *H. symmetrica*. A few individuals of almost all aquatic insects are also found (the small peaks are not always visible in the graphs because of their scale). These are probably the colonists from which the later populations descend. The arrival of adult *E. sticticus* is immediately followed by the appearance of its larvae. Surviving adult frogs and toads lay their eggs in the pond and tadpoles appear and develop rapidly.

Stage 2 - dominance by crustaceans (August-October)

Two species of cladocerans and one ostracod show peaks: *C. quadrangula*, *D. senegal* and *H. symmetrica*. The macro invertebrate fauna is dominated by *S. sudanicus* and by developing nymphal stages of *Anisops* species. Adult *E. sticticus* are sometimes observed and the corixids *A. senegalensis* and *Micronecta spp.* reach their highest densities during this stage.

Stage 3- shift towards insects (November - December)

This stage is characterized by the disappearance of *S. sudanicus*, high densities of all four species of ostracods and *Anisops spp.*, increasing densities of coleopterans and the appearance of the clam shrimp *C. hislopi*. The last cladocerans, *Alona spp*, peak.

Stage 4- dessication (January)

During this phase *C. hislopi* is the only remaining crustacean and it peaks shortly before the pond dries out. Small coleopterans that were hardly observed before this time also reach high densities. They disappear only a few days before final dessication.

In general the successional trend in the pond is from a community dominated by crustaceans towards insects. A similar kind of succession has also been reported by other authors (e.g. Sublette and Sublette 1967; Moore 1970; Meintjes 1996). It is assumed that fauna in the early stages of the pond is characterized by *r*-species, typically fast growing crustaceans with a short life-cycle whose dormant stages hatch immediately. These are the species that are also capable of colonizing much shorter-lived ponds and pools. The community later shifts towards domination by more K-oriented species which are better suited to compete under density-dependent circumstances.

What exactly causes the disappearance of organisms during the dessication phase was not finally determined. Several parameters change dramatically at the same time. Temperature fluctuations, turbidity and salt content of the water increase. In the meanwhile the oxygen concentration drops to zero. In short, the water in the pond becomes a very harsh environment. The peak densities of small coleopterans that are observed during this phase may be a concentration effect. These species are associated with the shallow fringes of the pond that were not sampled when the water levels were higher. The increase in *C. hislopi* also seems caused by concentration.

Peaks of different taxonomic groups may vary considerably from year to year and from pond to pond in the study area (especially for zooplankton species and chironomid larvae). This has already been demonstrated by Lahr and Diallo and by Lahr *et al.* 1995. The succession of species as pointed out in the scheme may therefore not always be similar. Nevertheless, the general trend described above was also observed in the earlier studies.

Development of populations of Anostraca and *Anisops spp.*

Both fairy shrimps, *B. chudeaui* and *S. sudanicus*, grew at a constant rate during their presence in the pond. We found no clear indication that a maximum size was reached. The maximum length observed was some 24mm for both species, but *B. chudeaui* grew approximately ten times as fast. Hamer and Appleton (1991b) measured average growth rates for several South African phyllopods. The fastest developing species was *Branchipodopsis sp.* (Anostraca) which grew 0.77mm/day, less than half as fast as *B. chudeaui* in our study. The growth rate of *S. sudanicus* in our study (0.18 mm/day) was in the order of magnitude of the slowest growing *Streptocephalus* species from the South African pools, *S. macrourus*. The life-history pattern of the latter was characterized by the authors as essentially *r*-selected, but with a tendency towards a more K-oriented strategy. The time to first reproduction observed for *S. sudanicus*, 1½-2 months, is considerably longer than the range reported by Hamer and Appleton (1991b) for comparable species in South Africa: 10-26 days.

The steady decline of the percentage of males in the population of *S. sudanicus* cannot be explained properly. Male fairy shrimps are often more active than females, especially because they are continuously seeking and courting mates (Wiman 1981, Belk 1991, Brendonck 1995). They might therefore die at a higher rate than the females. Another explanation may be the method of sampling. Pelagic macro invertebrate samples were taken near the surface of the water. Since both sexes in *Streptocephalus* react differently to light (Brendonck *et al.* 1995) sexual stratification may occur. This would obviously have had consequences for the sex ratios encountered in the samples when water levels changed during the season.

Differences in sex ratios were also observed for *A. sardeus*. In this species the percentage of males was lower than 50% throughout the rainy season. *A. debilis perplexus* by contrast showed an almost equal

ratio between males and females.

One may also speculate on the stimulus that triggers the migration of these aquatic insects from permanent waters where they aestivate to temporary ponds when the rainy season begins. Temperatures, a drop in air pressure, lower salt concentrations at the water surface after rainstorms? We have no clue. Since most of these species are vigorous flyers they may also drop into temporary ponds by chance. But for several species of hemipterans, especially *Anisops spp.* and *A. senegalensis*, their high nymphal and adult densities suggest that migration to the relatively predator free environment of temporary ponds such as Passoula for reproduction may be an important stage in their life cycle. Random dispersal to populate these ponds would therefore mean a waste of energy that may better be used for reproduction itself.

Trophic relationships

Lahr (1997a) discussed the special trophic relationships that may prevail in turbid temporary ponds in arid zones. Food webs in these argillotrophic systems (Daborn 1975) may be based on organic matter that enters the ponds from the surrounding land. High turbidities can significantly alter the composition of plankton communities (Arruda *et al.* 1983, Jack *et al.* 1993). Bacteria and protozoans may be important primary producers (Rzóska 1984) and particle feeders are numerous. It can be speculated that such conditions also played a role in the pond in this study. The water was very opaque, especially at the beginning and at the end of the wet season (Fig 4) and most zooplankton and phyllopod species found in Passoula are considered particle feeders, although algae, bacteria and yeasts are also known to be part of their diet. But there was also a period from August until the end of October during which the water was clearer (Fig 4). In such circumstances primary production by phytoplankton would have been possible. Unfortunately we have no complete series of DO-measurements from this period, although a tendency for higher DO-levels was observed (Fig 4).

Macrophytes were almost absent from Passoula and did not therefore play a major role in the pond. But in other parts of Senegal a rich community is sometimes found in these habitats (*e.g.* Vanden Berghen 1990).

Higher trophic levels in the pond include predatory macro invertebrates, tadpoles, water turtles and water birds. Some of the trophic relationships for these groups could be derived from unpublished observations that we made in the field and in aquariums with different biota from temporary ponds in the area around Nioro du Rip. Fairy shrimps were eaten by large predatory coleopterans (*Eretes* sp., *Cybister sp.*), by coleopteran larvae and by water turtles (*Pelomedusa subrufa*). *Anisops spp.* were also seen eating fairy shrimps, but only when they were kept together in an aquarium for several days. These species may have a preference for smaller prey such as zooplankton. Cannibalism was also observed in these Notonectidae. Tadpoles may live on the detritus in the pond. In turn they are eaten by large coleopterans and *Agraptacorixa senegalensis*. *Anisops spp.* were never seen attacking tadpoles. *A. senegalensis* has a benthic lifestyle. Maybe it also feeds on chironomid larvae and ostracods. These are mostly benthic too.

Other benthic species are *Cyclestheria hislopi* and *Triops sp.* The first is usually associated with particulate environments (Roessler 1995). The latter is a scavenger that also predates on mosquito larvae (Maffi 1962).

Many of the larger invertebrate predators were regularly observed in Passoula, but the numbers caught with our sampling methods were too low to assess their population densities. These species include *Cybister sp.* (Dytiscidae, Coleoptera) and *Laccotrephes fabricii* and *Lethocerus cordofanus* (Nepidae, Hemiptera). Such invertebrate predators may greatly reduce the densities of their macro fauna prey at the end of the wet period when their numbers increase (Prejs and Prejs 1992). *Pelomedusa subrufa* in aquariums and in the field feeds on almost anything from ostracods and phyllopods to insects and tadpoles. The many species of water birds observed around Passoula included stints and many egrets and herons. One species of kingfisher (*Halcyon senegalensis*) was always present and fishing actively in the water. On one occasion its stomach contents were investigated. It contained resting eggs of fairy shrimps and the remains of several terrestrial insects.

The trophic relationships outlined here show much resemblance to the food web Weir (1969) postulated for similar ponds in Zimbabwe. The only real difference is that fish are absent from Passoula and other temporary ponds in the Nioro area. Their role may be taken over by the (slower) water turtles.

Indicator species for side-effect assessment of insecticides

Lahr and Diallo (1993) and Lahr *et al.* (1995) executed experimental trials with insecticides in temporary ponds in the area of Nioro du Rip. The most sensitive groups of invertebrates to these treatments were cladocerans, fairy shrimps (*Streptocephalus spp.*) and backswimmers (*Anisops spp.*). The ecological importance of these biota in temporary ponds was investigated in this study.

Cladocera (*Moina micrura* and *Diaphanosoma senegal*) were the most numerous zooplankton species in this study (Fig 6). The fact that they show distinct peak densities (Fig 8) during the season may imply that it will not always be possible to observe insecticide effects on these species in the field for the simple reason that they may not be present. At later stages of temporary ponds copepods may be a good substitute (Fig 11). Since *Daphnia magna* is not always a suitable standard test species to predict the effects of insecticides in Senegalese temporary ponds (Lahr 1997b), it should be considered to develop a toxicity test with one of the indigenous cladoceran species mentioned above.

Combined, *Streptocephalus spp.* and *Anisops spp.* account for more than 50% of the macro invertebrate biomass for most of the season (Fig 19). Their role in these systems thus clearly justifies their use in toxicity tests for Sahelian temporary ponds (Lahr *et al.* 1996, Marquenie *et al.* 1997).

Cyclestheria hislopi, the only other macro invertebrate species that was very numerous in Passoula may be less suitable as an indicator or test species. Their high abundance may largely have been a local phenomenon of the pond, i.e. they were never found in such large numbers in other ponds in the area around Nioro du Rip or in Passoula in other years (see Lahr and Diallo 1993, Lahr *et al.* 1995).

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FIGURES LIST

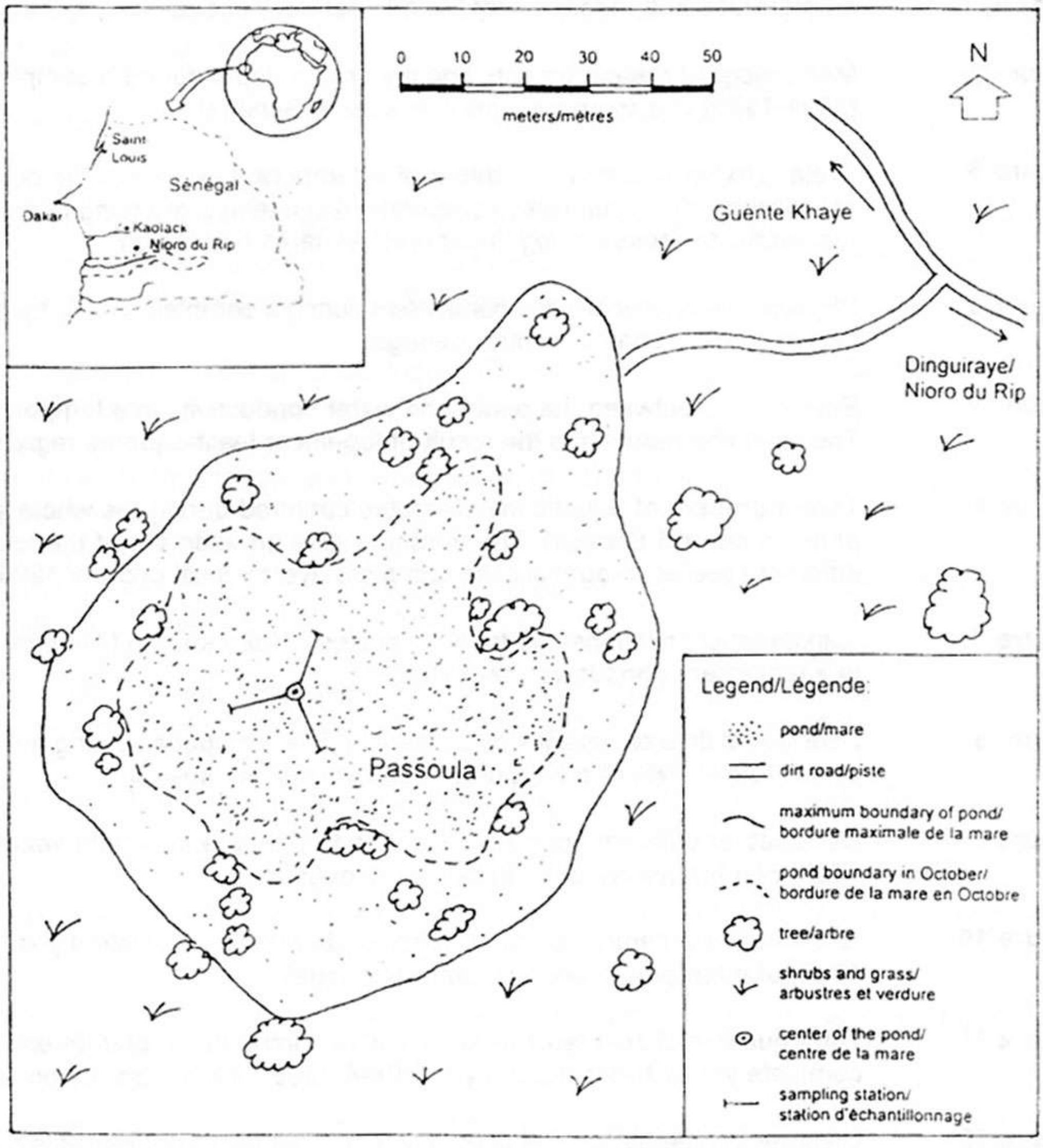

Saint Louis
Sénégal
Dakar
Kaolack
Nioro du Rip
0 10 20 30 40 50
meters/mètres
N
Guente Khaye
Dinguiraye/
Nioro du Rip
Passoula
Legend/Légende:
pond/mare
dirt road/piste
maximum boundary of pond/
bordure maximale de la mare
pond boundary in October/
bordure de la mare en Octobre
tree/arbre
shrubs and grass/
arbustres et verdure
center of the pond/
centre de la mare
sampling station/
station d'échantillonnage

FIGURE N° 2

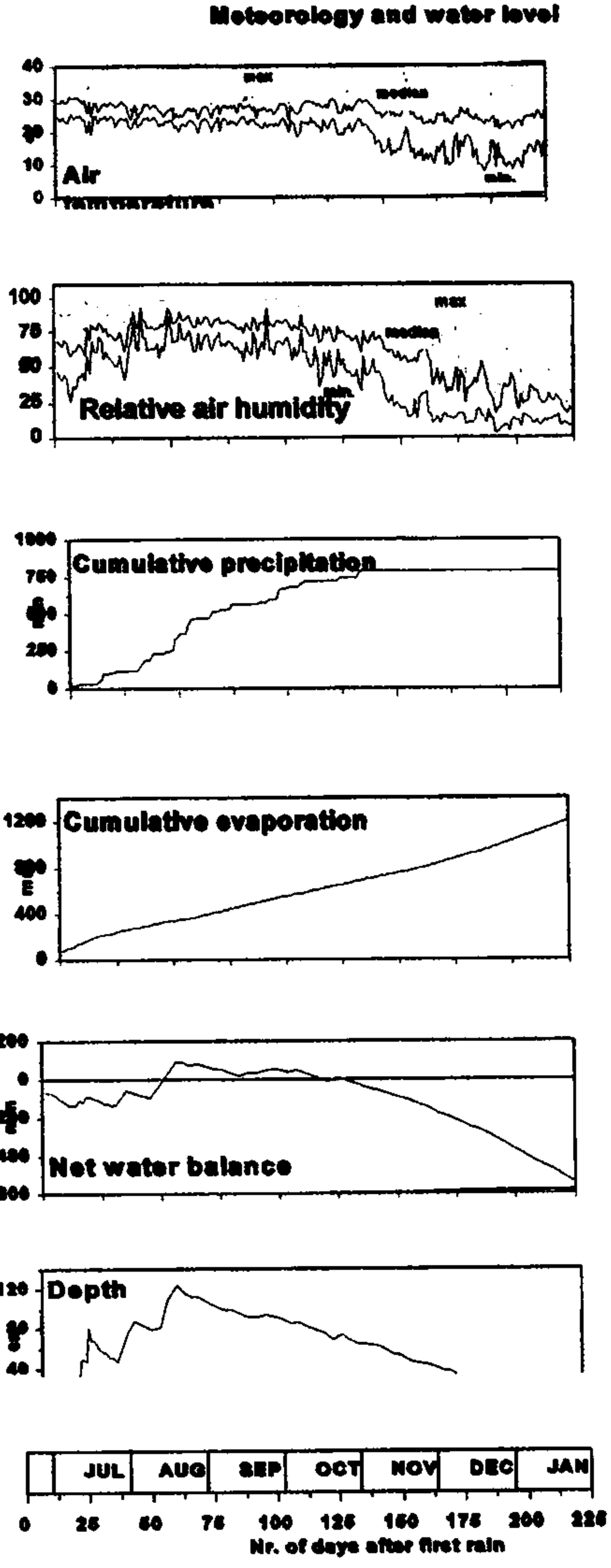

Meteorology and water level
40
30
20
10
0
max
median
min.
Air
100
75
50
25
0
max
median
min.
Relative air humidity
1000
750
500
250
0
Cumulative precipitation
1200
800
400
0
Cumulative evaporation
200
0
-200
-400
-600
Net water balance
120
80
40
Depth
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 3

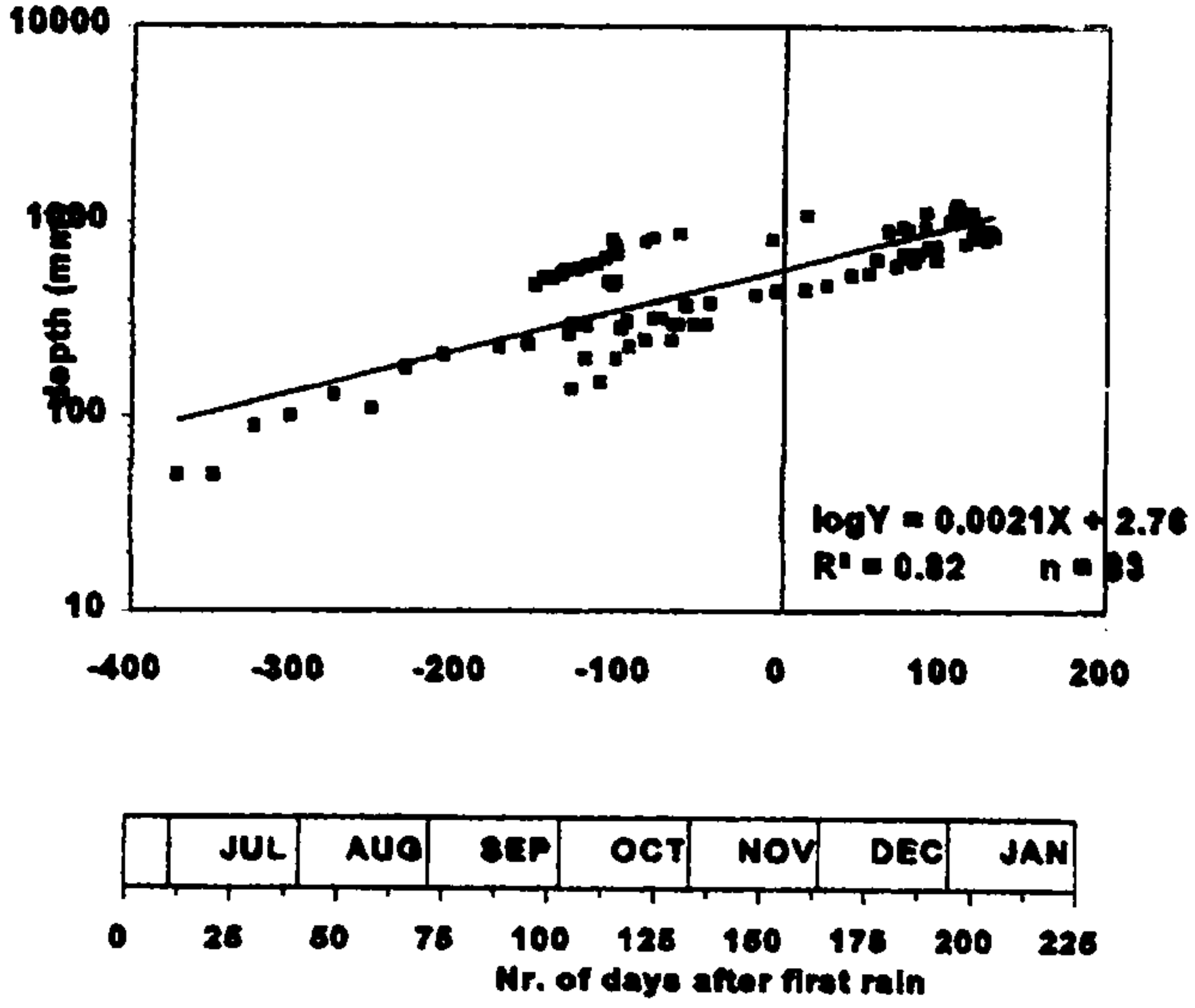

10000
1000
100
10
depth (m)
logY = 0.0021X + 2.76
R² = 0.82
n = 93
-400
-300
-200
-100
0
100
200
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 4

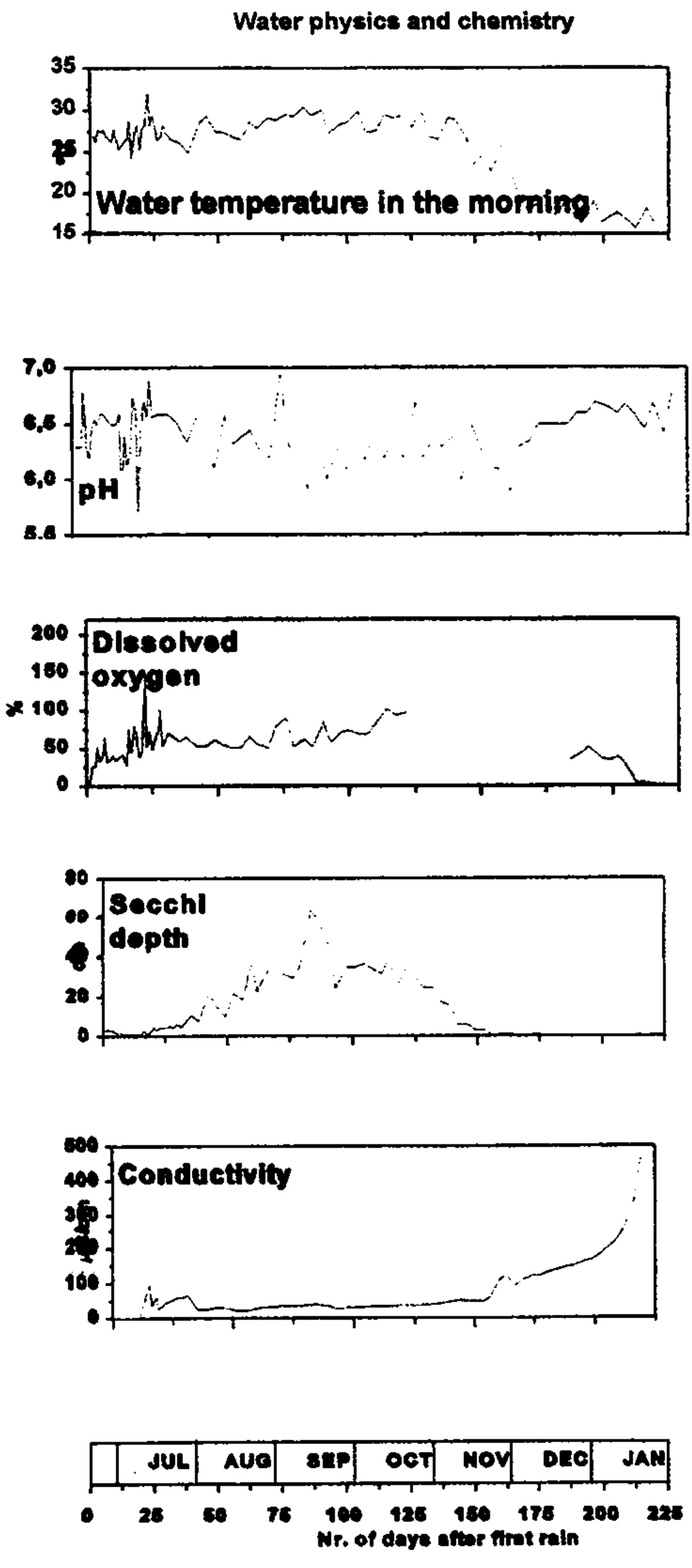

Water physics and chemistry
35
30
25
20
15
Water temperature in the morning
7,0
6,5
6,0
5,5
pH
200
150
100
50
0
Dissolved oxygen
80
40
20
0
Secchi depth
500
400
300
200
100
0
Conductivity
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE 5

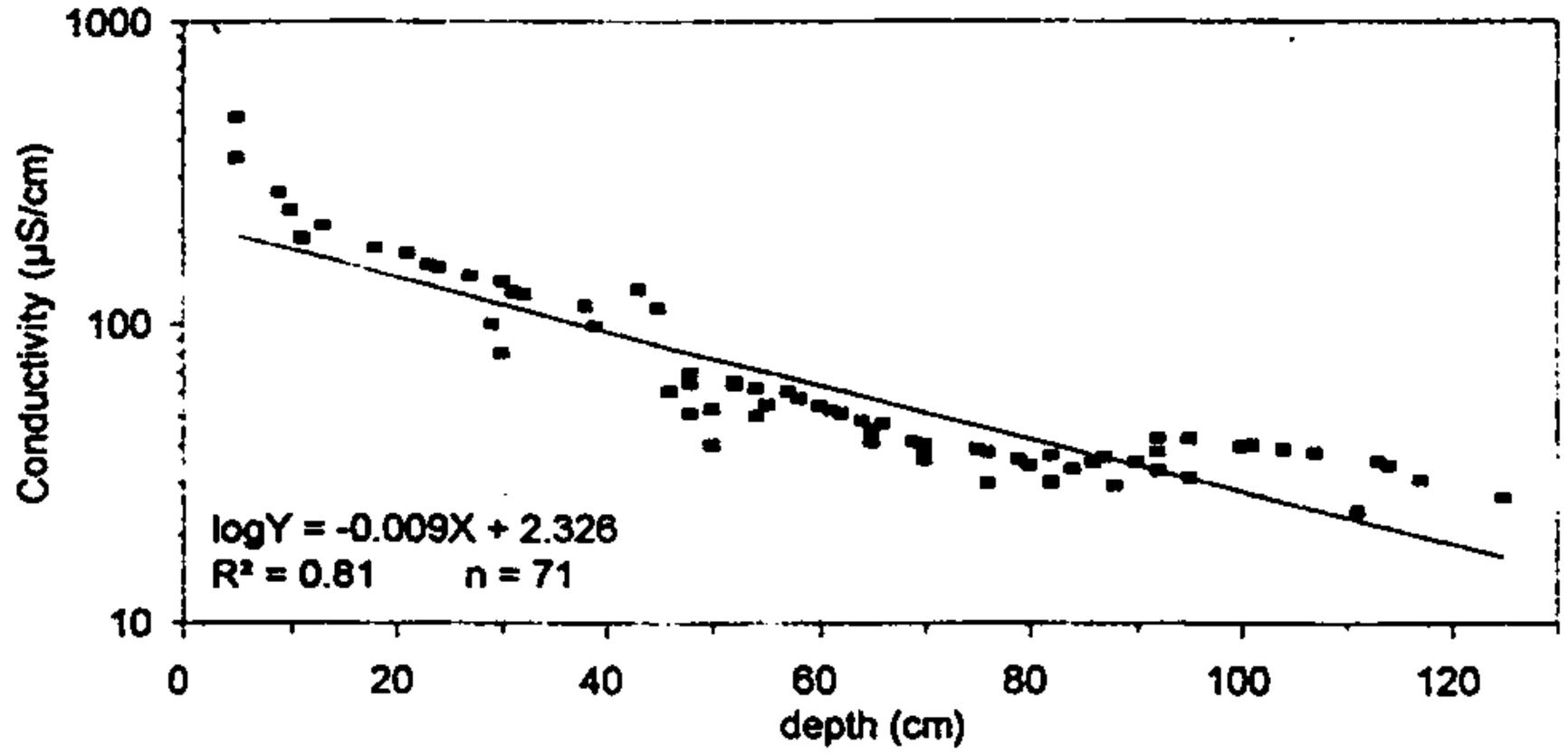


FIGURE N° 6

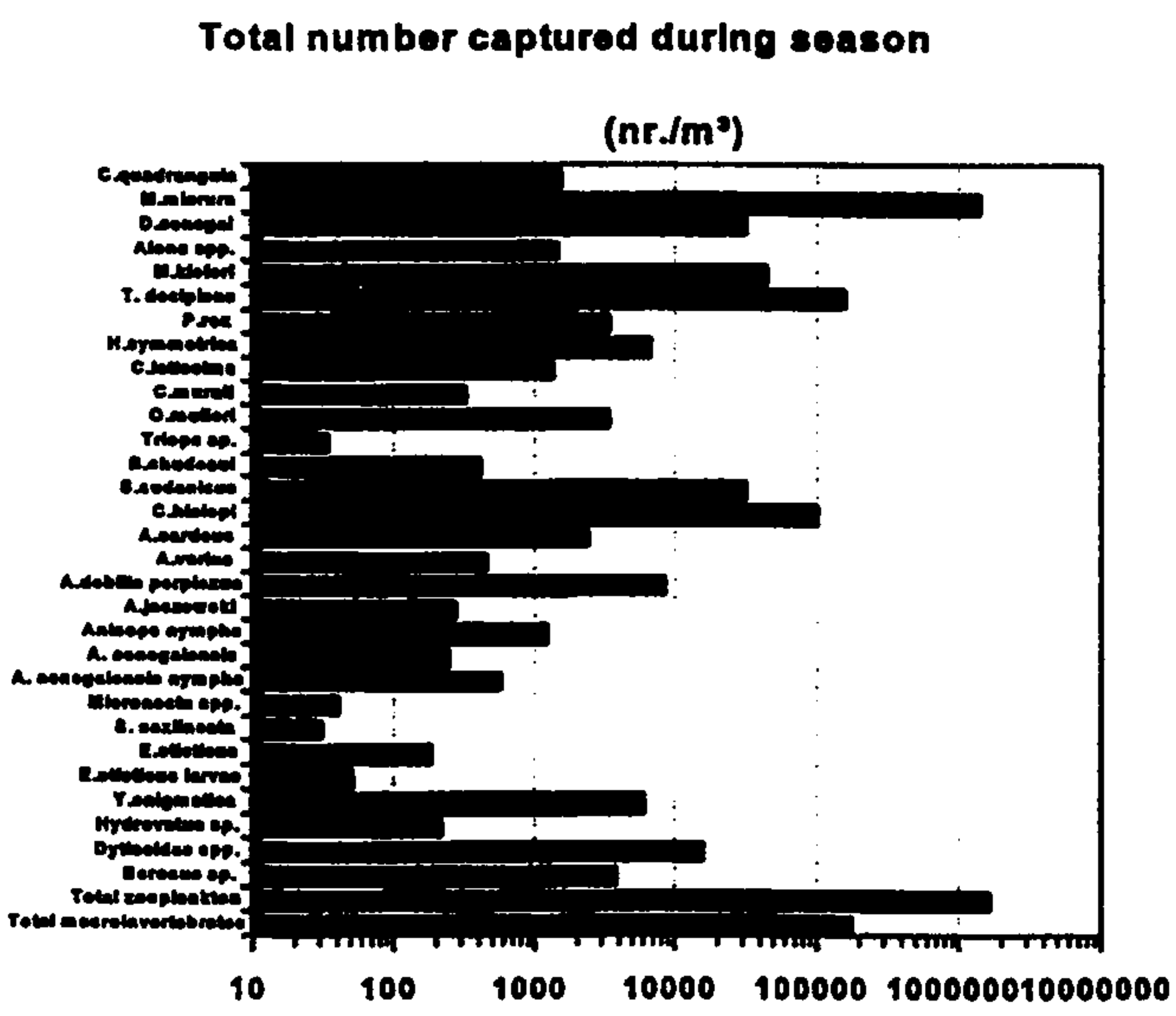


FIGURE N° 7

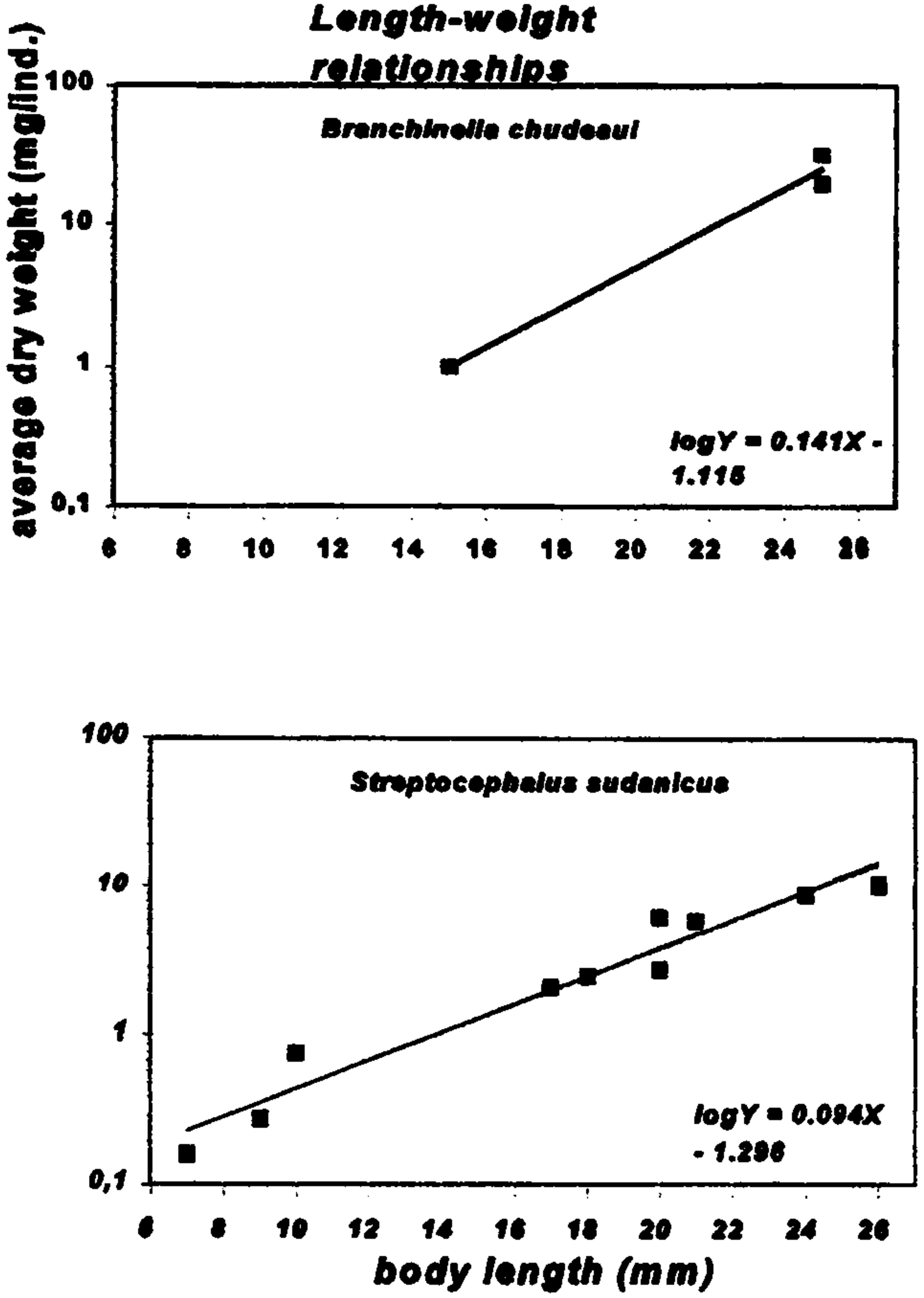

Length-weight relationships
Branchinella chudeaui
average dry weight (mg/ind
100
10
1
0,1
logY = 0.141X - 1.116
6
8
10
12
14
16
18
20
22
24
26
Streptocephalus sudanicus
100
10
1
0,1
logY = 0.094X - 1.296
6
8
10
12
14
16
18
20
22
24
26
body length (mm)

FIGURE N° 8

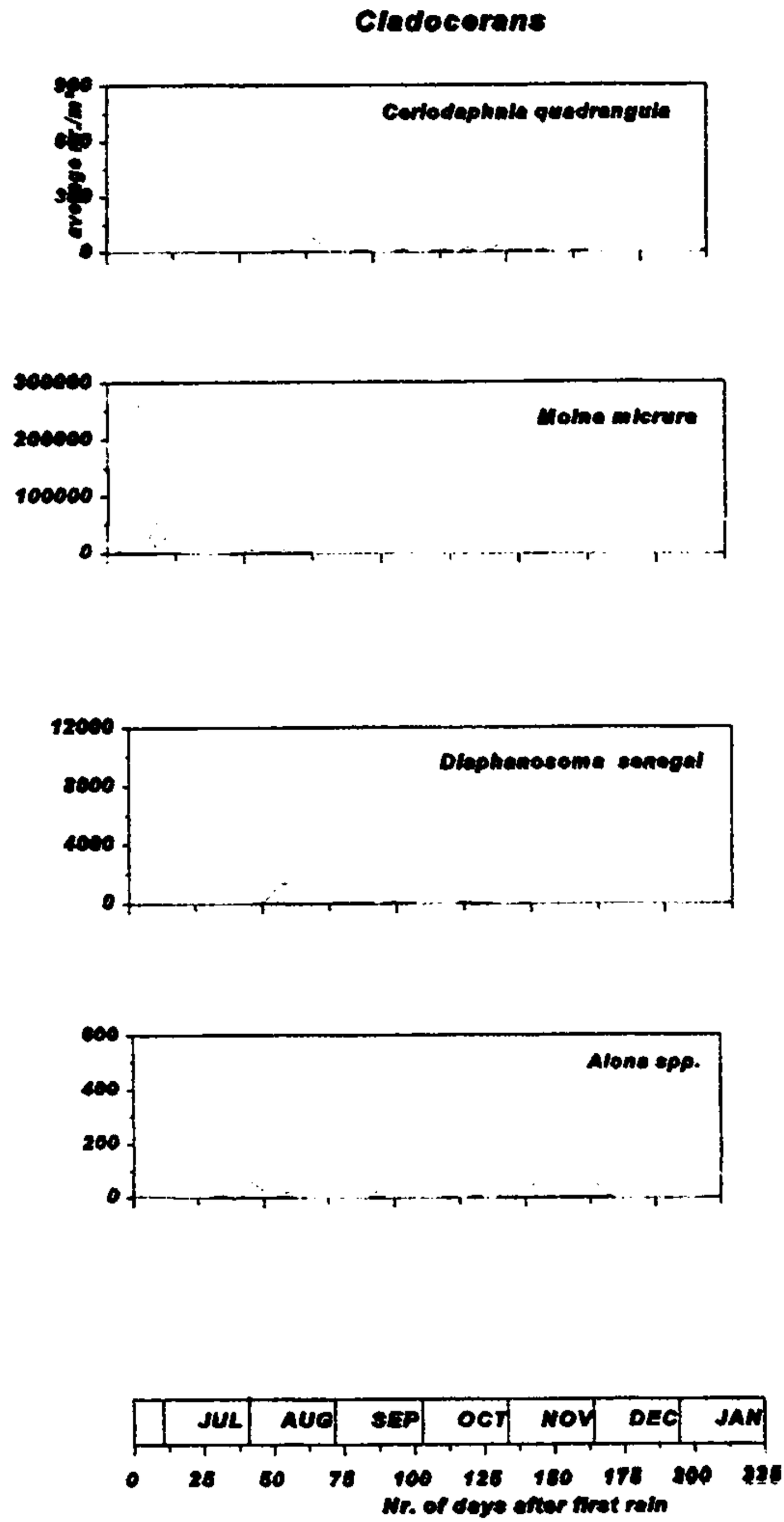

Cladocerans
Ceriodaphnia quadrangula
300000
200000
100000
0
Moina micrura
12000
8000
4000
0
Diaphanosoma senegal
800
400
200
0
Alona spp.
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 9

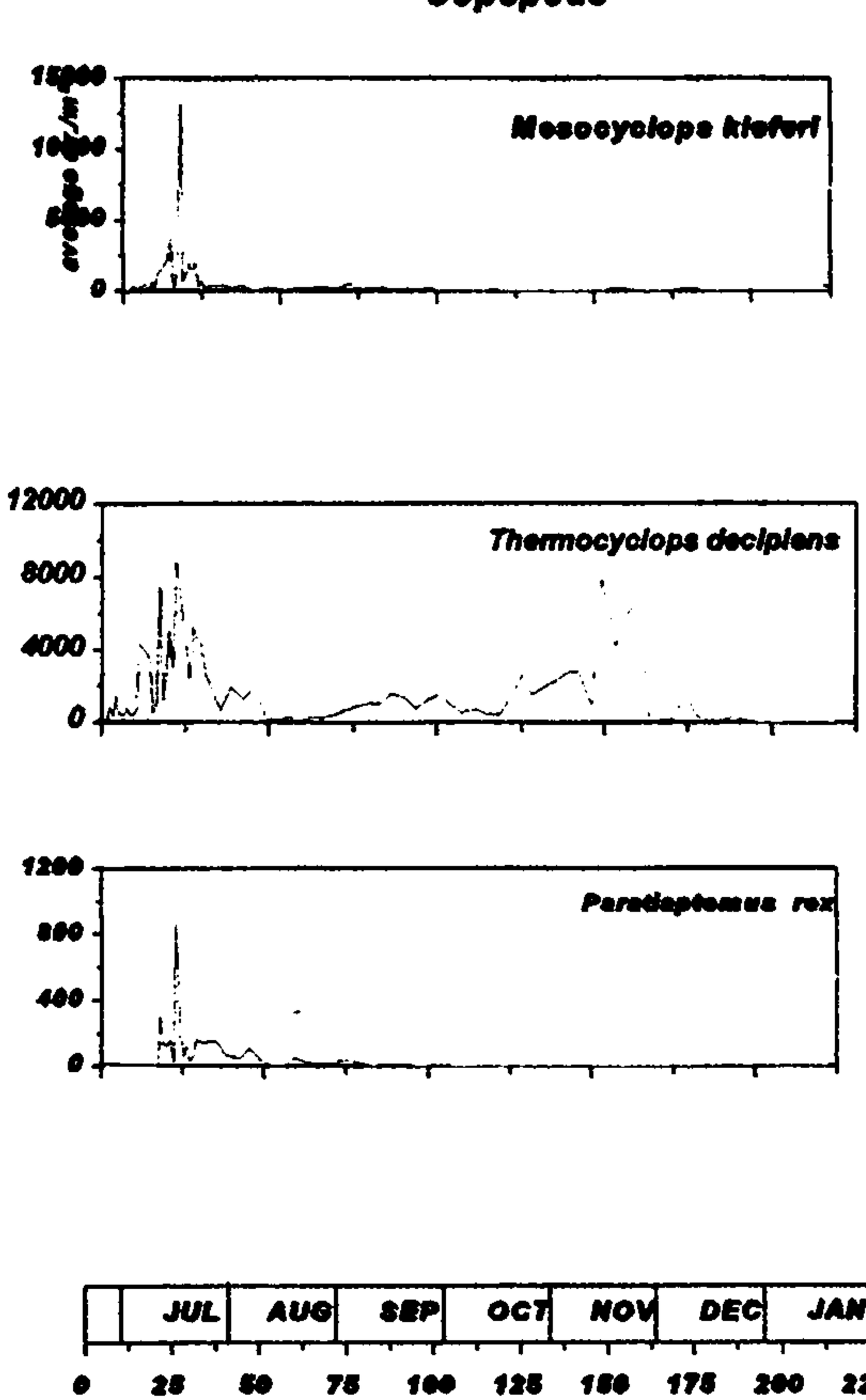

Copepods
Mesocyclops kieferi
Thermocyclops decipiens
Paradiaptomus rex
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 10

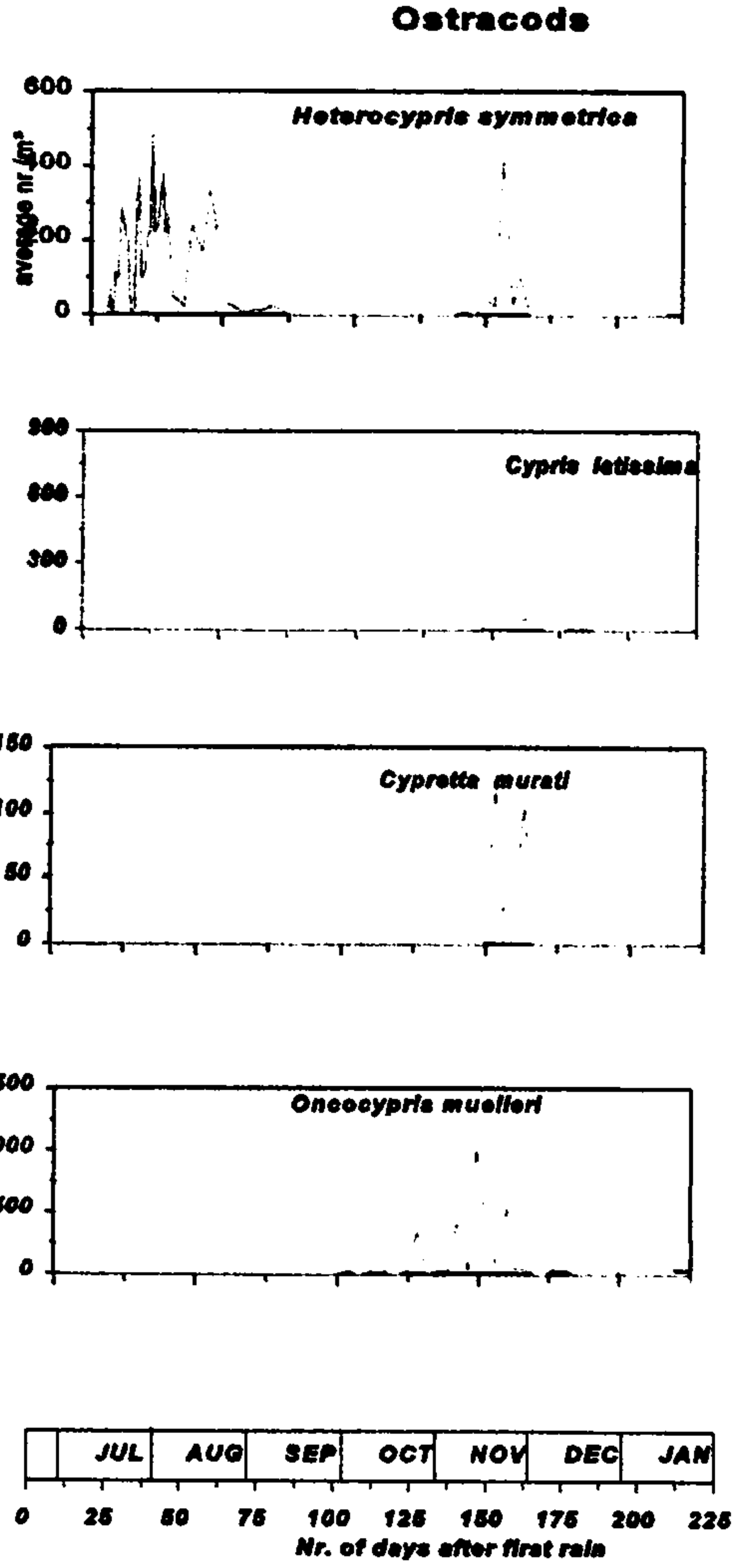

Ostracods
Heterocypris symmetrica
600
400
200
0
average nr./sq.m.
Cypris fatiscima
300
200
100
0
Cypretta muratl
150
100
50
0
Oncocypris mueller!
1500
1000
500
0
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 11

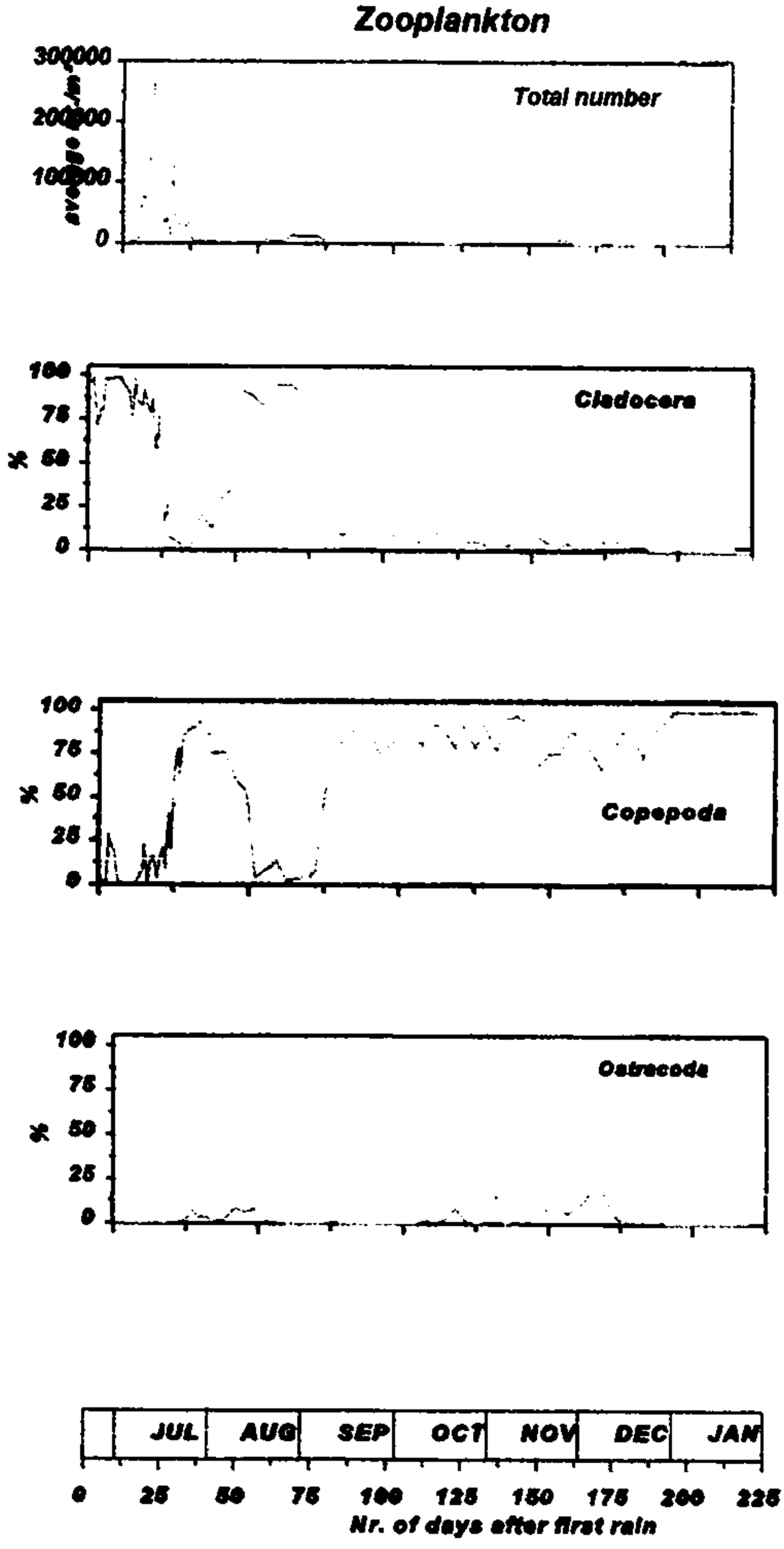

Zooplankton
Total number
300000
200000
100000
0
Cladocera
Copepoda
Ostracoda
100
75
50
25
0
%
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 12

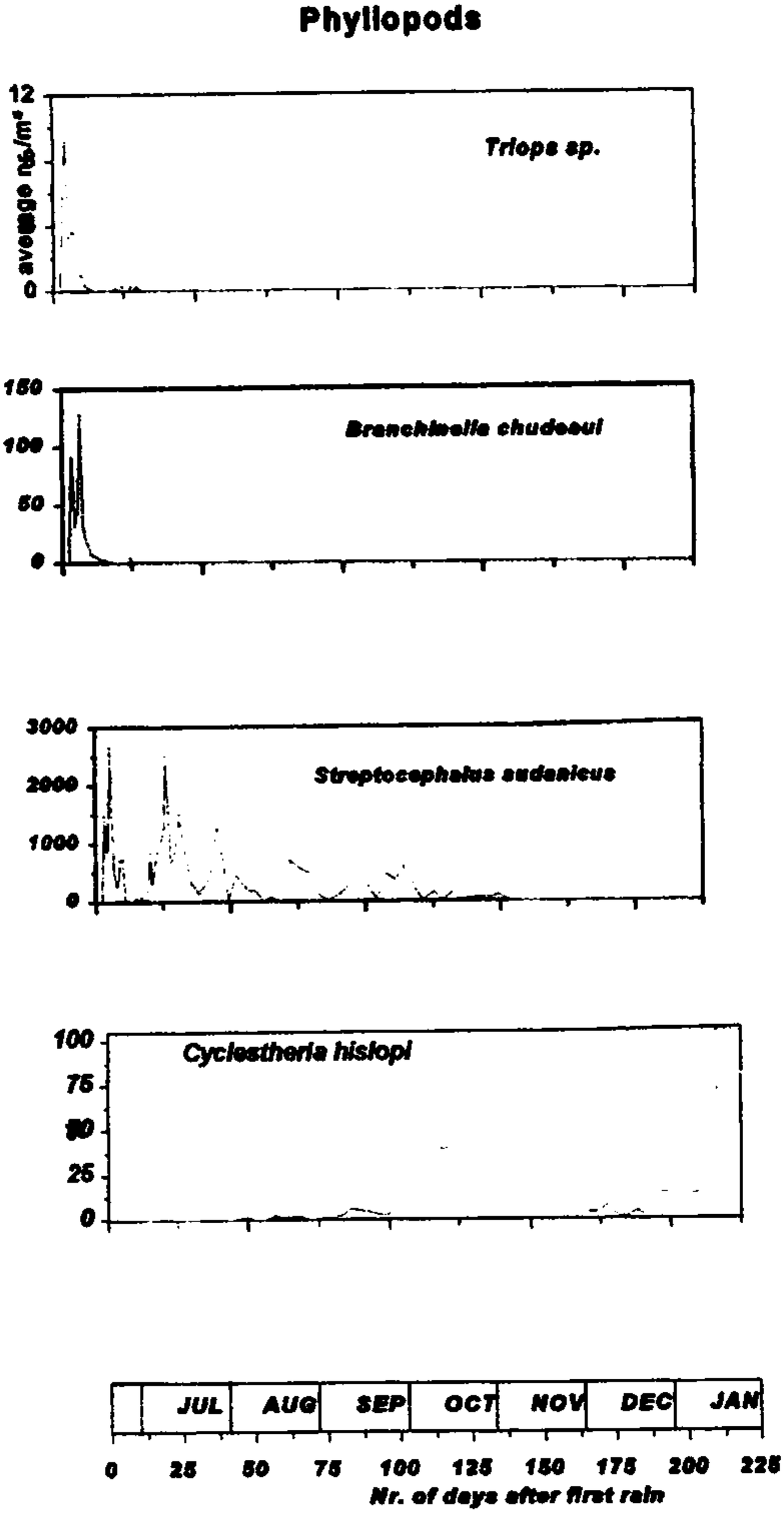

Phyllopods
12
0
average no/m²
Triops sp.
150
100
50
0
Branchinella chudeaui
3000
2000
1000
0
Streptocephalus sudanicus
100
75
50
25
0
Cyclestheria hislopi
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 13

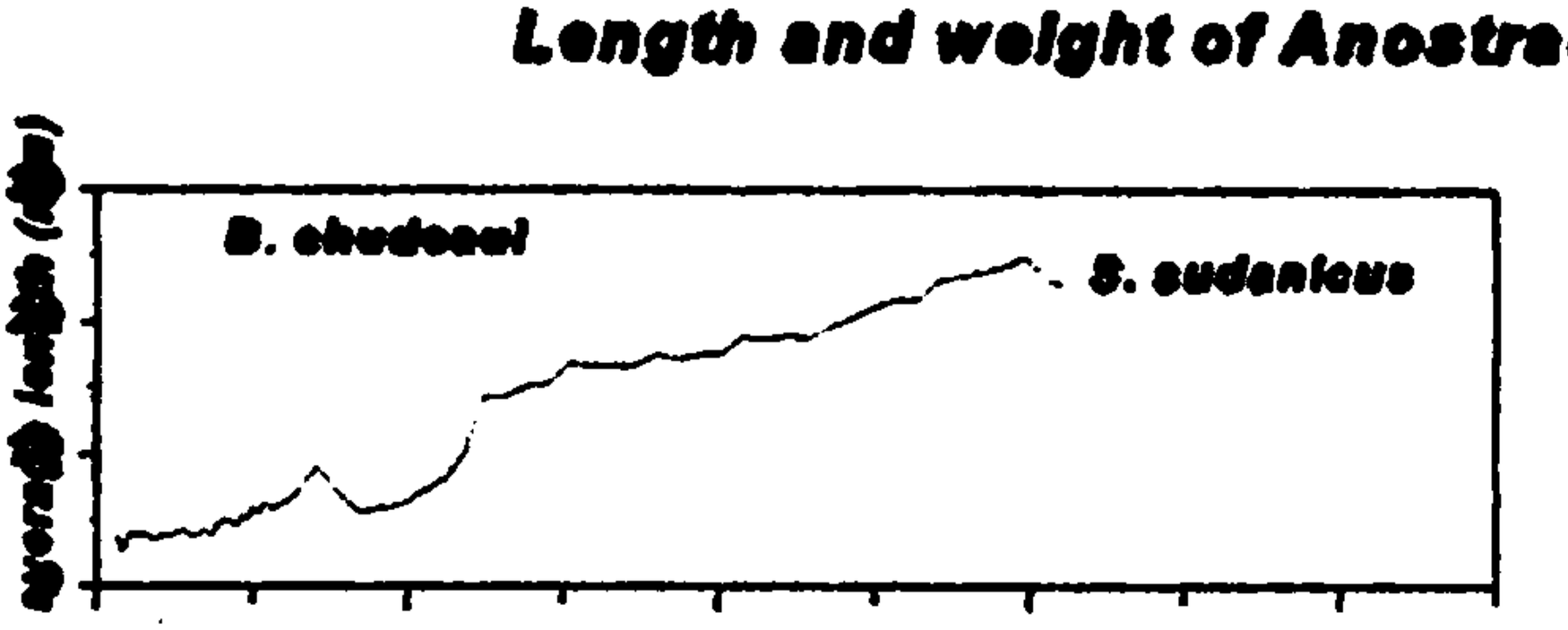

Length and weight of Anostraca
B. chudeaui
S. sudanicus
B. chudeaui
S. sudanicus
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 14

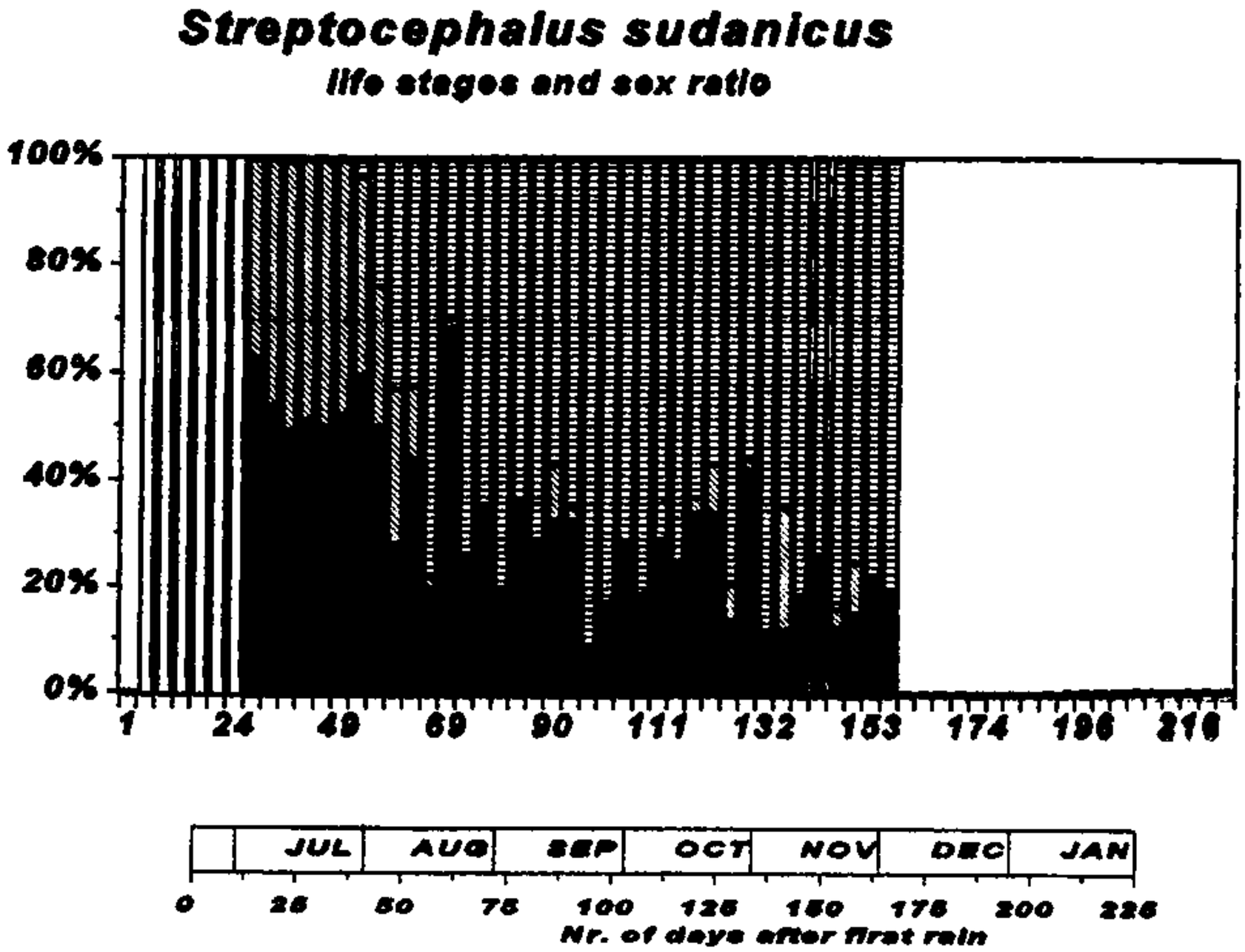


FIGURE N° 15

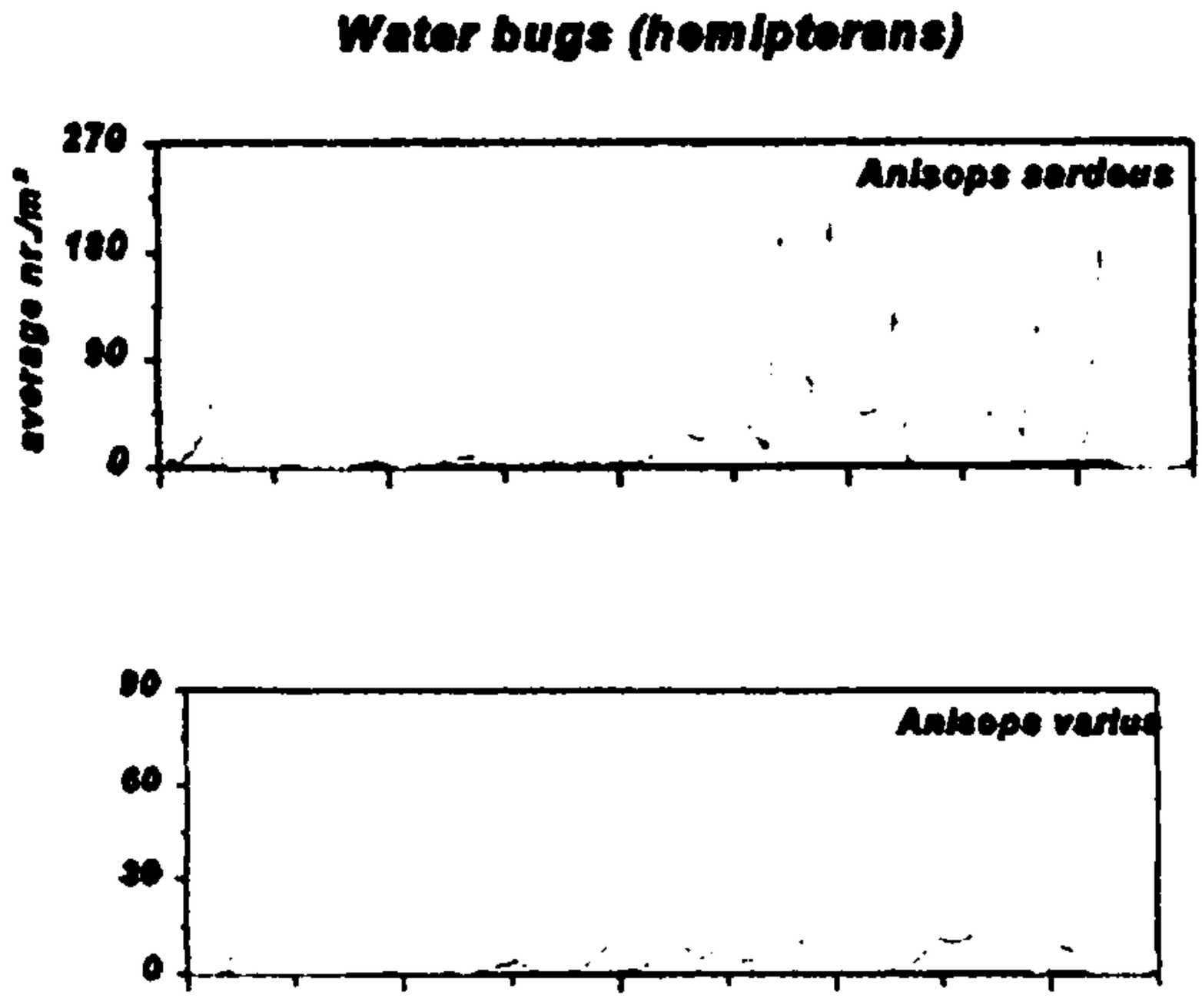
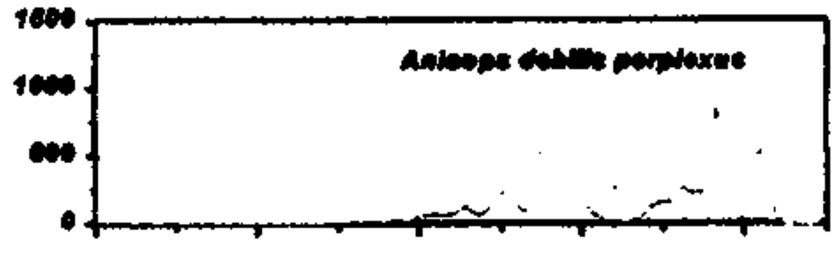
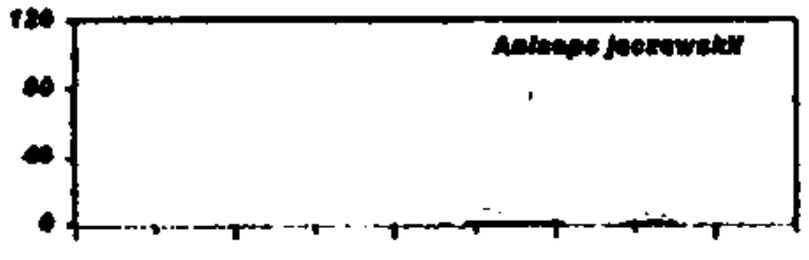
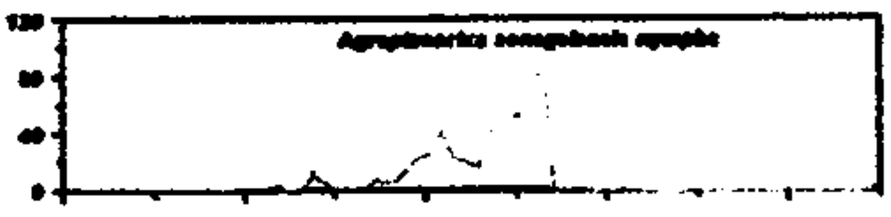
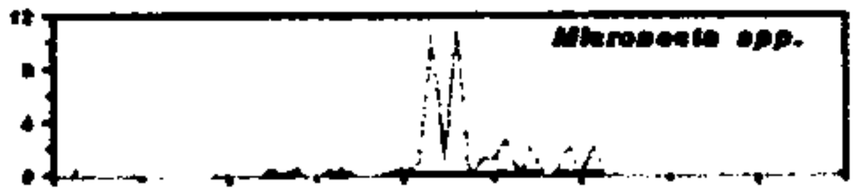


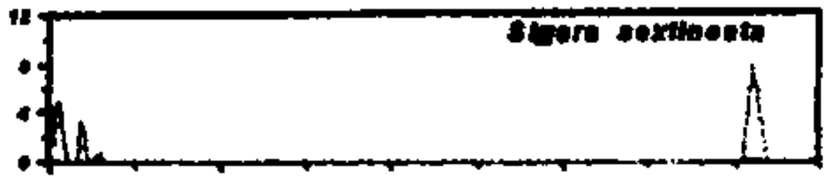
FIGURE N° 15 (suite)











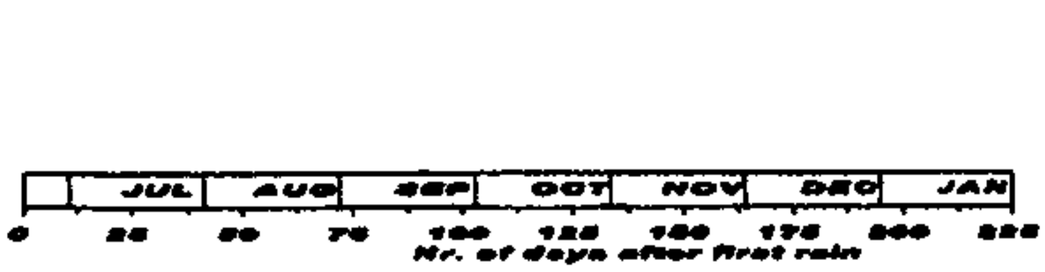


FIGURE N° 16

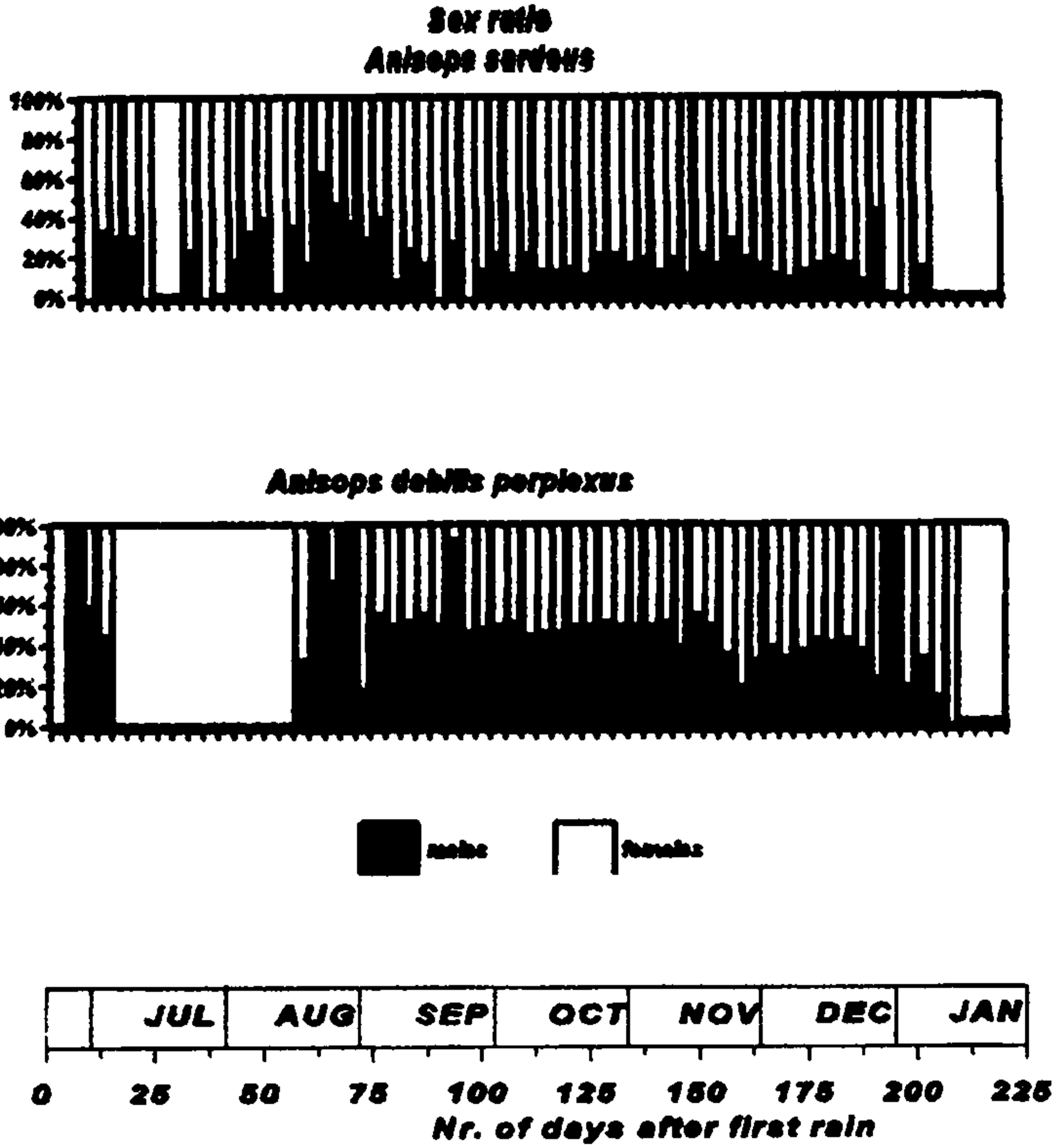

Sex ratio
Anisops serrulus
100%
80%
60%
40%
20%
0%
Anisops debilis perplexus
100%
80%
60%
40%
20%
0%
male
female
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 17

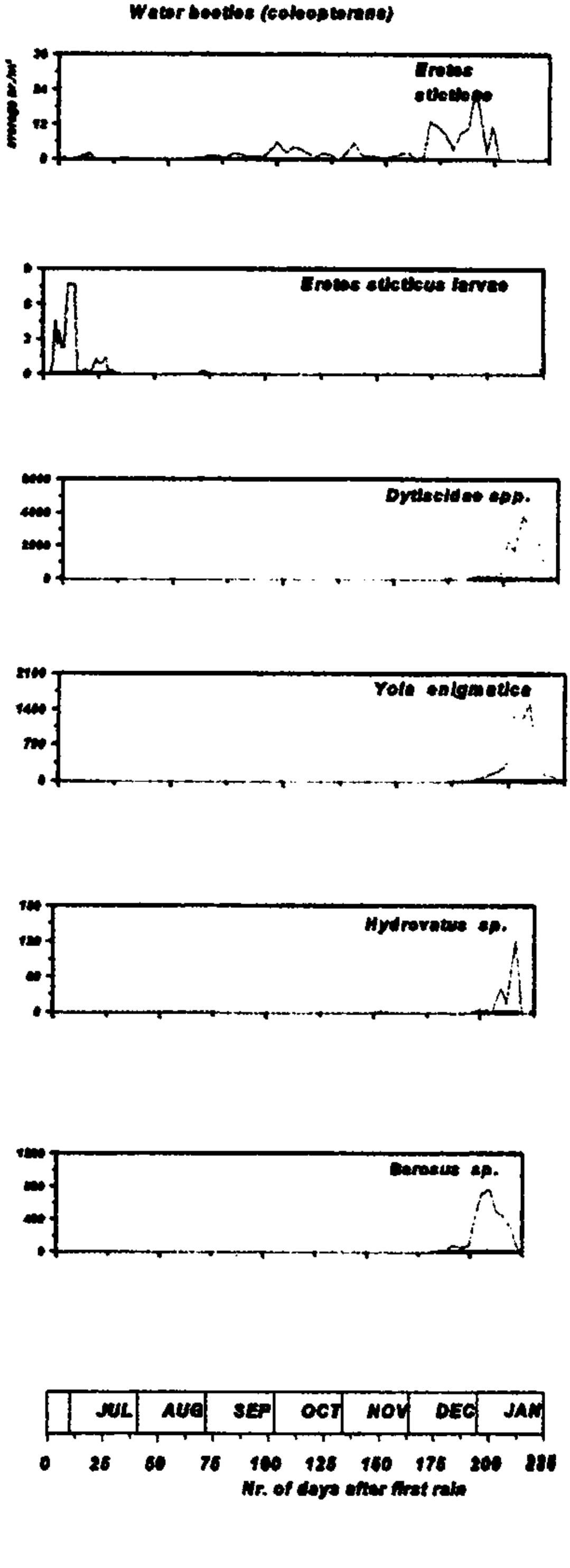

Water beetles (coleopterans)
Eretes sticticus larvae
Dytiscidae spp.
Yola enigmatica
Hydrovatus sp.
Berosus sp.
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 18

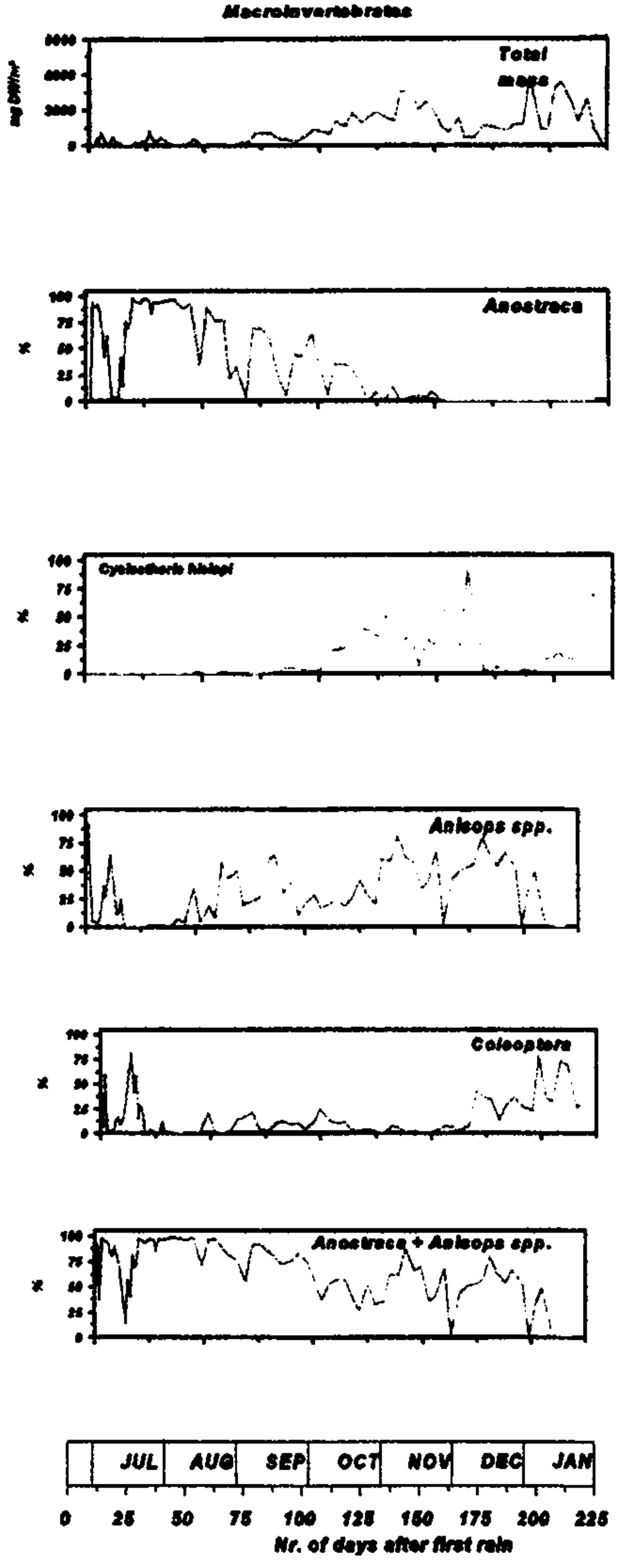

Macroinvertebrates
Total mass
Anostraca
Cyclestheria hislopi
Anisops spp.
Coleoptera
Anostraca + Anisops spp.
JUL
AUG
SEP
OCT
NOV
DEC
JAN
0
25
50
75
100
125
150
175
200
225
Nr. of days after first rain

FIGURE N° 19

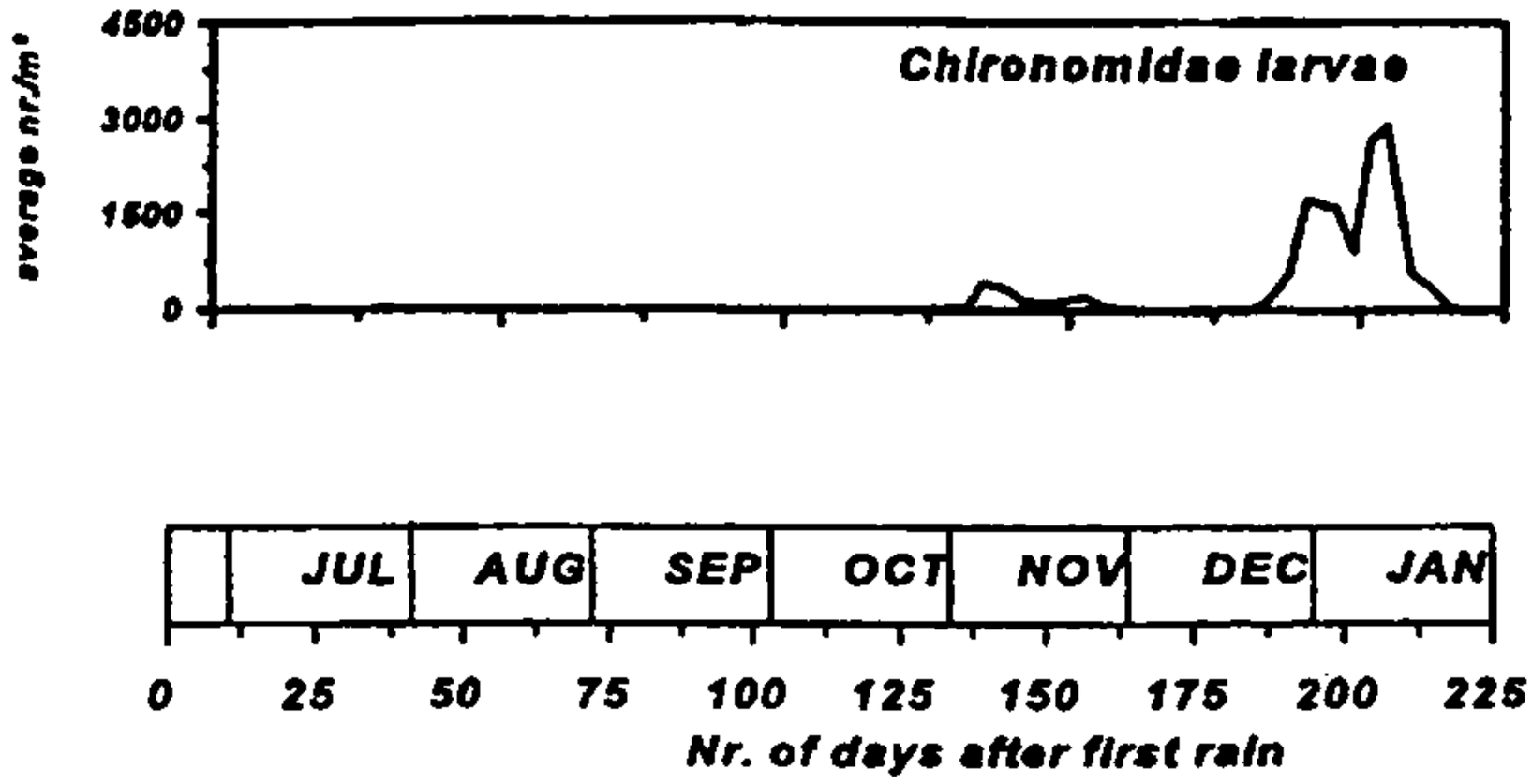
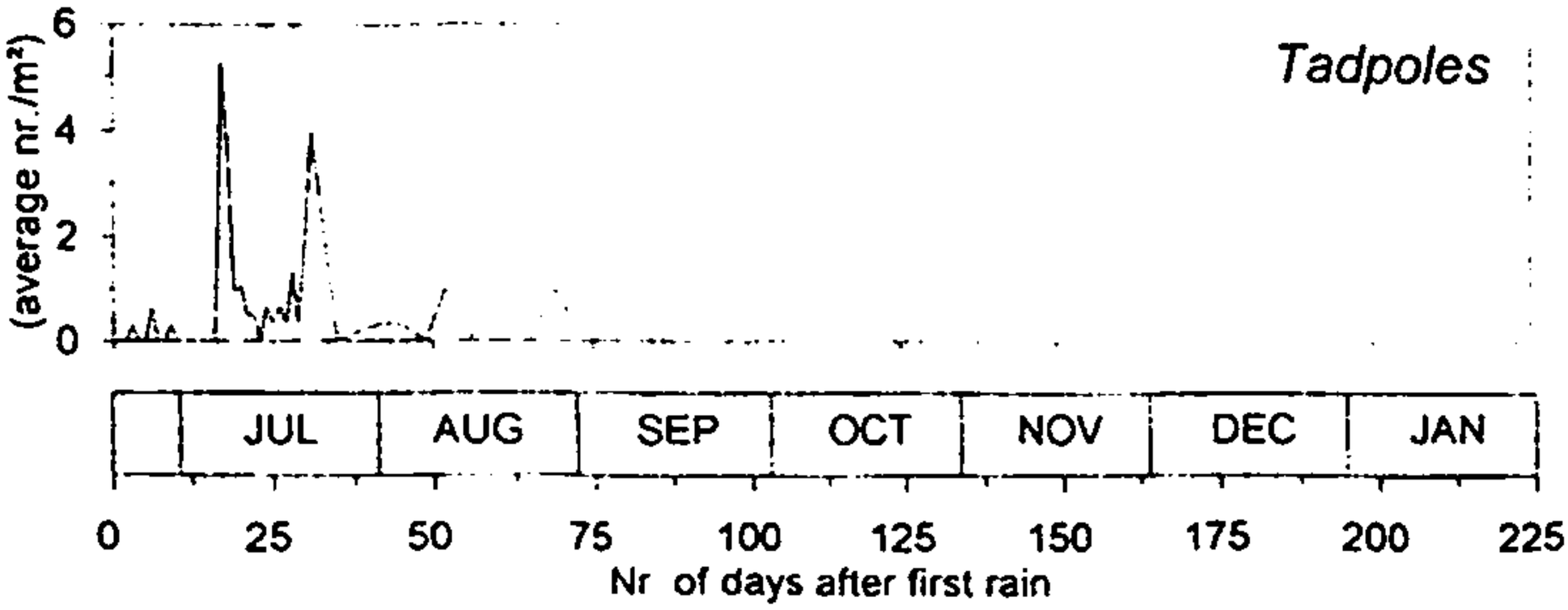


FIGURE N° 20



CHAPTER 2:

An ecological assessment of the hazard of insecticides used in desert locust control, to invertebrates in temporary ponds in the sahel

Joost Lahr

SUMMARY

Temporary ponds are important surface waters during the rainy season in dry regions such as the Sahel. Because such ponds are relatively small, they may easily be contaminated by downwind drift or accidental overspraying during insecticide applications against desert locust. The possible impact of several insecticides on these waters has been assessed in the past by experimental trials in natural ponds in Senegal. Invertebrate populations were affected in a species-specific manner, according to the insecticide used. Fairy shrimps (Branchiopoda, Anostraca), backswimmers (Hemiptera, Notonectidae) and cladocerans (Branchiopoda) represent the most important and most sensitive taxa to insecticides tested in the field. Populations of these three groups each recover after a fixed and characteristic time which can be linked to specific life-history traits. Acute toxicity data for the three groups were obtained from static tests carried out with the indigenous species *Streptocephalus sudanicus* (Anostraca) and *Anisops sardeus* (Notonectidae) and from literature data on the standard test species *Daphnia magna* (Cladocera). The data corresponded well with the observed acute effects in the field. A simple method for hazard ranking and risk analysis for temporary ponds, based on these species, was applied to antilocust insecticides. The method is based on operational application rates of the insecticides and their acute toxicity, but also on the characteristic population recovery speed for each group of species. Based on this method, the ranking in order of increasing adverse effects in the ponds was: fipronil < bendiocarb < fenitrothion < malathion < diflubenzuron < triflumuron < teflubenzuron < chlorpyrifos < deltamethrin < lambdacyhalothrin. Because similar groups of species have a much wider distribution than only Senegal and the Sahel, the data may also be useful for risk assessment in other regions with a comparable arid climate.

INTRODUCTION

In has been shown in various field experiments that the use of insecticides to combat Desert Locust *Schistocerca gregaria* may have side-effects on different non-target organisms in the Sahel (*e.g.* Everts, 1990; Everts & Ba, 1997). Insecticides are regularly evaluated with respect to their efficacy against desert locusts. The latest published evaluation (FAO, 1998) lists effective application rates for eleven insecticides, all in Ultra Low Volume (ULV) formulations, against hoppers or adults in barrier or blanket treatments. This evaluation also includes an environmental risk evaluation with respect to aquatic organisms, wildlife and beneficial arthropods. Efforts to establish a wider comprehensive framework for hazard and risk analysis of pesticide use in locust control are continuing.

Control operations against desert locust often take place in arid zones and recently it has been argued that risk assessment of chemicals for such areas should take their special physical and ecological features into account (SETAC 1996; Everts, 1997; Van der Valk, 1997). In this study an ecological method is described to classify the hazard and assess the risk of listed insecticides to one particular and characteristic aquatic habitat in dry regions: temporary freshwater ponds. These ponds, present during and shortly after the annual rainy season in the region, may be more at risk than larger water bodies because their small size makes them more prone to accidental spraying.

Hazard is defined here as the set of inherent properties of a chemical which makes it capable of causing adverse effects when a particular level of exposure occurs (Van Leeuwen & Hermens, 1995). The risk of insecticides is the probability of an adverse effect at a given exposure level (Van Leeuwen & Hermens, 1995).

APPROACH

The hazard ranking method proposed here is based on three parameters, viz. the application rates used for desert locust control, results of acute toxicity tests (LC_{50} or EC_{50} values), and characteristic recovery rates of important groups of invertebrates (indicators). The method and the assumptions necessary to establish the ranking will be discussed and supported with field- and laboratory data. The basic assumptions are:

1- initial environmental concentrations (IECs) in the water of the ponds are proportional to and can approximately be estimated from the application rate of the insecticides and water depths,

2- effects of the insecticides on invertebrate populations occur in a matter of hours or days and depend largely on the IEC; none of the compounds is persistent enough in pond water to cause substantial residual effects,

3- the results of acute toxicity tests with three species have an adequate predictive potential for the effects on the populations of indicator species in the field, and

4- there are three main groups of aquatic invertebrate indicator species for side-effects of insecticides in the ponds and each group has its own characteristic potential recovery time when populations are reduced or eradicated; since the insecticides are not persistent, population recovery depends on life-history traits of the species.

As a complement to the hazard ranking the risk to individual invertebrate taxa was established using the quotients of predicted initial environmental concentrations and LC_{50}- or EC_{50}-values.

BASIC DATA AND PREVIOUS EXPERIMENTS

The insecticides evaluated for the ranking are the organophosphates fenitrothion, malathion and chlorpyrifos, the carbamate bendiocarb, the synthetic pyrethroids deltamethrin and lambdacyhalothrin, the benzoyl urea compounds diflubenzuron, teflubenzuron and triflumuron, the phenyl pyrazole fipronil, and the fungus *Metarhizium anisopliae* var. *acridum*. The suggested application rates for these compounds are given by the FAO (1998) and can also be found in Table 6.

Four of these insecticides, fenitrothion, bendiocarb, deltamethrin and diflubenzuron, have previously been applied at the suggested rates to natural temporary ponds in Senegal during experimental trials to assess the impact on non-target aquatic invertebrates (Gadji B, 1993). Data from these studies will be used to verify some of the assumptions mentioned above.

Based on literature data, most synthetic insecticides evaluated here are generally considered degradable (half-life, DT_{50} = 2-15 days) or readily degradable (DT_{50}< 2 days) in the aquatic environment under natural circumstances (classification according to Van Rijn *et al.*, 1995). During the field experiments fenitrothion, diflubenzuron and deltamethrin disappeared in a matter of hours to days. Only bendiocarb disappeared more slowly from the ponds compared to the other three substances. Its average DT_{50} was approximately 17 days. The remaining seven compounds were not tested under Sahelian conditions. Under temperate climate conditions a DT_{50} value for malathion, determined in natural fresh water, was less than one week (Miles & Takashima, 1991). Chlorpyrifos also disappeared rapidly from artificial outdoor ditches (Van Wijngaarden *et al.*, 1996) and indoor model ecosystems (Brock *et al.*, 1992). Lambdacyhalothrin was moderately persistent (DT_{50} 20 days) in a laboratory water/ sludge system (Van Rijn *et al.*, 1995), but initial concentrations rapidly declined in pond mesocosms (Farmer *et al.*, 1995). Teflubenzuron was persistent in a laboratory water /sediment system (average DT_{50} 76 days), but was degraded rapidly in the presence of activated sludge (DT_{50} 6.5 days) (CTB, 1999). At present, no data on fipronil have been published in the public domain, but according to the manufacturer it is not persistent (K. Romijn, Rhône-Poulenc, pers. comm.). There is no data in the public domain on the persistence of triflumuron and the biopesticide *M. anisopliae* var. *acridum* in surface waters.

The trials in Senegal also revealed important information about groups of indicator species for side-effects of insecticides. Each of the four insecticides reduced populations of aquatic invertebrates, but the kind and number of affected groups was different for each compound. The most affected groups of species were fairy shrimps (Branchiopoda, Anostraca), backswimmers (Hemiptera, Notonectidae) and cladocerans (Branchiopoda). The three groups are very abundant, constitute a major part of the invertebrate biomass in the ponds and likely occupy important ecological niches. Because of their abundance, the groups could be easily monitored for the detection of side-effects. The effects on backswimmers were also indicative for most other pelagic aquatic insects. Whenever backswimmers were killed by insecticides, all other groups of insects were affected. The proposed method for hazard ranking and risk characterization of insecticides in temporary ponds is therefore based on the three groups mentioned.

Different groups of organisms in temporary ponds are in various ways adapted to survive dry periods when water is not present in the ponds. These adaptations clearly affect the recovery potential of the three major groups distinguished here. Table 1 shows recovery times for different species in each group observed in the field experiments (summarized from Table 4.3). When wiped out by insecticides, fairy shrimps (*Streptocephalus* spp.) do not recover until the next rainy season because these organisms produce drought-resistant resting eggs (or cysts) that presumably need at least one dry period to hatch. Backswimmers of the genus *Anisops* are very active dispersers. When ponds dry out they fly away to seek refuge in larger (permanent) waters until the next monsoon season. Because of their active flying behaviour, adults usually recolonize affected ponds in a matter of one to four weeks (Table 1). Like fairy shrimps, cladocerans do produce resting eggs (ephippia), but they are also capable of immediate reproduction through parthenogenesis. Not all ephippial resting eggs of Cladocera necessarily hatch immediately after the inundation of sediments. Certain clones may hatch later than others. Both possible mechanisms may explain why the three cladoceran species in Table 1, *Ceriodaphnia quadrangula* (Cladocera, Daphniidae), *Moina micrura* (Cladocera, Moinidae) and *Diaphanosoma senegal* (Cladocera, Sididae), usually recover during the same season, after 3-6 weeks. In general each of the indicator

groups seems to have a characteristic average recovery time that fluctuates around one value: >6-8 weeks (one year, until the next wet season) for fairy shrimps, approximately 2½ weeks for backswimmers, and an estimated 5 weeks for cladocerans.

Table 6.1 Time to full recovery of affected populations of invertebrates in natural temporary ponds in Senegal after treatments with several insecticides used in desert locust control . The average application rates of the treatments are given in Table 3.

Insecticide	Recovery time after treatment (weeks)						
	Streptocephalus spp.	*Anisops sardeus*	*Anisops debilis perplexus*	*Anisops varius*	*Ceriodaphnia quadrangula*	*Moina micrura*	*Diaphanosoma senegal*
Fenitrothion	n.s.e.	0.5	3	2-4[a]	n.a.	3.5	4
Bendiocarb	n.s.e.	n.s.e.	n.s.e.	n.s.e.	5.5	4	6
Deltamethrin	>8[b]	1	3.5	4	5.5	n.s.e.	n.s.e.
Diflubenzuron	>6[b]	n.s.e	n.s.e.	n.a.	6	3	6

n.s.e.= no significant effects; n.a. = not applicable because species was not present in ponds
[a] possible effect, and casualties found [b] effect lasts until ponds dry out, recovery in following rainy season, one year later

Acute toxicity data for two of the indicator groups were obtained from static tests with indigenous species in a field laboratory in Nioro du Rip, Senegal: *Anisops sardeus* (Hemiptera, Notonectidae) represented the backswimmers, and *Streptocephalus sudanicus* (Branchiopoda, Anostraca, Streptocephalidae) the fairy shrimps . These were usually the most numerous macroinvertebrate species in the study area around Nioro du Rip. No tests were conducted with indigenous cladocerans because the development of complex rearing and maintenance methods for these species was beyond the scope of the work. Instead, the geometric mean of available literature data on the standard test species *Daphnia magna* (Branchiopoda, Cladocera, Daphniidae) were used for the hazard ranking exercise. Because they were of the same order of magnitude LC_{50}- and EC_{50}-values for *D. magna* were lumped. The data are summarized in Table 2.

Table Acute toxicity of insecticides used in desert locust control to three species of aquatic invertebrates

Insecticide	*Anisops sardeus* LC_{50} (µg/L)	*Streptocephalus sudanicus* EC_{50} (µg/L)	*Daphnia magna*[a] $L(E)C_{50}$ (µg/L)	References *D. magna*
Fenitrothion	8.61	1,230	11	Sanders *et al.* (1983); LeBlanc (1984)
Chlorpyrifos	0.90	3.48	1.3	Barron & Woodburn (1995); Kersting & Van Wijngaarden (1992)
Malathion	42.2	67,750	1	Van Rijn *et al.* (1995)
Bendiocarb	373	41.0	74	Visser & Linders (1992)
Deltamethrin	0.012	0.018	0.8	Van Rijn *et al.* (1995)
Lambdacyhalothrin	0.025	0.028	0.35	Hill 1989; Mokry & Hoagland (1990); Van Rijn *et al.* (1995)
Diflubenzuron	1,937	0.74	8.0	Hansen & Garton 1982; Mayer & Ellersieck (1986)
Teflubenzuron	249	0.59	0.37	Cyanamid Agro B.V. (J. Anthonissen, pers. comm.); CTB (1999)

Triflumuron	189	0.21	225	Tomlin (1994)
Fipronil	9.06	9.94	190	Tomlin (1994)
Metarhizium anisopliae var. *acridum*	11,400	3,000	-	-

[a] geometric mean from values in the references, see Table 5.5

Table 3 Initial Environmental Concentrations (IECs) and Predicted Initial Environmental Concentrations (PIECs) of four insecticides experimentally applied to temporary ponds in Senegal; Gadji, 1993). PIECs were calculated assuming a uniform depth equal to the depth in the centre of the ponds ($PIEC_{min}$) or from a conical shape ($PIEC_{max}$).

Insecticide	Nominal application rate (g a.i. ha^{-1})	Pond (no. and name)	Actual application rate (g a.i. ha^{-1})	Depth in the centre (cm)	IEC ($\mu g\ l^{-1}$)	$PIEC_{min}$ ($\mu g\ l^{-1}$)	$PIEC_{max}$ ($\mu g\ l^{-1}$)
Fenitrothion	500	2.Koudote	356	40	34	89	178
		5.Daladiam	509	20	163	254	508
		6.Wimbody	490	39	27	126	252
		11.Mbambah	601	50	145	120	240
		15.Gandiang	575	28	31	205	410
		average:	**506**	**35**	**80**	**159**	**318**
Deltamethrin	15	2.Koudote	14.5	61	0.27	2.38	4.76
		9. Kourène	16.6	67	1.28	2.48	4.96
		12. Fana awa	15.1	46	0.01	3.28	6.56
		16. Debreye	16.2	44	0.26	3.68	7.36
		average:	**15.6**	**55**	**0.46**	**2.96**	**5.91**
Bendiocarb	100	3.Sadiowar	99	60	15	17	34
		5.Daladiam	100	61	13	16	32
		7.Palagne	89	68	45	13	26
		11.Mbambah	80	85	26	10	20
		15.Gandiang	102	36	21	28	56
		average:	**94**	**62**	**24**	**17**	**34**
Diflubenzuron	60	3.Sadiowar	69	45	18	15	30
		7.Palagne	104	70	18	15	30
		9.Kourène	60	47	7	13	26
		12.Fana awa	76	37	5	21	42
		16.Debreye	67	52	4	13	26
		average:	**75**	**50**	**10**	**15**	**31**

METHODS

Verification of approaches and assumptions

For each of the four compounds tested during the field experiments, Initial Environmental Concentrations or IECs (samples taken one hour after application) in pond water were determined using chromatographical analysis methods. These data are here compared to the predicted IECs (PIECs) which were estimated from the application rate and the measured depth in individual ponds at the time of the treatments assuming complete mixing (Table 3). The insecticides in the trials were directly applied to the water surface to simulate overspraying by aircraft. The assumed deposition was therefore 100%. Since the exact morphometry of the ponds was not known, a minimum and a maximum PIEC were estimated. The first, $PIEC_{min}$, was calculated assuming a uniform depth of the ponds equal to the maximum depth which was measured in the centre of the ponds. $PIEC_{max}$ was assessed from the maximum depth and a conical shape of the depth profile. Because most ponds had a bowl-like shape the actual IEC was supposed to be somewhere between these two values.*

To compare the acute toxicity derived with laboratory tests to population reductions in the field, the classification system for ratios between environmental concentrations and acute toxicity data proposed by Canton *et al.* (1991) was used (also see Van Rijn *et al.*, 1995). In this system, predicted or measured environmental concentrations are simply divided by the LC_{50}- or EC_{50}-values and the corresponding risk of acute effects is estimated from the quotient (>10 very large risk; 1-10 large risk; 0.1-1 risk present; 0.01-0.1 small risk; <0.01 negligible risk). This method was verified for the four insecticides studied in the field trials. The risk, derived from the IECs and LC_{50} or EC_{50} values, was compared to measured population effects on the most important species in each of the three indicator groups shown in Table 1.

Hazard ranking and risk characterization

Insecticides were ranked in order of increasing hazard to temporary pond inhabitants. Initially, only the four compounds previously tested in the field were ranked. This was done according to their effects on populations of species in the three indicator groups. Whenever a single species in one of the groups was affected by one of the insecticides in the field experiments, a certain number of points was allocated to the particular compound. It was shown above that recovery times of separate indicator groups are more or less constant and that these times are different for each indicator group. The principle underlying the allocation of the points is that an effect on a population is considered more serious when it lasts longer. Therefore the number of points represented by each affected indicator group was chosen proportionally to the characteristic recovery time: 3 points when fairy shrimps were affected (recovery time longer than 6-8 weeks), 2 points for one or more affected species of cladocerans (recovery in approximately 5 weeks), and 1 point for reductions of backswimmers (recovery after 2½ weeks on the average). The larger the number of total ranking points an insecticide received, the more serious was the combined impact on the indicator groups.

The final ranking method, that also included insecticides that had not been tested in the field, was based on results of acute laboratory toxicity tests instead of field trials. PIECs are directly proportional to the application rate (assumption 1). Therefore, the application rate can be used as a relative measure of exposure (*i.e.*, the ranking is the same for any given depth). A hazard ranking quotient (HRQ) was calculated for each compound by dividing the nominal application rate used in desert locust control by the LC_{50} or EC_{50} of each compound for the appropriate test species (data on toxicity in Table 2). Thus, different rankings of the insecticides were obtained for each of the three test species. Low rank numbers correspond to lower HRQs and hence to compounds that are less harmful to one group of species. An overall ranking was obtained through multiplication of these individual rank numbers with a weight factor for the corresponding indicator group which was equal to the number of points used for the ranking derived from the field experiments in the previous section (3 for *Streptocephalus* spp., 2 for Cladocera, and 1 for *Anisops* spp.). These values were then added for each insecticide. The resulting total number of ranking points was used to establish a final ranking. The lower this number, the less hazardous the

compound is on the whole, and the lower the overall rank number.

The classification only describes the relative hazard of insecticides when compared to each other, but does not reveal the actual threat the insecticides pose to each indicator group. For example, the ranking does not tell us if a hazardous insecticide is likely to cause effects on all three groups of species or on none of them. This risk is not only a function of the application rate which was used for the ranking, but also depends on the depth of the ponds concerned. During the field experiments the average depth of all treated ponds was approximately 59 cm. These treatments, however, were conducted halfway the annual rainy season, when water levels in the ponds are often at their highest. For a more general approach to the risk in temporary ponds, a lower average depth seems more realistic. It is proposed to use a uniform depth of 25 cm because this would be more in agreement with the average value that can be expected during one wet season. PIECs were calculated from the recommended application rates in desert locust control and this standard depth. These values were divided by the LC_{50} or EC_{50} values to obtain the PIEC/L(E)C_{50} ratio for each of the three test species. The corresponding risk was again derived with the classification of Canton *et al.* (1991).

RESULTS

Verification of approaches and assumptions with field data

The PIECs and IECs for the field trials were highly variable (Table3). Variability in PIECs is caused by differences in application rates and water depths between ponds. The even higher variability in IECs may be explained by horizontal and vertical variation in insecticide concentration in the water column which is usually largest during the first day after application (*e.g.* Van Wijngaarden *et al.*, 1996). The average IEC for bendiocarb was the only one between the minimum and maximum PIECs. For fenitrothion and diflubenzuron IECs were lower than the $PIEC_{min}$, but still of the same order of magnitude. The IECs for deltamethrin were much lower than predicted.

Table 4 Classification of the estimated risk of acute effects of insecticides used in desert locust control to three test species and comparison to effects observed on indicator groups during experimental trials in natural temporary ponds. The average application rates of the treatments are given in Table 3.

Insecticide	IEC/EC_{50} *Streptocephalus sudanicus*	risk to fairy shrimps	effects on *Streptocephalus* spp.[a] observed	IEC/LC_{50} *Anisops sardeus*	risk to backswimmers	effects on *Anisops sardeus* observed	effects on *Anisops debilis perplexus* observed	effects on *Anisops varius* observed	IEC/L(E)C_{50} *Daphnia magna*	risk to cladocerans	effects on *Ceriodaphnia quadrangula* observed	effects on *Moina micrura* observed	effects on *Diaphanosoma senegal* observed
Fenitrothion	0.07	small	no	9.3	large	yes	yes	yes	7.3	large	n.a.	yes	yes
Bendiocarb	0.59	present	no	0.06	small	no	no	no	0.32	present	yes	yes	yes
Deltamethrin	25.6	very large	yes	38.3	very large	yes	yes	yes	0.58	present	yes	no	no
Diflubenzuron	14.1	very large	yes	0.005	negligible	no	no	no	1.30	large	n.a.	yes	yes

n.a. = not applicable because species was not present in ponds

[a] *Streptocephalus spp.* contains a mixture of *S. sudanicus* and *S. zeltneri*

Table 4 shows the effects observed in the field in comparison to the risk that was expected based on the measured IECs and acute toxicity to the indicator species. When the estimated risk for a specific group was large or very large, significant reduction of populations in the field were observed. Similarly, when the estimated risk was small or negligible, no effects occurred. The intermediate 'risk present' category proved true for bendiocarb and cladocerans, but false for the same compound and fairy shrimps. For deltamethrin which poses a present risk to cladocerans, only one of the three abundant species (*C. quadrangula*) was affected in the pond experiments. It can be concluded that the classification system has a good predictive value for the cases shown here.

Table 5 Ranking of four insecticides tested in field experiments in temporary ponds in increasing order of effects on groups of indicator species. When one or more of the species in an indicator group were seriously reduced (see Table 1), the insecticide that caused this effect received the corresponding number of points for a particular indicator group. The points are proportional to characteristic recovery rates (Table 1).

Insecticide	Indicator groups			Total of field ranking points	Rank number
	fairy shrimps (3 points)	cladocerans (2 points)	beckswimmers (1 point)		
Fenitrothion	0	2	1	3	2
Bendiocarb	0	2	0	2	1
Deltamethrin	3	2	1	6	4
Diflubenzuron	3	2	0	5	3

Hazard ranking

The ranking of the four insecticides that was obtained for the field is shown in Table 5. The ranking in order of increasing adverse effects in the ponds was: bendiocarb < fenitrothion < diflubenzuron < deltamethrin.

Table 6 Overall laboratory ranking of insecticides used in Desert Locust control according to their relative hazard to groups of indicator species in temporary ponds in the Sahel. Low rank numbers correspond to low relative hazard. Weight factors are equal to the number of points used in Table 5.

Insecticide	Operational application rate	fairy shrimps weight factor = 3		cladocerans w.f. = 2		backswimmers w.f. = 1		Total of laboratory ranking points	Overall rank number
	(g a.i. ha^{-1})	HRQ	Rank nr.	HRQ	Rank nr.	HRQ	Rank nr.		
Fenitrothion	450	0.37	2	40.9	6	52.3	7	25	3
Chlorpyrifos	240	69.0	6	185	9	267	8	44	8
Malathion	925	0.01	1	925	10	21.9	6	29	4
Bendiocarb	100	2.44	4	1.35	3	0.27	4	22	2
Deltamethrin	12.5	694	9	15.6	5	1042	10	47	9
Lambdacyhalothrin	20	714	10	57.1	7	800	9	53	10
Diflubenzuron	60	81.1	7	7.50	4	0.03	1	30	5
Teflubenzuron	30	50.9	5	81.1	8	0.12	2	33	7
Triflumuron	37.5	179	8	0.17	2	0.20	3	31	6
Fipronil	6.25	0.63	3	0.03	1	0.69	5	16	1

The results of the final ranking based on laboratory toxicity tests is shown in Table 6. At their operational application rates, the two synthetic pyrethroids are the most hazardous compounds to fairy shrimps and backswimmers and somewhat less so to cladocerans to whom the organophosphorous compounds are relatively harmful. Diflubenzuron and triflumuron are also rather hazardous to fairy shrimps. On the whole, the pyrethroids pose the largest hazard to invertebrates in the ponds, followed by chlorpyrifos, teflubenzuron, triflumuron, diflubenzuron, malathion and fenitrothion. Bendiocarb and fipronil are the least hazardous. Because there is no data on its toxicity to *D. magna*, *M. anisopliae* var. *acridum* could not be included in the ranking.

Given that only part of the total applied quantity of deltamethrin was recovered from the water after treatments in the field (approximately 20%), it could be argued that the two pyrethroids are unjustly classified as the most hazardous. However, even when the HRQs for the two compounds would be 20% of those given in Table 6, they would still be ranked as the second and third most hazardous insecticides behind chlorpyriphos.

Risk for separate indicator groups

The results of risk analysis are shown in Table 7. It was found that even the least hazardous insecticides, fipronil and bendiocarb, may still present a danger to several of the indicator groups at desert locust application rates. Deltamethrin, lambdacyhalothrin and chlorpyrifos are likely to wipe out all groups of indicator species and all other compounds may seriously affect one or more groups. *M. anisopliae* var. *acridum* seems safe to fairy shrimp and to backswimmers.

Table 7: Predicted risk of insecticides used in desert locust control to individual groups of indicator organisms in temporary ponds receiving a full dose of treatments at recommended dose rates in 25 cm deep water.

Insecticide	fairy shrimps		cladocerans		backswimmers	
	$PIEC/EC_{50}$	risk	$PIEC/L(E)C_{50}$	risk	$PIEC/LC_{50}$	risk
Fenitrothion	0.146	present	16.3	very large	20.9	very large
Chlorpyrifos	27.5	very large	73.8	very large	107	very large
Malathion	0.006	negligible	370	very large	8.77	large
Bendiocarb	0.976	present	0.541	present	0.107	present
Deltamethrin	278	very large	6.25	large	417	very large
Lambdacyhalothrin	286	very large	22.9	very large	320	very large
Diflubenzuron	32.4	very large	3.00	large	0.012	small
Teflubenzuron	20.3	very large	31.4	very large	0.048	small
Triflumuron	71.4	very large	0.067	small	0.079	small
Fipronil	0.251	present	0.013	small	0.276	present
Metarhizium anisopliae var. *acridum*[a]	0.013	small	-	-	0.004	negligible

[a] application rate 100 g a.i./ha

DISCUSSION

The methods for hazard ranking and risk analysis that were used here allowed the prediction of the relative danger and possible effects of desert locust insecticides on the most important groups of invertebrates in temporary ponds by simple means. The field ranking that was obtained for the insecticides that were tested in natural temporary ponds (Table 5) was confirmed by the overall ranking based on laboratory data (Table 6).

From field results it was also shown that the necessary assumptions for the application of the methods were met on most occasions. Reasonable PIECs for fenitrothion, diflubenzuron and bendiocarb could be derived from the application rate and the depth of the ponds (assumption 1).. It was supposed that after the treatments most of the active ingredient of deltamethrin remained in a microlayer of the formulation on the surface of the water. This layer was possibly blown away by the wind. It can be argued, however, that under different circumstances, *i.e.*, no or less wind or strong wave action, deltamethrin concentrations may have been higher than those found and would have approached the predicted value. Furthermore, the classification scheme of Canton *et al.* (1991), when applied to IECs from field experiments, was found to have a good predictive potential for the effects that actually occurred in these trials (assumptions 2 and 3). Furthermore, it was also substantiated that most synthetic insecticides are likely to degrade rapidly in surface water (assumption 2). However, the persistence of teflubenzuron, triflumuron and fipronil should be studied into more detail, preferably in temporary ponds. Recovery times of different affected populations of indicator species could be linked to specific life-history traits and did not seem to depend on residual activity of insecticides tested in the field (assumption 4).

For a first screening, the methods that were used here may therefore be applied when data on novel desert locust insecticides become available, but for each new compound it should first be verified if the basic assumptions apply. Substances with deviant properties should be subjected to further scrutiny.

Future improvements of this simple model should include more accurate predictions of IECs. Important processes such as volatilization, dissolution and sorption are currently not included in the estimates. For instance, the PIEC/IEC ratios that can be derived from Table 3 show a trend of lower values for less soluble compounds. Sorption may also reduce the availability of insecticides to swimming invertebrates. However, despite adsorption to suspended matter in the ponds (deltamethrin and bendiocarb, this process had no dramatic consequences, *i.e.*, effects observed in field trials were in agreement with the predictions. The method may further be extended with data on other important characteristic organisms from temporary ponds such as diving beetles, copepods, ostracods, bacteria and water turtles. Some of the data presented here (deltamethrin) also support toxicity testing with more sensitive indigenous water fleas instead of using *D. magna* as the standard cladoceran.

A novelty of the ranking method is that the recovery potential of populations is explicitly taken into account. Until now standard hazard and risk estimation procedures are generally based on the comparison of Predicted Environmental Concentrations (PECs) with toxicity data such as No Effect Concentrations (NECs) or LC_{50}-values (see for instance Van Leeuwen & Hermens, 1995), *i.e.*, only on the possible (initial) effects on populations and not on the time these effects may last. Compared to the ranking that should have been obtained when differences between recovery rates of groups of indicator species are not taken into account (*i.e.*, when equal weight factors would have been applied), the current method classifies substances that are most toxic to the more slowly recovering crustaceans, such as the growth regulators diflubenzuron, teflubenzuron and triflumuron at a relatively higher rank (more hazardous).

Since similar species assemblages as those in the Sahel are found in temporary ponds all over the world (Williams, 1987), the results of this exercise may be applied to other dry regions as well. The ranking of insecticides according to their relative ecological hazard provides a tool for decision makers, campaign managers and applicators in these areas who are willing to reduce side-effects in temporary ponds through selection of appropriate compounds, but it can only be applied when there is a *de facto* possibility to choose between different insecticides during desert locust control campaigns. It should be realized that at present, often for reasons of limited availability of suitable insecticides for desert locust control in a given country or the high costs of alternative insecticides, such a choice cannot always be made.

Moreover, temporary ponds will not be the only ecosystems that need to be protected. Side-effects of desert locust insecticides have been reported on many other non-target organisms, both in the aquatic and terrestrial environment and inside and outside of the target zones themselves: birds, reptiles, livestock, fish etc. (see Everts, 1997, for an overview). Besides its efficacy against the target, the ideal insecticide for desert locust control should not be harmful to all these groups combined. It is unlikely that such a substance will be found. The risk of individual insecticides will undoubtedly vary from one group to another. Therefore the choice of the appropriate insecticide for a given desert locust control campaign should be based on the non-target organisms or ecosystems that do actually occur in the target zone and on the specific importance that these systems and biota represent for the area. The availability of ecological maps indicating vulnerable zones would contribute greatly to making such decisions.

The results of risk analysis showed that even the least hazardous among the eleven insecticides may still provoke side-effects in temporary ponds. Therefore, until less hazardous insecticides become available, the protection of these ecosystems should not be based exclusively on the use of compounds that are less hazardous in the classification presented here. Because temporary ponds do not present a target for locust control, their contamination should be avoided as well, for example through the application of buffer zones.

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CHAPTER 3:

Estimation of buffer zones for the protection of temporary ponds against ground-based insecticide applications in Desert Locust control

Joost Lahr, Baba Gadji & Demba Dia

SUMMARY

Temporary ponds are important surface waters during the rainy season in dry regions such as the Sahel. Because they are relatively small they may easily be contaminated by downwind drift or accidental spraying during insecticide applications against Desert Locust. Side-effects of operational applications against locusts could be reduced through the application of buffer zones.

Several experimental trials with two ground-based sprayers for ULV-insecticides, a hand-held Micro-Ulva® and a vehicle-mounted Ulva-Mast® X15 were carried out in order to establish the horizontal deposition of Sumithion® L50 (fenitrothion 500 g a.i./L ULV) and its deposition on water at different distances downwind. The treatments were carried out in flat fields without any vegetation. Deposition profiles for each sprayer were obtained for a single track and a multiple-track application. In addition, water from glass containers exposed to the multiple-track treatments was used for bioassays with *Anisops sardeus* (Hemiptera, Notonectidae) in the laboratory.

The buffer zone widths derived from the 48h-LC_{10} in the bioassays and from the deposition profiles were 74m for the Micro-Ulva and 114m for the Ulva-mast at wind speeds of 3.7 and 2.6 m/s respectively. These values were in reasonable agreement with those estimated from the single-track data and routine toxicity tests with *A. sardeus*.

The deposition profiles from the multiple-track applications were subsequently used to estimate buffer zones for eight insecticides currently used in Desert Locust control. The profiles were combined with the lowest LC_{10} or EC_{10} value for each compound calculated from the results of previous acute toxicity tests in the laboratory with three sensitive indicator species: *A. sardeus*, *Streptocephalus sudanicus* (Branchiopoda, Anostraca) or *Daphnia magna* (Branchiopoda, Cladocera). Buffer zones ranged from 0 to 155m for the Micro-Ulva sprayer and from 0 to 382m for the Ulva-mast for similar wind speeds as in the experimental trials.

It is argued that at moderate wind speeds (2-4 m/s) a buffer zone width of 200m for the Micro-Ulva and 400m for the Ulva-Mast will usually be sufficient for each of the eight insecticides.

INTRODUCTION

In has been shown in various field experiments that the use of insecticides to combat the Desert Locust *Schistocerca gregaria* may may have considerable negative effects on non-target aquatic organisms in the Sahel when surface waters in this region are contaminated. Because of their small size, temporary ponds, which are present during and shortly after the annual rainy season in the area, may be more at risk than larger water bodies because they can not easily be spotted by applicators. Moreover, the use of drift spraying techniques in locust control, in which concentrated, Ultra Low Volume (ULV) formulations of insecticides are applied as a mist of very small droplets using rotary atomizers increases this risk even more because the compounds can be transported by wind over long, downwind distances.

In the past, experimental studies with four acridicides in natural temporary ponds in central Senegal have shown that, at application rates used in Desert Locust control, in particular non-target aquatic invertebrates are impacted (Lahr and Diallo 1993; Lahr *et al.* 1995). Lahr *et al.* (1996) developed static acute toxicity tests with two of the most abundant macroinvertebrates in Senegalese temporary ponds: *Anisops sardeus* (Hemiptera, Notonectidae), a backswimmer, and *Streptocephalus sudanicus* (Streptocephalidae), a fairy shrimp. All eight insecticides mentioned above were screened with these tests.

Contamination of non-target areas and side-effects from downwind drift are well known for a large variety of pesticide uses: for instance in agriculture (Bird *et al.* 1996; Frank *et al.* 1991; Riley and Wiesner 1989; Shires and Bennett 1985), in orchards (Hall *et al.* 1996), in forestry (Ernst *et al.* 1991a, 1991b; Hatakeyama *et al.* 1990; Payne *et al.* 1991; Payne and Thompson 1992; Payne 1994) and in mosquito control (Tietze *et al.* 1994). Lahr and Banister reported mortality of fish and aquatic invertebrates from insecticide drift following the application of insecticides against Desert Locusts near the River Senegal.

The risk to surface waters of side-effects of insecticides used in locust control can be reduced in two ways. First, it may be diminished by using insecticides that are less harmful to temporary ponds. A hazard ranking for this purpose was established by Lahr (1997). An other way to reduce side-effects in temporary ponds may be to avoid contamination of ponds, either by not applying any insecticides in areas where these ponds are present or by respecting safe buffer zones when treatments take place.

Buffer zones are upwind distances from sensitive areas needed to protect these from hazardous levels of downwind drift from pesticide applications. They receive increasing attention as tools to avoid side-effects from agricultural and silvicultural practices (*e.g.* Kingsbury and Trial 1987; Muscutt *et al.* 1993). Pesticide deposition and therefore buffer zones may vary according to the formulation of the insecticide, its application rate, meteorological conditions (wind speed, turbulence, temperature), type of sprayer used, and the ecotoxicological treshold that is applied (*i.e.* the toxicity of a compound to non-target organisms).

This report deals with a method to establish buffer zones for temporary ponds in the Sahel for ground-based applications and it gives a first estimation of these widths for the eight compounds mentioned. The experimental approach was loosely based on similar experiments in the past with aquatic animals (Ernst *et al.* 1991; Helson *et al.* 1993; Payne *et al.* 1988), with honeybees and butterflies (Çilgi and Jepson 1995; Davis and Williams 1990; Davis *et al.* 1991), and with plants (Marrs *et al.* 1993). Downwind profiles of pesticide deposition are measured using various techniques. These data are combined with results from *in situ* bioassays and/or laboratory toxicity tests with sensitive species. We applied this approach to experimental treatments with two ground-based rotary atomizers, the hand-held Micron-Ulva® and a vehicle mounted Ulva-Mast® X15. The first sprayer is used by local village control brigades in the Sahel, the second by governmental and international control organizations. Fenitrothion, one of the most widely used compounds in Desert Locust control, was used as a model insecticide and *Anisops sardeus* (Hemiptera, Notonectidae), a typical inhabitant of temporary ponds in the Sahel, as a model organism in bioassays.

METHODS AND MATERIALS

For each sprayer two applications were carried out. During a preliminary experiment the horizontal deposition from a single track was measured as a function of downwind distance. These data were used to establish the appropriate distances for sampling sites and bioassays in the final experiments in which five spaced tracks were sprayed. The results of the preliminary applications were also applied to predict those of the final treatments.

Experimental sites

The choice of the sites was based on a worst case scenario, *i.e.* they consisted of bare, flat fields with no vegetation that would reduce downwind drift.

All experiments were conducted in Senegal, West Africa. The preliminary treatment with the Micro-Ulva was carried out in an uncultivated field on a farm near Sebikotane (14°45N, 17°10W), not far from the capital Dakar. The field had been recently harvested and was slightly tilled before the treatment. The final treatment took place near Keur Lougé, 3 km east of Nioro du Rip (13°46N, 15°46W,) in a peanut field that had been harvested the days before.

Both the preliminary and the final treatment with the Ulva-Mast were carried out in a harvested peanut field near Keur Bidji Awa (13°43N, 15°52W), approximately 10 km to the east of Nioro du Rip.

Insecticide and sprayers

The insecticide used in the field experiments was Sumithion® L50 (ULV, 500 g a.i./L fenitrothion), (batch nr. 4025C ; February 1996), manufactured by Sumitomo Chemical Company Ltd. In Osaka, Japan.

The hand-held Micronair Micro-Ulva® rotary atomizer was charged with five 1.5V monocel LR20 batteries. The red nozzle was used for both treatments. The spinning disk was held at a height of approximately 1.5m during the treatments. The insecticide was transferred from the barrel to 1L plastic bottles which can be connected to the atomizer head.

The Micronair Ulva-Mast® X-15 stacked spinning-disc atomizer was mounted on a four wheel drive Toyota pick-up truck with the atomizer at a height of 2.7 m. Power was supplied from the 12V battery of the vehicle and the insecticide was pumped to the atomizer head from the sprayer's 250L tank.

The flow rates of the insecticide were calibrated prior to each treatment with the spinning-discs turned off. Rotation speed of the atomizers was measured without insecticide using a tachometer.

Experimental design and treatments

The sprayers were calibrated for fenitrothion treatments at the application rate recommended in Desert Locust control, 450 g.a.i./ha (FAO 1996), and the track spacings used. In order to reduce large variations in deposition, instead of applying a single dose each track was sprayed five consecutive times. All deposition data were later corrected for applications at single dose rates by dividing them by five (section 2.5). Track spacings during the final treatments were 10m for the Micro-Ulva and 30m for the Ulva-Mast. All treatments were carried out in the morning to avoid high temperatures during application.

As is usual for drift spraying, spraying tracks during the field trials were perpendicular to the direction of the wind. Downwind sampling stations for deposition measurements and bioassays were placed in straight lines parallel to the wind, downwards from the centre of the first or single track. The position of these stations in each experiment is given below. Two series of replicate stations were used. The spacings between these lines were 10 and 20m for the Micron-Ulva and Ulva-Mast respectively The length of the treated tracks was 100m for the Micron-Ulva treatments and 200m for the Ulva-Mast.. Only the average values for measurements of replicate stations will be presented in the results.

Meteorological measurements

All meteorological measurements were carried out next to the treated tracks at heights between 1.5 and 2m. Wind speed was measured five times during each single sprayed track with a portable hand-held anemometer. For each treatment the average of these measurements was calculated. The direction of the wind was established with a weather vane mounted on a tripod. Air temperatures at the beginning and the end of the treatments were measured with a rotating thermometer. The relative humidity was derived from the difference between the dry and wet temperature.

Sampling of deposition

Each sampling station consisted of a 2 x 2 m sheet of transparent plastic to mimic a water surface (Payne *et al.* 1988) which was attached to the ground with tent-pegs.

Horizontal deposition of droplets was established using a single 8 x 5 cm oil-sensible paper (manufacturer Ciba-Geigy) that was placed in a holder near the centre of each sheet. After treatment, the number of droplets in five separate 1cm² squares on each card was counted under a binocularly microscope at between 7.5 and 64x magnifications. The average number of droplets per square centimetre was calculated from the five counted squares on each paper and the two replicate stations. These numbers were divided by five to correct for the overdosing used.

Horizontal deposition of the active ingredient was assessed using 10x10cm bare glass plates. Four of these were placed at each sampling station at approximately 50cm from the centre of the sheets. After analysis (section 2.6) the average deposition (of the replicate stations) was calculated, divided by five and expressed in µg a.i./cm².

During the multi-track treatments the sampling stations also contained 20L rectangular glass containers (HxLxW = 26x36x23cm) which were placed in a hole in the centre of each sheet with only 1cm of the vessel above the level of the soil. The containers were filled with *Anisops* test water (Lahr *et al.* 1996) to 1cm below the ridge (20L). After treatment the water was gently stirred with a stainless steel baton with a teflon knob in order to distribute deposited insecticide evenly through the containers. The water was then used for residue analysis and bioassays.

Chemical analyses

Horizontal deposition of the active ingredient

The four glass plates recovered from each sampling station were rinsed into a single 500 mL glass flask using four times 20 mL of a 1:3 v/v acetone/hexane mixture and a glass funnel (diameter 15cm). The flasks were closed with a teflon cap and immediately transported to the field laboratory. All samples were prepared for analysis on the day of the trials. In the laboratory another 20 mL of the solvent mixture was added to the 80 mL sample. The solution was then transferred to a 100 mL bulb and evaporated dry in a rotary evaporator with a water bath adjusted to 40°C. The residue was then dissolved in 5 mL hexane in an ultrasonic bath.

Water samples

After the multi-track applications at each sampling station a glass flask of 500 mL was carefully filled with water from the glass containers using a 200 mL glass beaker. After immediate transportation to the laboratory the samples were extracted by means of Solid Phase Extraction (SPE). Prior to extraction SPE cartridges (C8 filling, 3mL, 500mg) were conditioned by consecutively passing 5mL methanol and 5mL of distilled water. The samples were little by little transferred into the reservoir mounted on the cartridges. The flow rate was 10 mL/min. The absorbed insecticide was released into a 100 mL evaporator bulb by passing 100 mL of a 1:1 v/v acetone/hexane mixture through each cartridge. The extract was then evaporated dry similarly to the extracts from the glass plates and dissolved in 5 mL hexane.

GLC analysis

The hexane extracts of fenitrothion were injected in a Gas Liquid Chromatograph (GLC) equipped with an Electron Capture Detector (ECD).

Injector:	temperature 240°C
Column:	OV101 5% in glass, length 2m, temperature 235°C
EC	I_R 0.09 nA, $I_{min.}$ 0.23 nA, $I_{max.}$ 2.43 nA, slope 100-400, temperature 260°C
Carrier:	nitrogen (N_2), flow rate 24 mL/min.
Retention time:	±4.2 min.
Detection limit:	0.02 µg a.i./mL in extract, 0.2 µg/mL in original water sample

Recovery of the compound from spiked samples in tap water varied between 95 and 105%.

Bioassays

Water samples from the glass jars at the sampling stations were used for bioassays with *Anisops sardeus* in the LOCUSTOX laboratory at the field base of the DPV in Nioro du Rip. Samples were taken in the field by carefully transferring the water from the containers into 0.5L dark glass bottles using a 200mL glass beaker. Bottles were closed with a cap with a teflon coating and immediately transported to the laboratory.

Female *A. sardeus* were captured in temporary ponds in the vicinity of Nioro du Rip. For each bioassay ten individuals were put in a 3L glass beaker containing 1.6L of test water. To this, 0.4L water from the sampling stations was added. This dilution therefore resulted in exposure of the insects to concentrations from similar treatments without five times overdosing. The bioassays were further conducted according to the protocol described by Lahr *et al.* (1996) and lasted 48h. The concentrations of fenitrothion in the vessels were calculated from the chemical analyses.

Statistical analyses

To establish the relation between horizontal deposition from the final two treatments and distance, data for droplet deposition, deposition of the active ingredient (a.i.) on glass plates or concentrations of the insecticide in the water of the containers were transformed using natural logarithms. Least-squares linear regression was applied to these data pairs (deposition or concentration vs. distance) using the module available in the Quattro Pro spreadsheet programme v.6.0 for Windows (Novell Applications Group, Orem, Utah). Significance of the correlation coefficients R was tested using a one-sided test according to Steel and Torrie (1980) at $\alpha = 0.05$.

Relationships between droplet deposition, deposition of the a.i. and concentrations in water were established without transformation using the same linear regression method. The constant C was set at zero.

LC_{10} or EC_{10} values were calculated with the survival module of the DEBtox programme (Kooijman and Bedaux 1996). LC_{50} or EC_{50} were calculated with the RIZA-programme based on the method by Kooijman (1981).

Calculation and estimation of buffer zones

We agreed with Payne *et al.* (1988) to use the LC_{10} for sensitive species as the ecotoxicological treshold for the calculation of buffer zone widths. This means that 10% reductions of invertebrate populations in the field are accepted.

Buffer zones from the final multiple track field experiments were calculated using the 48h-LC_{10} for *A. sardeus* derived from the mortality in the bioassays and the fenitrothion concentrations measured in the glass containers. The corresponding downwind distance for this LC_{10} was calculated from the relationship between the water concentrations and the downwind distance established with ln-linear regression.

Buffer zones for the final multiple track tests were also predicted using the results from the preliminary single track experiments. Downwind water concentrations for the five-track treatments were estimated by superposition of the droplet deposition profiles for five single tracks. The estimated downwind profile of water concentrations for 25cm depth was established using the relationship between droplet numbers and water concentrations from the final tests with each sprayer (these relationships showed the highest correlation in the multiple track experiments). Buffer zones were calculated using the average 48h-LC_{10} value for *A. sardeus* and fenitrothion derived from previous laboratory tests by Lahr *et al.* (1996) and Marquenie *et al.* (1997).

Finally, buffer zones for the eight insecticides mentioned were estimated using the water concentration profiles of the two final tests divided by the application rate for fenitrothion. With the recommended application rates for other compound this profile could be translated into the water concentrations for each insecticide at similar distances and under the same circumstances. 48h-LC_{10} values for the most sensitive invertebrate indicator species for temporary ponds were used to estimate the widths. These values were either calculated from the original test data by Lahr *et al.* (1996) (for *A. sardeus* and *S. sudanicus*) or estimated from the LC_{50} or EC_{50} found in the literature (for *D. magna*).

RESULTS

Treatments

The treatment characteristics and the position of sampling stations for the four experiments are shown in Table 1. Preliminary and final treatments with each sprayer were carried out under comparable circumstances except for relative air humidity and wind speed in the case of the Micron-Ulva treatments. Since ULV-formulations are little volatile, the difference in air humidity is thought to matter little for deposition of the active ingredient. The difference in wind speed between the two treatments with this sprayer obviously had consequences for the deposition profiles.

Micro-Ulva

Preliminary single track treatment

The results of this single track experiment are shown in Fig. 1. Deposition of droplets and the active ingredient was highest at 10m downwind of the track and declined rapidly with increasing distance (Figs. 1a and 1b). There was a positive and significant correlation between the deposition of the droplets and the active ingredient (Fig. 1c). R^2 was 0.93. The estimated deposition of a treatment under similar conditions (wind speed 2.8 m/s) using five tracks with 10m spacing is shown in Figs. 1d and 1e. The cumulative effect of the separate tracks results in a good cover of the treated area.

Final multiple-track treatment

The final treatment was conducted at a higher wind speed than the preliminary treatment (3.7 m/s compared to 2.8 m/s). The deposition was therefore further than predicted from the superposition of the single track results. Deposition could still be measured at 100m. The negative correlation between the ln-transformed deposition results or water concentrations and the downwind distance from the treatment was very significant (Figs 2a-c). The best correlation was obtained for the deposition of droplets (R^2= 0.97) and the water concentration in the containers (R^2= 0.93). The regression line for the deposition of the active ingredient against the distance underestimated the deposition at short downwind distances (Fig. 2b). Relationships between droplet deposition, deposition of the active ingredient and the water concentrations were also highly significant (Fig. 2d-f). The best correlation between the water concentrations and horizontal deposition was obtained for the droplets (R^2= 0.98).

Mortality in the bioassays decreased with the downwind distance. After 24h this relationship was somewhat irregular, but after 48h a smoother curve was obtained (Fig. 2g).

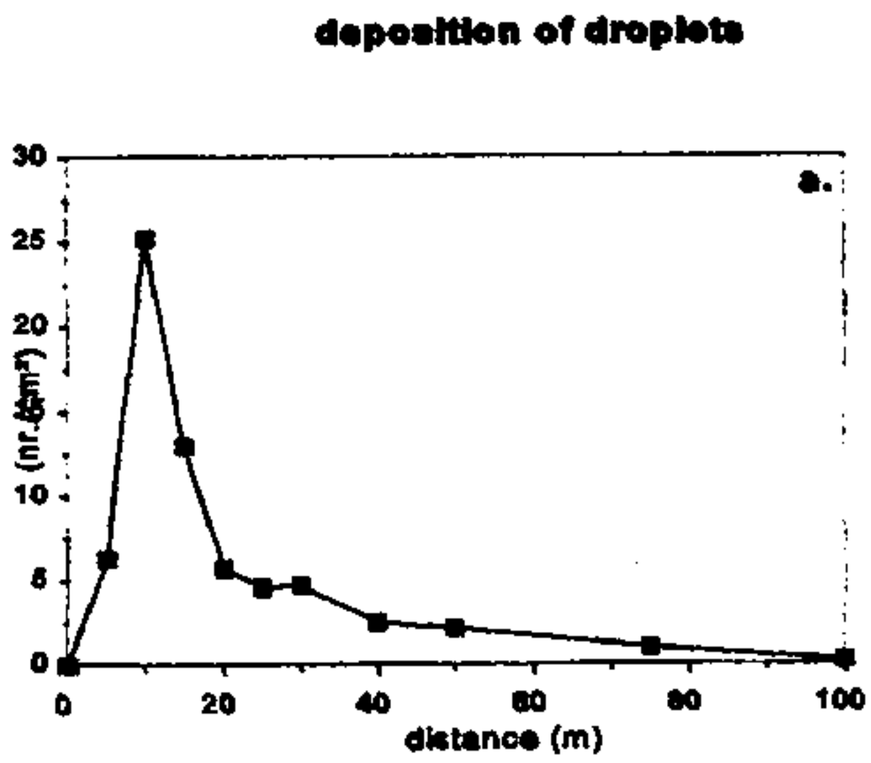
Table 1: Treatment characteristics of preliminary (single track) and final (multiple, spaced tracks) experiments with a ULV-formulation of fenitrothion using a hand-held Micron-Ulva or a vehicle-mounted Ulva-Mast sprayer. The nominal application rate was 450 g a.i./ha as recommended for Desert locust control.

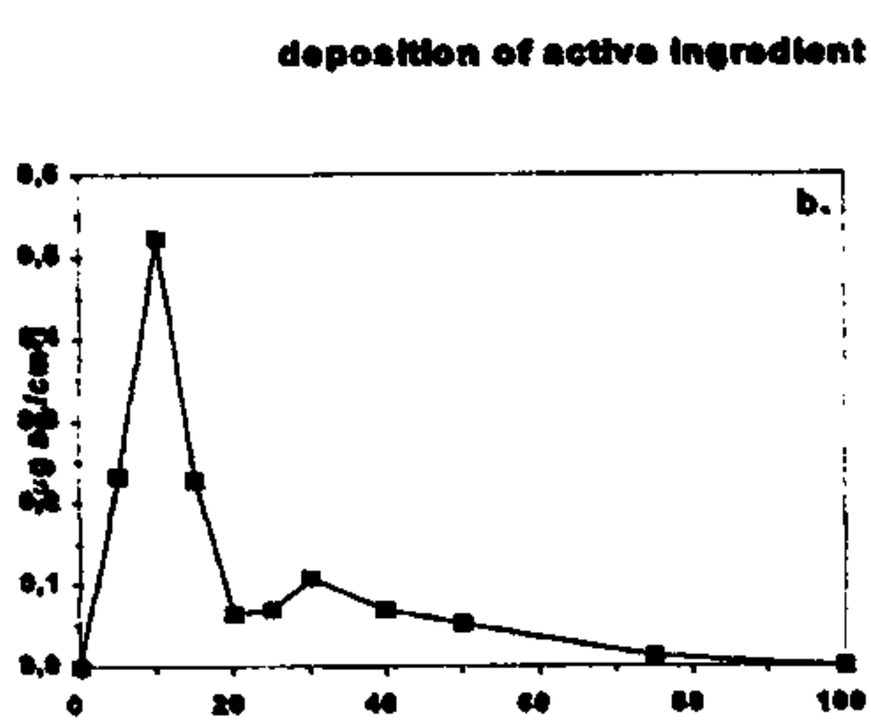
	Micron-Ulva		Ulva-Mast	
	Preliminary	Final	Preliminary	Final
Experimental site	Ranch Filifili	Keur Lougé	Keur Bidji Awa	Keur Bidji Awa
Date	5/10/96	15/11'96	21/11/96	22/11/96
Time (start-end)	9.29-9.48	10.20-11.07	9.13-9.20	9.46-10.22
Calibrated flow rate (mL/min)	77	80	613	600
Rotation speed of disc (rpm)	9 000	8 500	5 500	5 500
Average speed of sprayer (m/min)	78	74	205	198
Number of tracks	5x1	5x5	5x1	5x5
Track length (m)	100	100	200	200
Track spacing (m)	-	10	-	30
Position of downwind sampling stations (m)	5 10 15 20 25 30 40 50 75 100	12½ 25 37½ 50 62½ 75 87½ 100	10 20 40 60 80 100 130 160 200 240	25 50 75 100 125 150 175 200
Volume of insecticide applied (mL)	440	2 335	2 900	13 000
Strength of the line source/5 (mL/m)	0.88	0.93	2.9	2.6
Application rate/5 (g a.i./ha)	-	467	-	433
Wind direction	N-NNW	NE	NE-ENE	NNE-NE
Wind speed (min.-max.) (m/s)	2.0-3.5	1.5-5.0	1.5-3.5	1.0-4.5
Average wind speed ± s.e (m/s)	2.8±0.5	3.7±0.8	2.4±0.6	2.6±0.8
Air temperature (start-end) (°C)	30.0-30.5	29.0-30.0	29.5-29.5	30.5-30.0
Relative air humidity (start-end) (%)	79-76	36-35	29-32	26-30

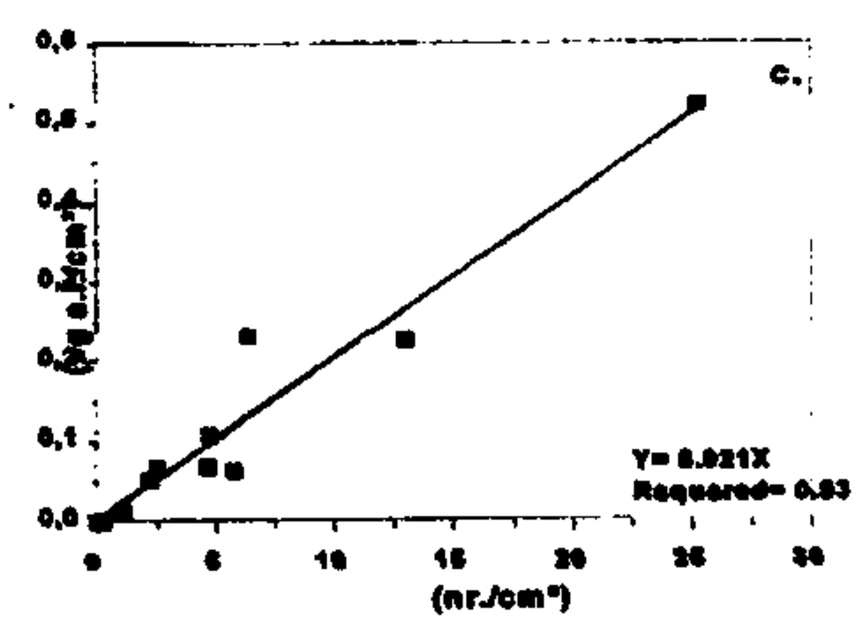
Ulva-Mast

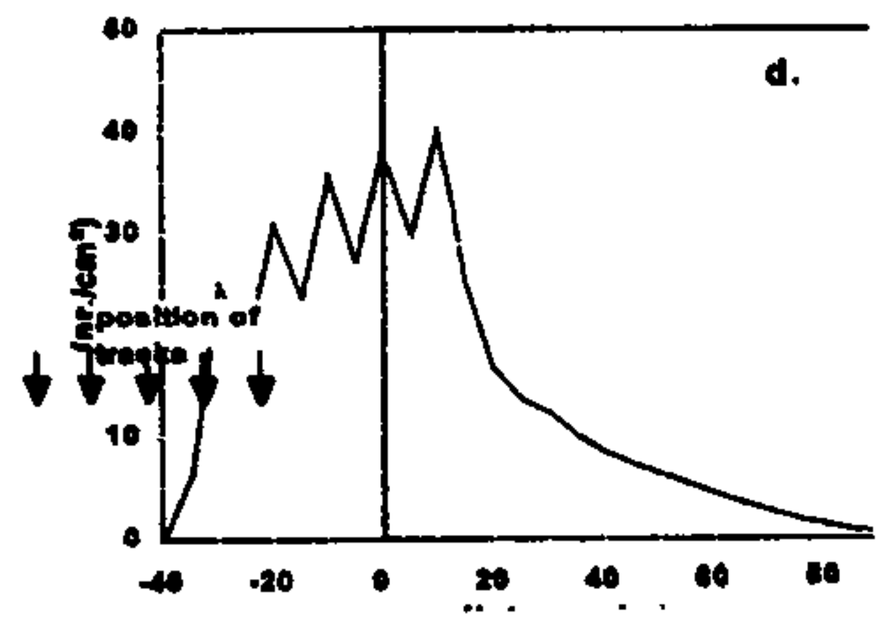
Preliminary single track treatment

The deposition from the single track Ulva-Mast treatment showed high deposition levels at short downwind distances and low levels further away (Figs. 3a,b). Most of the insecticide was found at 10m downwind, right next to the spraying track. The correlation between the horizontal deposition of the active ingredient and that of droplets was also highly significant for this sprayer (Fig. 3c) (R^2= 0.98). The deposition pattern that was derived from the superposition of five single tracks however, showed that the resulting pesticide cover would be very irregular as a consequence of the high level of insecticide that deposits at short distances (Fig. 3d and 3e).









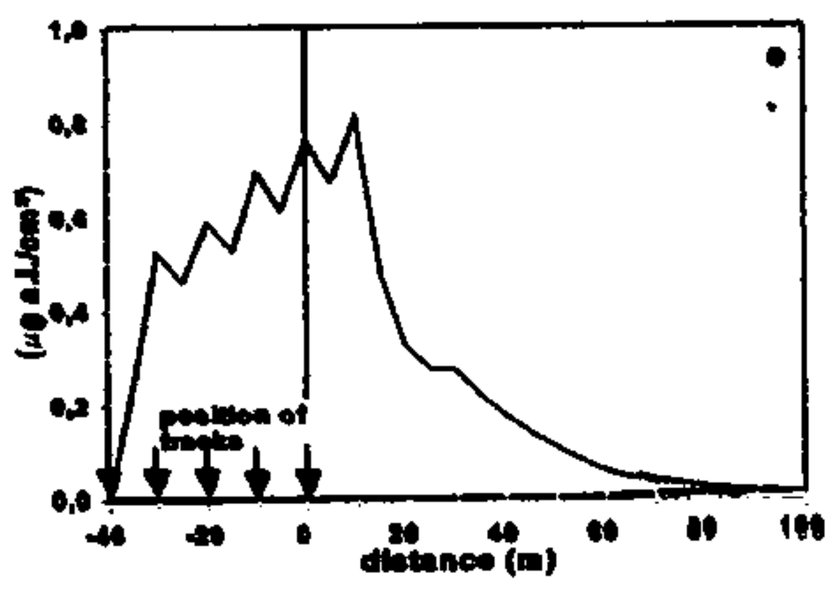
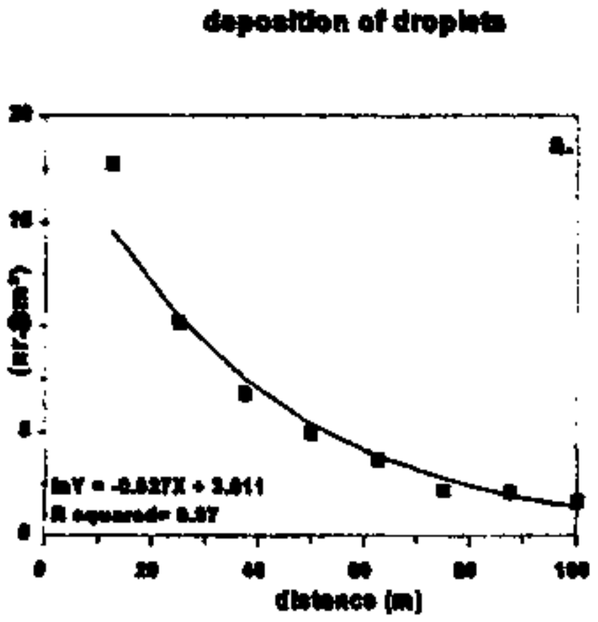
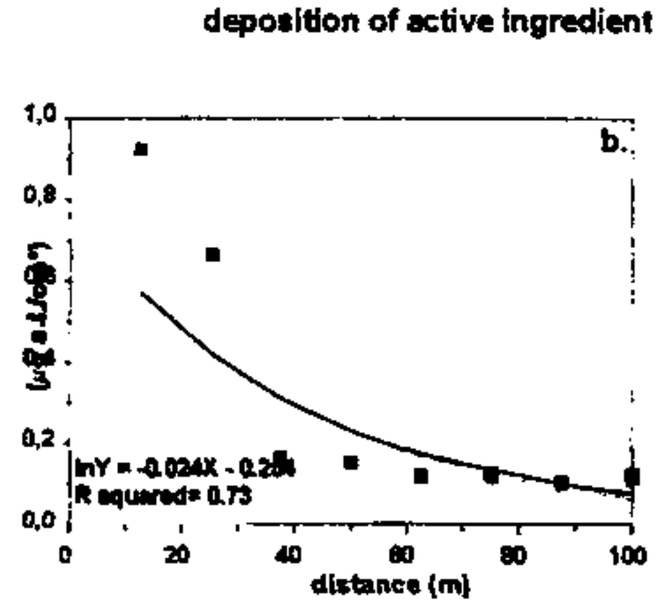
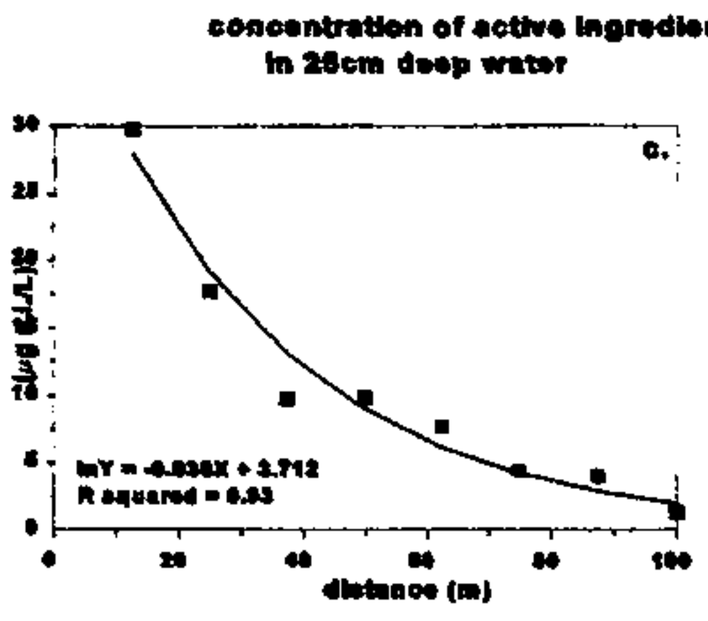
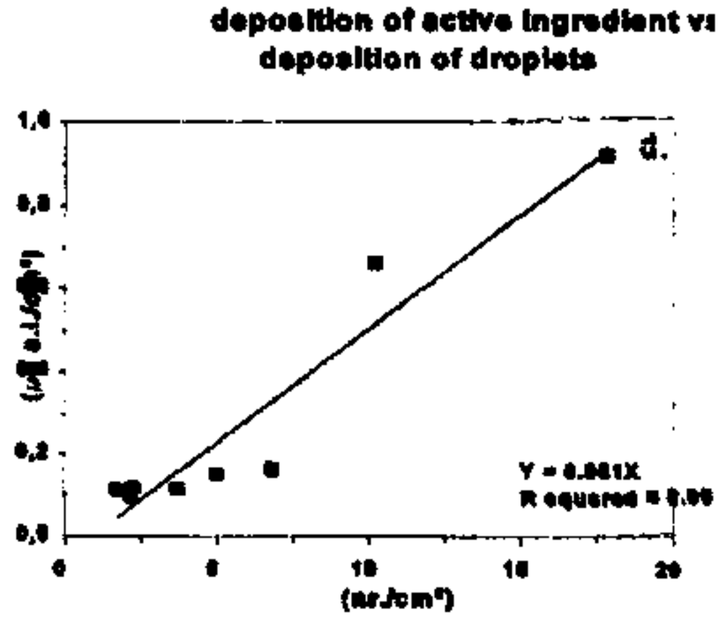


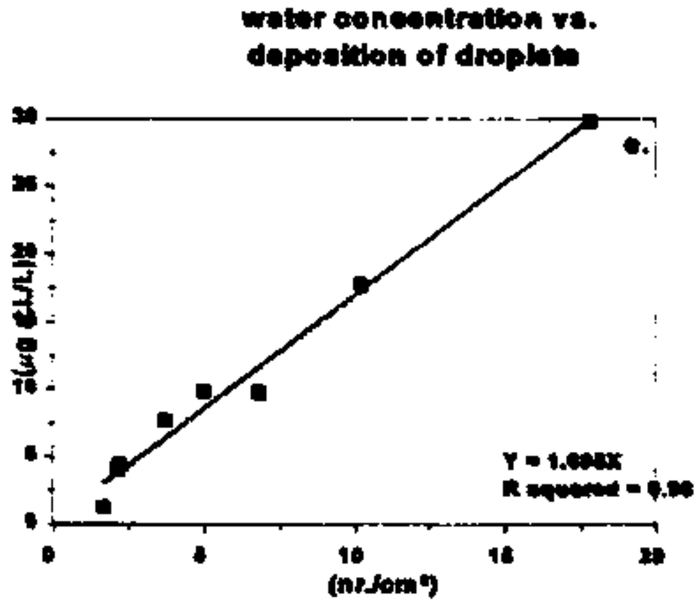
Fig. 1: Results from a single track application of Sumithion® L50 (500 g a.i./L ULV) with a hand-held Micron-Ulva® sprayer at a nominal application rate of 450 g a.i./ha: (a) horizontal droplet deposits on oil-sensible papers at downwind sampling sites, (b) deposition of active ingredient on horizontal glass plates, (c)relationship between deposits of droplets and deposition of active ingredient, (d) predicted droplet deposits from a treatment with five 10m spaced tracks, (e) predicted deposition of active ingredient from a treatment with five 10m spaced tracks. Wind speed 2.8±0.5 m/s.

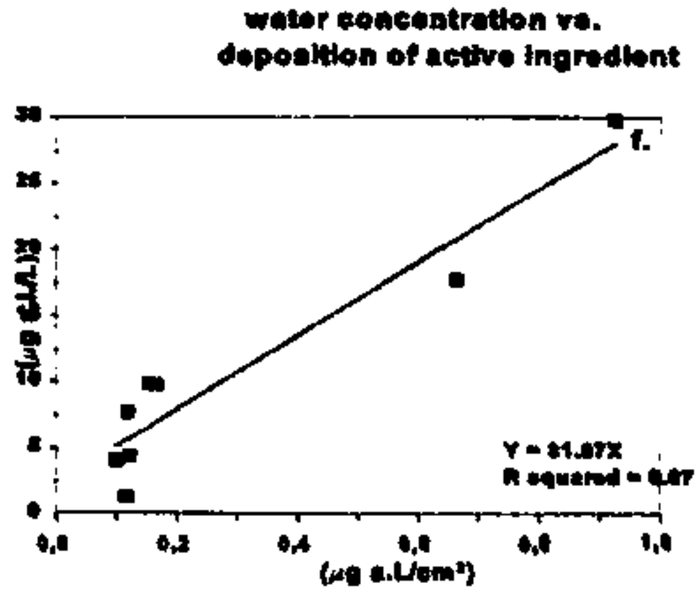












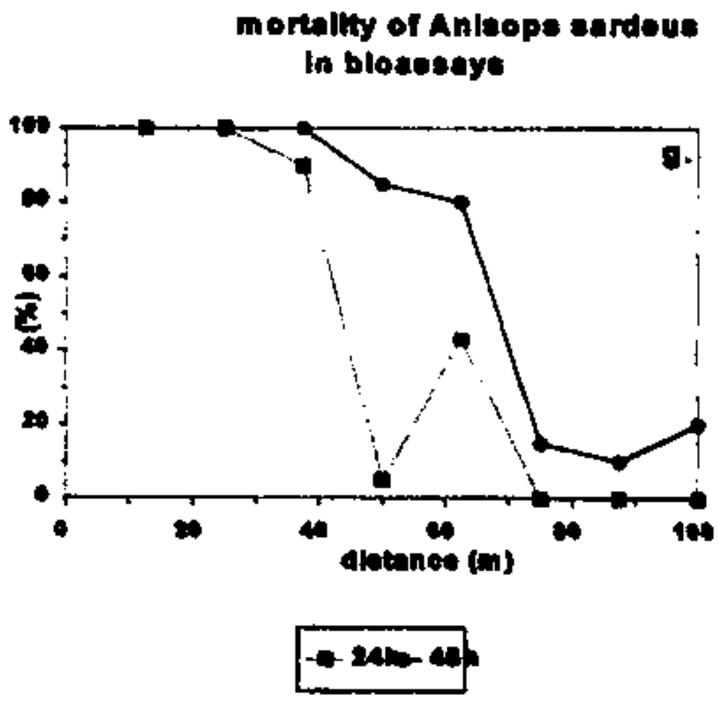
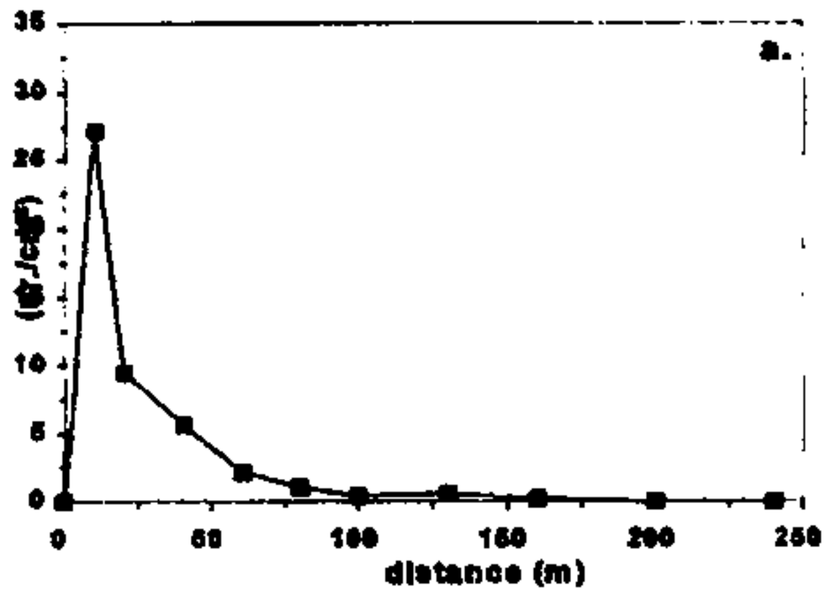
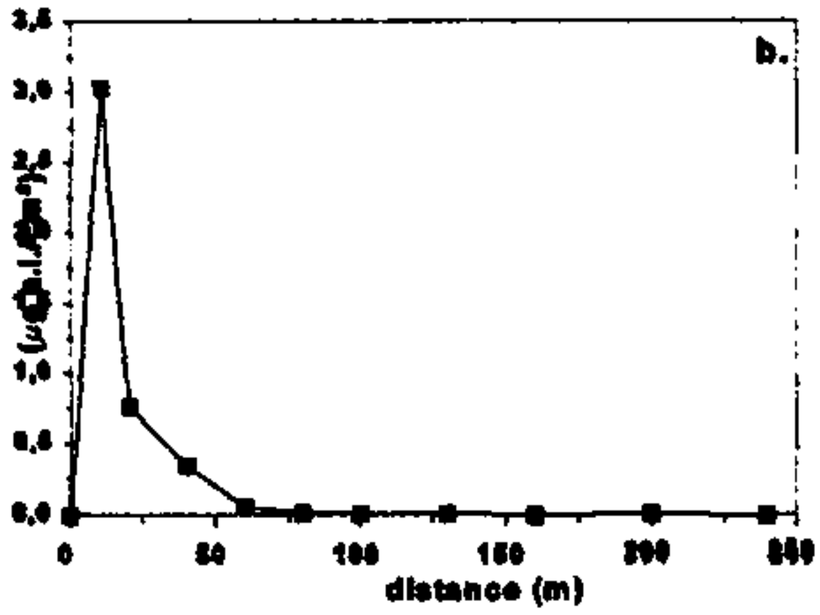
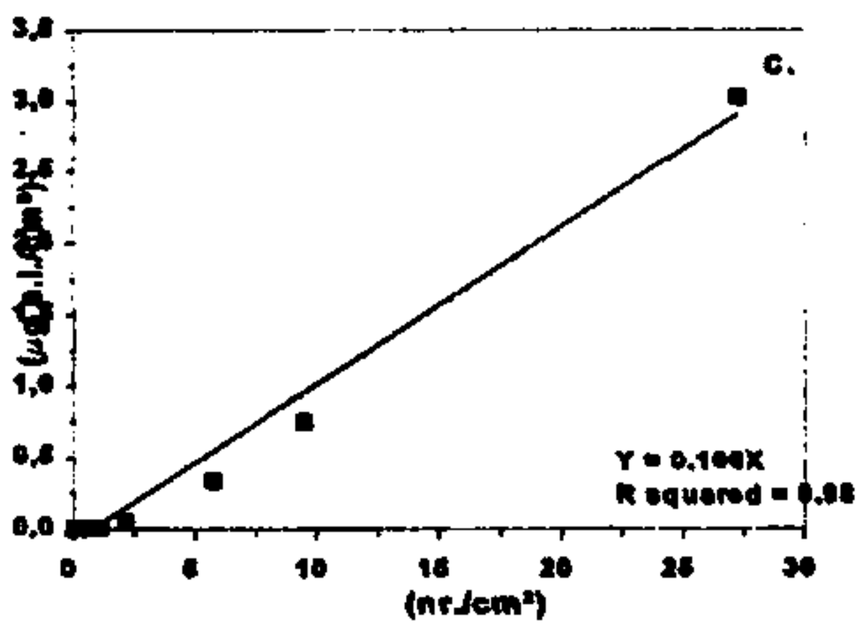
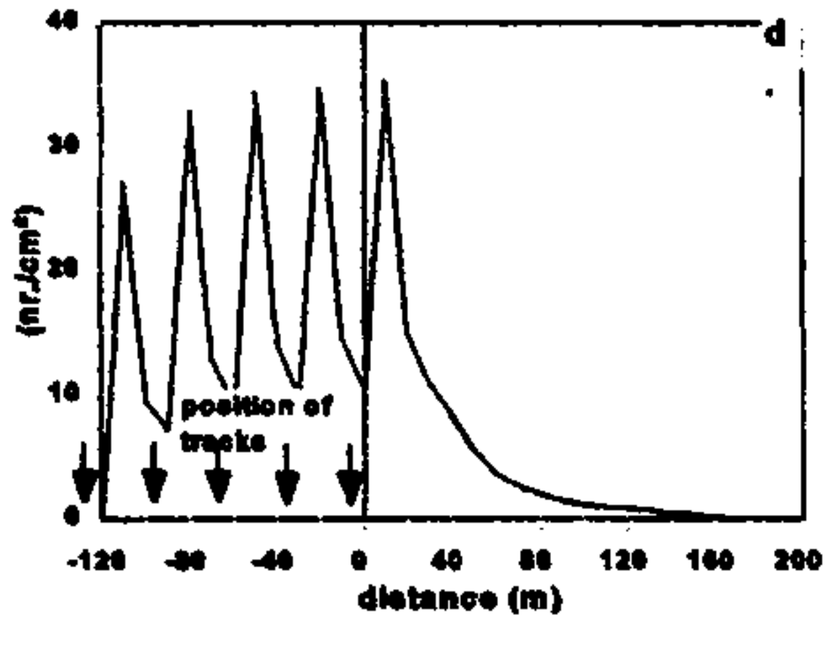


Fig. 2: Results from a multiple-track application of Sumithion® L50 (500 g a.i./L ULV) with a hand-held Micron-Ulva® sprayer at a nominal application rate of 450 g a.i./ha: (a) horizontal droplet deposits on oil-sensible papers at downwind sampling sites, (b) deposition of active ingredient on horizontal glass plates, (c)concentration of the active ingredient in 20L glass containers containing water, (d) relationship between deposits of droplets and deposition of active ingredient, (e)relationship between concentration in water and droplet deposits, (f) relationship between concentration in water and deposition of the active ingredient, (g) mortality of *Anisops sardeus* exposed to water from the glass containers. Nr. of tracks: 5; track spacing: 10m; wind speed 3.7±0.8 m/s.









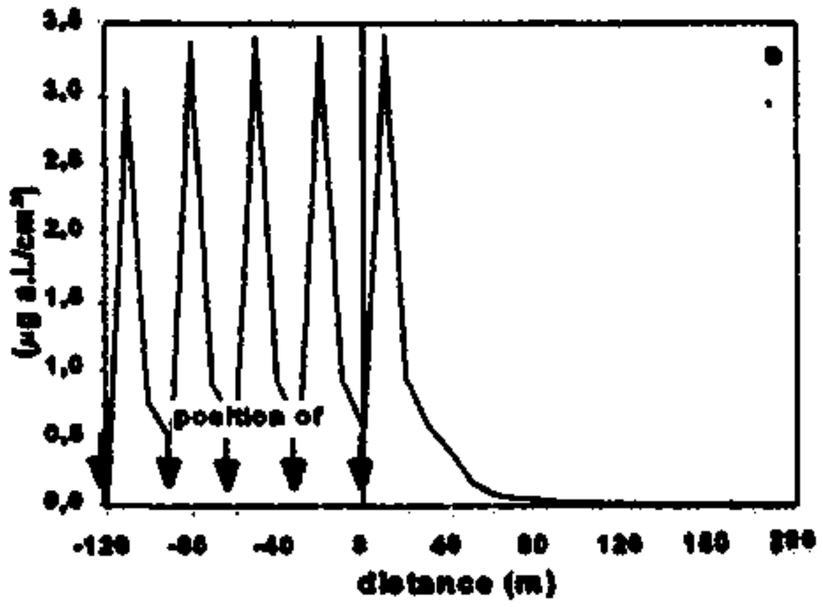


Fig. 3: Results from a single track application of Sumithion® L50 (500 g a.i./L ULV) with a vehicle-mounted Ulva-Mast® sprayer at a nominal application rate of 450 g a.i./ha: (a) horizontal droplet deposits on oil-sensible papers at downwind sampling sites, (b) deposition of active ingredient on horizontal glass plates, (c)relationship between deposits of droplets and deposition of active ingredient, (d) predicted droplet deposits from a treatment with five 30m spaced tracks, (e) predicted deposition of active ingredient from a treatment with five 30m spaced tracks. Wind speed 2.4±0.6 m/s.

Final multiple-track treatment

The results of the final Ulva-mast treatment show a similar pattern as the multiple track Micron-Ulva treatment. The natural logarithms of the deposition and water concentrations decreased significantly with the downwind distance (Figs. 4a-c). The best correlation was again obtained for droplet deposition (R^2= 0.97). Deposition of the insecticide and water concentrations also correlated well among each other (Figs. 4d-e). The measured water concentrations were equally well explained by the deposition measured in numbers of droplets or as the amount of active ingredient (R^2= 0.92).

In the bioassays average mortality gradually decreased from 100 to 0% along the downwind line.

Toxicity data

The toxicity of fenitrothion to *A. sardeus* in the bioassays from the field trials with the two sprayers was compared to previously obtained results from the laboratory (Lahr *et al.* 1996; Marquenie *et al.* 1997). The 48h-LC50 and -LC_{10} values for the bioassays were somewhat lower than those derived from the laboratory tests, but not more than twice as low (Table 2). The LC_{10} values are only slightly different from the LC_{50} values, an indication for a steep dose-response curve for *A. sardeus* and fenitrothion. The 48h-LC_{10} values are used for the calculation and estimation of buffer zones in the next paragraph.

Table 2: Acute toxicity of fenitrothion to *Anisops sardeus* (Hemiptera, Notonectidae) in laboratory tests and in bioassays from field trials with two different sprayers. The RIZA-programme is based on Kooijman (1981), the DEBtox-programme on Kooijman and Bedaux (1996).

Experiments	48h-LC_{50} (µg/L) (RIZA-programme)	48h-LC_{10} (µg/L) (DEBtox-programme)
average of laboratory tests ± s.e.	8.5±0.6[1)]	7.8±0.4[2)]
Micron-Ulva bioassays	5.7	4.3
Ulva-Mast X15 bioassays	6.9	7.5

[1)]average from three tests reported by Lahr *et al.* (1996) and two from Marquenie *et al.* (1997)
[2)]average calculated from raw data of Lahr *et al.* (1996) and Marquenie *et al.* (1997)

Lahr (1997) distinguished three groups of indicator species for side-effects of insecticides in temporary ponds in the Sahel: fairy shrimps (Branchiopoda, Anostraca), backswimmers (Hemiptera, Notonectidae) and water fleas (Branchiopoda, Cladocera). These could be represented by the test species *Streptocephalus sudanicus*, *A. sardeus* and *Daphnia magna* respectively. The acute toxicity of the eight insecticides mentioned in Chapter 1 to these three species were reported by the same author. Table 3 lists the most sensitive species for each insecticide and their 48h-LC_{50} or -EC_{50}. LC_{10} values were calculated from the raw data by Lahr *et al.* (1996) when the most sensitive species was either *S. sudanicus* or *A. sardeus*. LC_{50} values for *Daphnia* were found in the literature (see Lahr *et al.* 1996). LC_{10} or EC_{10} values for this species were estimated from the LC_{50} or EC_{50} using the lowest ratio found between the LC_{10} and the LC_{50} for the other two species (0.46 for *A. sardeus* and fipronil, Table 3). Note that *D. magna* and not *A. sardeus* is the most sensitive species to fenitrothion. *A. sardeus* was used in the field trials as a model organism because bioassays with this species could be easily executed at the field station in Nioro du Rip, not because it was the most sensitive to fenitrothion.

Table 3: Acute toxicity of eight insecticides used in Desert Locust control to the most sensitive of three indicator species for side-effects in temporary ponds in the Sahel. Data from Lahr *et al.* (1996), Tomlin (1994) and Van Rijn *et al.* (1995).

Insecticide	Most sensitive test species	48h-LC_{50} or -EC_{50} (µg/L)	48h-LC_{10} or -EC_{10} (µg/L)	ratio 48h-LC_{10}/LC_{50} or 48h-EC_{10}/EC_{50}
fenitrothion	*Daphnia magna*	1.6	0.73[1)]	0.46[2)]
chlorpyrifos	*Anisops sardeus*	0.90	0.77	0.86
malathion	*Daphnia magna*	1	0.46[1)]	0.46[2)]
bendiocarb	*Streptocephalus sudanicus*	42.1	30.7	0.73
deltamethrin	*Anisops sardeus*	0.012	0.010	0.83
lambda-cyhalothrin	*Anisops sardeus*	0.026	0.017	0.66
diflubenzuron	*Streptocephalus sudanicus*	0.75	0.49	0.65
fipronil	*Anisops sardeus*	9.25	4.21	0.46

1) estimated from lowest ratio 48h-LC_{10}/LC_{50} or 48h-EC_{10}/EC_{50} found (*A. sardeus* and fipronil)
2) lowest ratio 48h-LC_{10}/LC_{50} or 48h-EC_{10}/EC_{50} found for other insecticides (*A. sardeus* and fipronil)

Buffer zones

The buffer zones calculated for fenitrothion and *A. sardeus* were 74 and 114m for the Micro-Ulva and the Ulva-Mast respectively. These distances are fully based on the results of the field trials: e.g. they are calculated from the relation between the downwind distance and the measured water concentrations in the glass containers and the 48h-LC_{10} values derived from the bioassays. These data are only valid at the wind speeds at which the experiments were conducted, 3.7 m/s for the Micro-Ulva and 2.6 m/s for the Ulva-mast.

Buffer zone widths for both sprayers were also estimated from the single track experiments and previous routine laboratory toxicity data for *A. sardeus* and fenitrothion. The superposition of the droplet deposition profiles from the single track experiments combined with the relation between droplet numbers and water concentrations found during the multi-track trials gave an estimated buffer zone of 51m for the Micro-Ulva and 84 m for the Ulva-Mast respectively. These values are ±30% lower than the measured ones.

Table 4 shows the estimated buffer zone widths for the eight insecticides mentioned. The profiles of the water concentrations found during the multi-track trials, corrected for the application rate of each insecticide, were combined with the 48h-LC_{10} values for the most sensitive species given in Table 3. Most estimated buffer zones are wider than the ones obtained for *A. sardeus* because the species in Table 3 were usually more sensitive to the insecticides than *A. sardeus* to fenitrothion.

Table 4: Estimated buffer zone widths for eight insecticides used in Desert Locust control for the Micron-Ulva and Ulva-Mast X15 sprayers.

Insecticide	Application rate (g a.i./ha)	Estimated buffer zone width (m)	
		Micron-Ulva (wind speed 3.7 m/s)	Ulva-Mast (wind speed 2.6 m/s)
fenitrothion	450	132	293
chlorpyrifos	240	109	241
malathion	925	171	382
bendiocarb	100	0	0
deltamethrin	12.5	155	346
lambda-cyhalothrin	20	153	342
diflubenzuron	60	78	171
fipronil	6.25	0	0

DISCUSSION

The methods to measure the horizontal deposition of fenitrothion, droplet numbers on oil-sensitive papers and residues on glass plates, both proved to be adequate measures for the prediction of concentrations in water. The droplet counts in particular correlated well with downwind distance and water concentrations. This may be surprising given that it is often assumed that the size of deposited droplets decreases with the distance downwind of a treatment. Apparently the spectra of the sizes of the droplets were very narrow for both types of sprayers.

Because of the high deposits of fenitrothion close to the track in the preliminary experiment with the Ulva-Mast X-15, it can be deduced that the droplets from this machine must have been larger than for the Micro-Ulva. From the multi-track profiles in Figs. 3d and 3e it may even be questioned if this type of sprayer is appropriate for locust control with ULV formulations of fenitrothion. Symmons *et al.* (1989) used the same type of sprayer for treatments with a ULV formulation of bendiocarb. However the droplet deposit profile they found was considerably wider than ours. When the feed rate to the atomizer is increased, larger droplets are generally produced. Symmons *et al.* (1989) used a flow rate of 300 mL/min, ours was around 600 mL/min. This may explain the difference in droplet deposits. However, during the calibrations prior to the treatments in this study we found that flow rates from the X15 became very irregular at lower than 500 mL/min. Improved versions of the Ulva-Mast are currently available (*e.g.* Hewitt 1992) and have shown to give adequate deposition profiles (FAO 1994). These may prove more appropriate for further studies.

The combination of downwind deposition profiles with bioassays in multiple-track field trials was found to be a good approach for the calculation of buffer zones for such treatments. The buffer widths that were calculated for *A. sardeus* and fenitrothion were somewhat wider than the values that were predicted from the single track results and the laboratory toxicity data. For the Micron-Ulva this can be explained by the higher wind speed and the lower LC_{10} value for the multiple track trial. But for the Ulva-Mast the wind speed was only slightly higher during the final treatment and the LC_{10} from the bioassays was almost similar to the one calculated from the laboratory data. Other factors such as differences in turbulence between the preliminary and final trials may therefore have played a role as well. We feel however, that even though the predictions were 30% too low, they were reasonably adequate, and that therefore buffer zone widths may also be estimated by extrapolation from single track trial data.

Downwind deposition profiles may vary under different meteorological conditions (Frank *et al.* 1994; Lawson and Uk 1979). For precise predictions more field trials will be needed to investigate the variation between deposition profiles under different circumstances. These data may be fitted to theoretical models such as those by Bache and Sayer (1975) or Spillman (1983). Similar and repeated trials for aerial applications against Desert Locust may not seem logistically possible, but an adequate and validated model for such ULV treatments has been developed in the U.S. and is available on disk for use on personal computers (Bilanin *et al.* 1989; Teske *et al.* 1993; Rafferty and Bowers 1993).

The estimated buffer zone widths for eight locust insecticides in Table 4 represent worst case values in a sense that they were extrapolated from field trials carried out on surfaces without vegetation and for shallow water depths. At higher wind speeds the safe zones needed for protection may still be wider. In general, a buffer zone of 200m for the Micron-Ulva or 400m for the Ulva-Mast upwind from temporary ponds will be sufficient for any of the eight insecticides at moderate wind speeds such in our field trials (2-4 m/s). Since these distances are roughly proportional to the release heights of the atomizers (1.5m and 2.7m respectively) we may also make a preliminary estimate of a buffer zone under similar circumstances for an aerial treatment taking place at a height of 10m. This distance would approximately be 1 400m. These rough estimations may also be appropriate at higher wind speeds when vegetation is present, but this needs verification.

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CHAPTER 4:

Acute toxicity of five insecticides used in Desert Locust control to *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and *Anisops sardeus* (Hemiptera, Notonectidae)

Simone Marquenie, Egbertha Schuiling, Aliou Badji & Joost Lahr

SUMMARY

During campaigns to control Desert Locust and grasshopper plagues in West Africa large quantities of synthetic insecticides are applied. One of the activities of the FAO-LOCUSTOX Project in Senegal is to conduct research on environmental effects of these applications. Tests with two important indigenous macroinvertebrate species from temporary ponds for routine screening of the ecotoxicity of different compounds have recently been developed (Lahr *et al.* 1996). This report describes further tests using these species with compounds used for Desert Locust control.

The two species, the fairy shrimp *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and the backswimmer *Anisops sardeus* (Hemiptera, Notonectidae), were captured in natural temporary ponds. Four synthetic insecticides were tested: the carbamate-organophosphate mixture propoxur/phoxim, the benzoyl-ureas teflubenzuron and triflumuron (insect growth regulators) and the pyrethroid beta-cyfluthrin. In addition to these chemical insecticides, the effects of one biological insecticide developed to control Desert Locusts and grasshoppers, the fungus *Metarhizium flavoviride*, were tested.

The tests lasted 48 and 96 hours for the synthetic insecticides and *M. flavoviride* respectively. For all compounds the EC_{50} (*S. sudanicus*) or LC_{50} (*A. sardeus*) and the 95% confidence interval of these values were derived. Chemical parameters such as temperature, pH and dissolved oxygen were measured every 24 hours.

All synthetic insecticides tested were highly toxic to *S. sudanicus* and *A. sardeus*. Both species were most sensitive to beta-cyfluthrin.

The average 96h-EC_{50} and -LC_{50} values for *S. sudanicus* and *A. sardeus* and *M. flavoviride* were 2.5 and 15.4 mg of spores/L respectively. The fungus was much less harmful than the synthetic insecticides. Incubation of dead animals in petri-dishes did not reveal any clearly visible growth of *M. flavoviride*, although green spots and films were seen on many incubated *S. sudanicus*. The animals, especially *S. sudanicus*, would rapidly decompose in the dishes. It was not clear by which mechanism the fungus caused its effects.

INTRODUCTION

In several parts of the world locusts and grasshoppers are being treated with insecticides to protect crops. The insecticides do not only have an impact on the target animals, but may also have a detrimental effect on other, non-target, organisms in exposed ecosystems.

In West-Africa, the treated species are the Desert Locust *Schistocerca gregaria* and grasshoppers like *Oedaleus senegalesis*. Control of grasshoppers is more common and regular, especially during the rainy season. Both species can be treated with the same insecticides, but dose rates are usually higher in locust than in grasshopper control. Although large outbreaks occur irregularly, the areas treated and the amount of insecticides applied are considerable (Everts 1990). Most control occurs during the rainy season or shortly after it.

The aim of the LOCUSTOX Project is to reduce the environmental impact of insecticide use during campaigns against locusts and grasshoppers. New insecticides for locust and grasshopper control are continuously developed. The LOCUSTOX Project studies insecticides in both the aquatic and the terrestrial environment to investigate side-effects on non-target organisms in ecosystems in the Sahel.

Depending on the availability of trial data on the efficacy against Desert Locust, the Pesticide Referee Group, which advises FAO, identifies three categories of insecticides which are subsequently tabulated as Table 1, 2 or 3 compounds (FAO 1996). Table 1 contains insecticides with a verified dose rate for full cover sprays. Table 2 contains insecticides that have shown to be effective in full cover treatment, but which still need further verification of the suggested dose rates. The third table contains promising insecticides requiring additional information and/or trials for dose rate determination in barrier treatments. In 1997 a new system will be presented which gives the availability of data on the insecticides. The insecticides are not anymore divided in categories.

This report contains the results of further acute aquatic toxicity tests, conducted with two indigenous aquatic macroinvertebrates from temporary ponds in Senegal. These ponds are used by local people and their livestock, but are also important natural resources. They exist during and shortly after the annual rainy season, which is also the period of the year during which most of the treatments against locusts and grasshoppers are carried out. Temporary ponds may be contaminated with insecticides when these are sprayed in their vicinity. Methods for acute toxicity tests with the two species, the fairy shrimp *Streptocephalus sudanicus* (Branchiopoda, Anostraca, Streptocephalidae) and the backswimmer *Anisops sardeus* (Hemiptera, Notonectidae), were recently developed and applied to Table 1 insecticides (Lahr *et al.* 1996).

The tests were conducted with various new candidate insecticides for Desert Locust control. The propoxur/phoxim mixture (carbamate/organophosphate) is under investigation for treatments against swarms and hopper bands (Table 2, FAO 1996), while the benzoyl-urea insect growth regulators (IGR's) teflubenzuron and triflumuron are candidates for (barrier) applications against hopper bands (Table 3, FAO 1996). The pyrethroid beta-cyfluthrin has been submitted for evaluation by the Pesticide Referee Group but does not yet figure in one of its tables (H. van der Valk, pers. comm.). The fungus *Metarhizium anisopliae* (Deuteromycetes, Moniliales) is a recently developed biological control alternative to chemical locust insecticides. It is commonly found throughout Africa that oil-based formulations of spores give good control of locusts and grasshoppers in field trials. The host range of the acridid isolates is about 25 species of Acridoidea. The fungus is entomophagous and facultative (LUBILOSA 1994). The insecticide has already been tested on non-target arthropods and rats but no tests have been carried out on non-target aquatic organisms or birds.

METHODS AND MATERIALS

The tests were carried out in the rainy season between August and December 1996 in the laboratory of the Senegalese Crop Protection Directorate (DPV) in Nioro du Rip (13°45N, 15°46W). Organisms were collected in three of the ponds in this area that had been monitored during ecotoxicological field studies from 1991 to 1994 (Lahr and Diallo 1993; Lahr *et al.* 1995): Gandiang, Débreye and Kouthioum. Except for tests with *M. anisopliae* the methods used were similar to those described by Lahr *et al.* (1996).

Test water

For each species, a different type of test water was used. The water supplied to the laboratory in Nioro du Rip is groundwater from a depth of 40 meters. It contains no added chlorine and its conductivity is approximately 300 µS/cm. The conductivity of the water in natural temporary ponds varies between 50 and 100 µS/cm (Lahr and Diallo 1993; Lahr *et al.* 1995). For *A. sardeus*, this difference in conductivity is not a problem. It was sufficient to filter tap water with an active carbon-filter to obtain suitable test water. The pH of this water was almost neutral. *S. sudanicus* on the contrary is very sensitive to sudden changes in ionic strength. For these tests, water from the well was first demineralized by passing it through an ORC active carbon filter and two R_3 ion exchangers (supplied by Bioblock, France). The resulting water had a conductivity of less than 5 µS/cm. By adding 0.70 g Griffin Instant Pond Powder® (supplied by Fisons, UK) per 15L, the conductivity of the water was adjusted to approximately 100 µS/cm. As a result of this preparation, the test water for this species was somewhat acid (Annex 1).

Test water was prepared in 30L aquariums that had been washed and rinsed thoroughly. The water was aerated by means of small aquarium air pumps and porous stones prior to the tests until the dissolved oxygen level was at least 80% of complete saturation.

Biological material

The choice of the ponds from which the organisms were collected, depended on the available life stages of the organisms during the course of the rainy season: females with a visible ovisac of *S. sudanicus* or adult females of *A. sardeus*. Samples were taken in the center of the ponds to avoid possible interference with surrounding aquatic and terrestrial vegetation. Prior to each series of tests, the organisms were collected with a scoop net (diameter 35 cm, mesh width 1 mm) originally developed for quantitative sampling of pelagic macroinvertebrates (Lahr and Diallo 1993). One or more swaths, depending on the species abundance, were made with the upper part of the net approximately 5 cm below the water surface. The animals were carefully transferred to a 15L bucket which was covered by a lid. A sufficient level of dissolved oxygen during the transport to the laboratory was maintained through permanent aeration with portable battery-powered pumps. A tube with a porous stone at the end was placed in the water through a small hole in the lid of the bucket. In the laboratory, the animals were transferred to an aquarium containing 15L of test water. The organisms were acclimatized to the test water and laboratory conditions during at least two hours before the beginning of the tests.

For both species, only females were used. In the case of *S. sudanicus* these could easily be recognized by their well developed external ovisacs (a description of the species is given by Brendonck *et al.* 1992). The specimens used in the tests ranged in size from 15 to 22 mm. Females of *A. sardeus* are less easily distinguished than males which possess a very distinct rostrum (Nieser 1993), but females are nonetheless used in the tests because they are often more numerous in the ponds in the area.

Starting the tests

The tests were conducted using 3L glass beakers containing 2L of test water each. Ten organisms of one of the two species were selected and transferred from the aquarium into each test beaker.

For the tests with synthetic insecticides, two beakers, which only received a dose of the solvent used to dilute the insecticides, were used as controls. Between 5 and 10 beakers received logarithmically increasing concentrations of the compounds. The lowest and highest concentrations during the final tests were respectively the one that caused less than 10% mortality after 48 hours and the one that provoked 100% mortality after 24 hours in range finding tests. Final tests for each compound and each species were replicated with slightly different concentrations and fresh insecticide solutions until at least three reliable estimates of the toxicity parameters were obtained.

Contrary to the tests with synthetic insecticides, no acetone was used for assays with *M. anisopliae* . Instead, each control beaker received one drop of the liquid detergent used to prepare the suspension of this fungus (section 2.5). For the final tests with this agent, the intervals between different concentrations were equal and did not increase logarithmically.

Insecticides tested

The synthetic insecticides tested in this study were all solvent based ULV (Ultra Low Volume) formulations. These are commonly used at an application rate of 1 L/ha (FAO 1996). Table 1 gives an overview of the compounds. The products were preserved in a dark refrigerator at 5°C. *M. anisopliae* spores are a green powder which contains approximately $5*10^{10}$ spores/g (LUBILOSA 1994). For application in the field the spores are dispersed in a mixture of 70% light paraffin oil (like kerosene) and 30% vegetable oil. The fungus is produced from strain nr. IMI 330189, originally isolated from the grasshopper *Omithacris turbida cavroisi* in Niger (LUBILOSA 1994).

Table 1: Characteristics of insecticides used in acute toxicity tests.

Active ingredient	Chemical group	Commercial name	Conc. (g a.i./L)	Solubility in water (µg/L)	Formulation	Manufacturer	Year purchased	Batch numbers
propoxur+ phoxim	carbamate+ organophosphate	Volaton[6]+ Unden[3]	42+ 258= 300	$1.9x10^6$ / 1500	ULV	Bayer, Monheim, Germany	1996	BOQ5812315 0042/ SRA75020258 /PR003217, ANR020706 FL0039
teflubenzuron	benzoyl-urea	Nomolt[2]	50	19	ULV	Cyanamid, Schwabenheim, Germany	1996	SF09522 003 1996/2281 MEA29/96
triflumuron	benzoyl-urea	Asystin[5]	50	25	ULV	Bayer, Monheim, Germany	1996	PR04242874 ANR0892622
beta-cyfluthrin	pyrethroid	Bulldock[4]	12	2	ULV	Bayer, Monheim, Germany	1996	AB0101527 FL0212
Metarhizium flavoviride	fungus	Green Muscle[7]	$5*10^{10}$ (sp./g)	insoluble	none	IITA, Cotonou, Benin	1996	MP 191

Insecticide solutions/suspensions and dosing

The solutions of synthetic insecticides were prepared in acetone (analytical grade, min. 99.5% pure). For every test, a new series of solutions was made. First, 5 mL of the insecticide formulation was pipetted into a 50 mL glass volumetric flask. Acetone was then added. Further dilutions were prepared by pipetting 2.5 mL of each previous solution into 25 mL volumetric flasks and filling them up with acetone. The

number of dilutions that was prepared for any particular test depended on the desired concentrations in the test beakers.

Each beaker in tests with synthetic insecticides received a quantity of pure acetone equal to 1000 µL minus the volume of the insecticide solution to be added. Controls each received 1000 µL acetone. Depending on the concentration to be added, the other beakers received an amount between 100 and 1000 µL of the suitable dilution. Both the acetone and the insecticide solutions were added with a micropipette (range: 100-1000 µL). Following dosing, the water in the test beakers was stirred gently using a stainless steel baton with a teflon knob.

In tests with *M. anisopliae* no formulation was used. Our aim was to find out if the fungus alone would cause an effect. An initial suspension of the (very hydrophobic) spores was prepared by adding 1.6 gram powder to 2L test water. Ten drops of a liquid detergent were then added. The mixture was thoroughly stirred on a magnetic stirrer until, after approximately 2 hours, the suspension was sufficiently homogenized. The suspension was added to the test beakers in different quantities using micropipettes, ordinary glass pipettes, or small glass beakers.

Conditions and observations during tests

After dosing, the beakers containing the organisms were placed on a table in front of a window, out of direct sunlight, but exposing them to the natural light regime. The photoperiod was more or less constant throughout the testing period; day and night lasted approximately 13 and 11 hours respectively. During the tests, a constant temperature was maintained in the laboratory by an air conditioner equipped with a thermostat.

For tests with *A. sardeus* each beaker was covered tightly with a sheet of fine mosquito netting to prevent the insects from flying away. Backswimmers are very active flyers, especially at night. Beakers containing *S. sudanicus* were covered with loose mosquito netting, to prevent flies and other insects from falling in the beakers.

The duration of the tests was 48 and 96 hours for the synthetic insecticides and *M. anisopliae* respectively. Survival in each beaker was assessed every 24 hours by counting the numbers of normally behaving ("fit") animals.

For *A. sardeus* the animals were either alive or dead on all occasions. The toxicity parameter presented for this species is the LC_{50}.

S. sudanicus displays a range of reactions after exposure to different insecticides (Lahr *et al.* 1996). The principal effect prior to death is immobilization. Immobile organisms do not swim, but still move their thoracic appendages. An animal was called "immobile" when it would remain on the same spot during one minute or more of observation. Healthy organisms swim continuously. Recovery of immobile animals does hardly occur. When the organisms are exposed to pyrethroids, they initially swim frantically in circles at the water surface along the edge of the beakers, seemingly unable to orientate. These organisms are registered in a separate, "debilitated" category. Sometimes organisms recover from debilitation, but in general debilitation is followed by immobilization and finally by death. Debilitated and immobile organisms of *S. sudanicus* are included in the group of affected organisms ("not fit"). The toxicity parameters used for this species therefore is an EC_{50} (debilitation + immobilization + death).

Physical and chemical parameters were measured daily in the controls using a portable device. In general, temperature, pH and dissolved oxygen (DO, as % saturation) were determined at the start of each test (0h) and every 24 hours. Conductivity was only measured at 0h.

In order to check for possible external growth of the fungus, on several occasions, dead organisms in tests with *M. anisopliae* were carefully removed from the beakers and incubated at room temperature. They were placed on moist filter papers in petri-dishes, sealed with a piece of parafilm to prevent desiccation, and kept in the laboratory. After 20 h (the incubation time of *M. anisopliae*), the organisms were examined under a microscope (64x).

Statistical analyses and criteria for the acceptance of results

EC_{50} and LC_{50} values were calculated with the parametric method of Kooijman (1981) using the computer programme from the Institute for Inland Water Management and Waste Water Treatment (RIZA) in the Netherlands.

Test results were only accepted when the percentage of fit animals in the controls at the time of counting was 90% or more. In the lowest concentration 37% of the organisms at the most should be killed or affected and in the highest concentration 63% or more. These criteria are recommended by the ASTM (1989). Another criterium was the width of the 95% confidence interval (CI) of the EC_{50} or LC_{50} values derived from the calculations. Whenever the lower limit would be less than approximately half the calculated EC_{50} or LC_{50}, or if the upper limit would exceed twice this value, the results of a test were rejected.

RESULTS

Control mortalities in the tests and physical and chemical conditions were similar to the previous experiments described by Lahr *et al.* (1996) and will not be discussed here, except where relevant for explanation of the results. These data can be found in the annexes.

Synthetic insecticides

The data of all final tests with *S. sudanicus* and *A. sardeus* and the four synthetic compounds can be found in Annex 1 and 2 respectively.

Results from the tests with *S. sudanicus* and the pyrethroid beta-cyfluthrin were sometimes difficult to analyze. Effects between 0 and 100% could be observed over a large range of different concentrations and a classical dose-response relationship was difficult to establish. Because of this, the 95% CI's are rather wide. The same phenomenon had also be observed for other pyrethroids by Lahr *et al.* (1996). Additional experiments carried out to investigate the response of *S. sudanicus* to pyrethroids are reported by Schuiling *et al.* (1997).

For other tests with *S. sudanicus* and tests with *A. sardeus*, no particular problems were encountered. The average EC_{50} and LC_{50} values are given in Table 2.

Table 2: Average EC_{50} or LC_{50} values (µg/L) ± s.e. for four candidate synthetic insecticides for Desert Locust control, tested on *Streptocephalus sudanicus* and *Anisops sardeus* (n= number of tests).

Insecticide	*Streptocephalus sudanicus*		*Anisops sardeus*	
	EC_{50}		LC_{50}	
	24h	48h	24h	48h
propoxur+ phoxim	3.07±1.11 (n=6)	1.23±0.35 (n=6)	3.91±0.85 (n=4)	1.92±0.14 (n=4)
teflubenzuron	27.0±14.1 (n=5)	0.74±0.58 (n=5)	250±17.1 (n=3)	250±17.1 (n=3)
triflumuron	21.7±13.6 (n=3)	0.22±0.70 (n=3)	202±34.6 (n=4)	190±26.8 (n=4)
beta-cyfluthrin	0.010±0.007 (n=4)	0.004±0.001 (n=4)	0.020±0.004 (n=4)	0.020±0.004 (n=4)

The 48h-EC_{50} and -LC_{50} values in Table 2 were compared to the classification scheme for the toxicity of substances to aquatic organisms proposed by Canton *et al.* (1991) (Annex 3). All four insecticides were highly toxic to both *S. sudanicus* and *A. sardeus*.

Metarhizium anisopliae

For both species there was a clear relationship between the amount of spores in the test water and noxious effects. The results of the tests are given in Table 3. Detailed data can be found in Annex 4.

Table 3: Average EC_{50} and LC_{50} values (mg spores/L) ± s.e. of acute toxicity tests with *Streptocephalus sudanicus* and *Anisops sardeus* using the fungus *Metarhizium anisopliae* (n= 4 tests).

Organism	24h	48h	72h	96h
S. sudanicus (EC_{50})	>47.9	35.4 ± 56.6	4.8 ± 1.0	2.5 ± 1.0
A. sardeus (LC_{50})	>82.8	41.2 ± 42.8	25.1 ± 20.4	15.4 ± 12.4

The results of replicate tests were quite variable during the first 48h, but after 72h $E(L)C_{50}$ values started to converge. If the classification by Canton *et al.* (1991) would also be applied to *M. anisopliae* it would be considered moderately toxic to *S. sudanicus* and only slightly toxic to *A. sardeus* (96h data).

The incubation results for *S. sudanicus* are given in Fig. 1. The animals decomposed rapidly during incubation. Many showed green spots on their body or were completely covered by a slightly greenish film when they died in the tests. These spots and films did not look similar to the characteristic external sporulation observed in some terrestrial arthropods (Danfa 1994). During incubation the green spots would grow in some cases, but it could not be confirmed if this represented growth of the fungus. On some of the animals that did not display green spots or films after dying, these would appear after incubation. None of the incubated animals from controls had green spots or was covered with a green film, neither before nor after incubation.

For none of the incubated specimens of dead *A. sardeus* growth of the fungus was observed. The green spots and film observed with *S. sudanicus* were also absent.

To verify if *M. anisopliae* would provoke changes in pH or DO in the tests, in one test with *A. sardeus* (ANMT-6) these parameters were measured in all concentrations during 120h. These results are shown in Figs. 2 and 3.

The data show that there was hardly any variation of the pH between the different concentrations, neither was there an effect in time. DO decreased as usual during the test (Fig. 3), but there was no additional effect of the concentration.

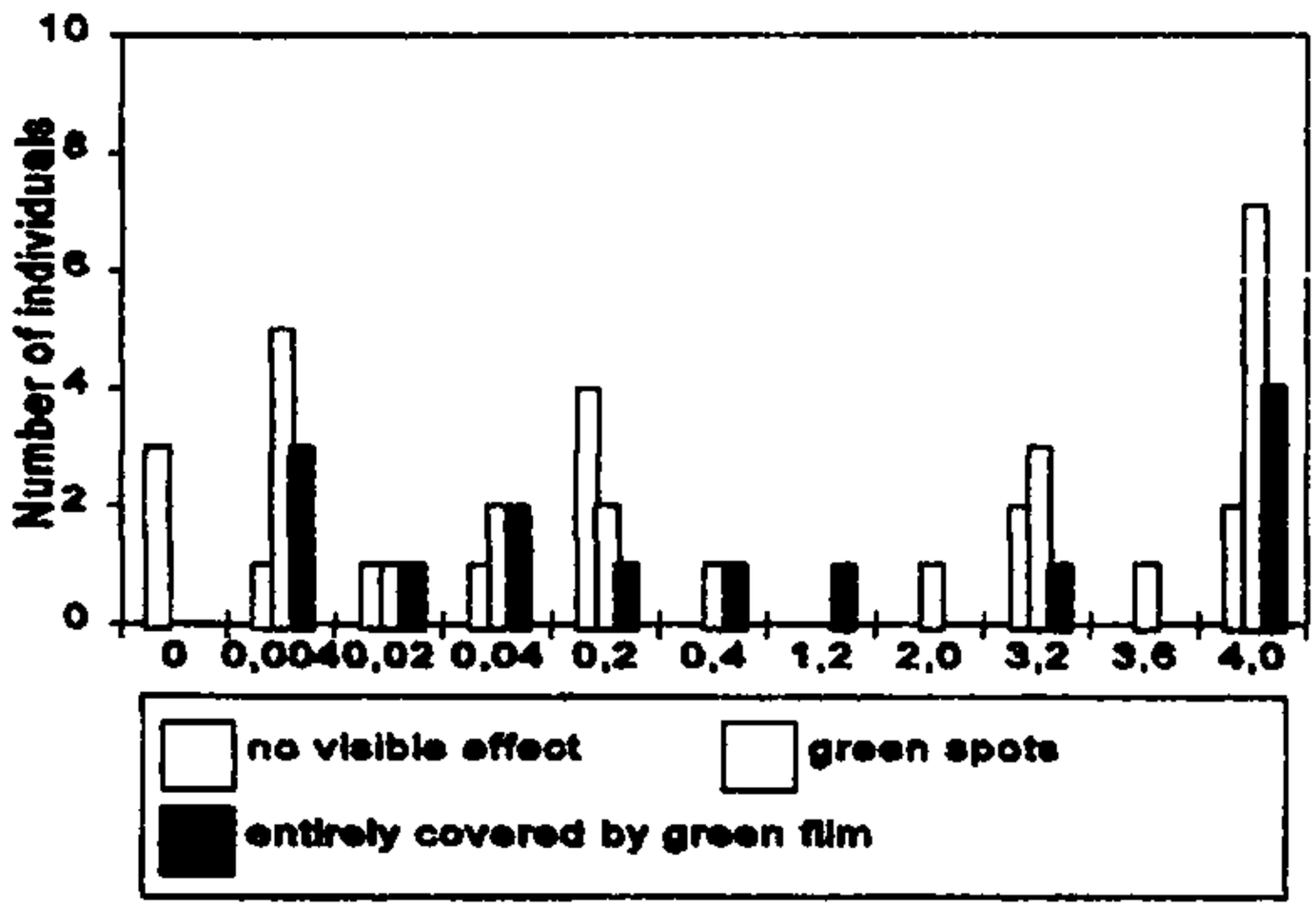

Figure 1: Presence of green spots or a green film after 20 hours of incubation on *Streptocephalus sudanicus* that died in toxicity tests with *Metarhizium anisopliae* . The data represent the combined results of four seperate tests.

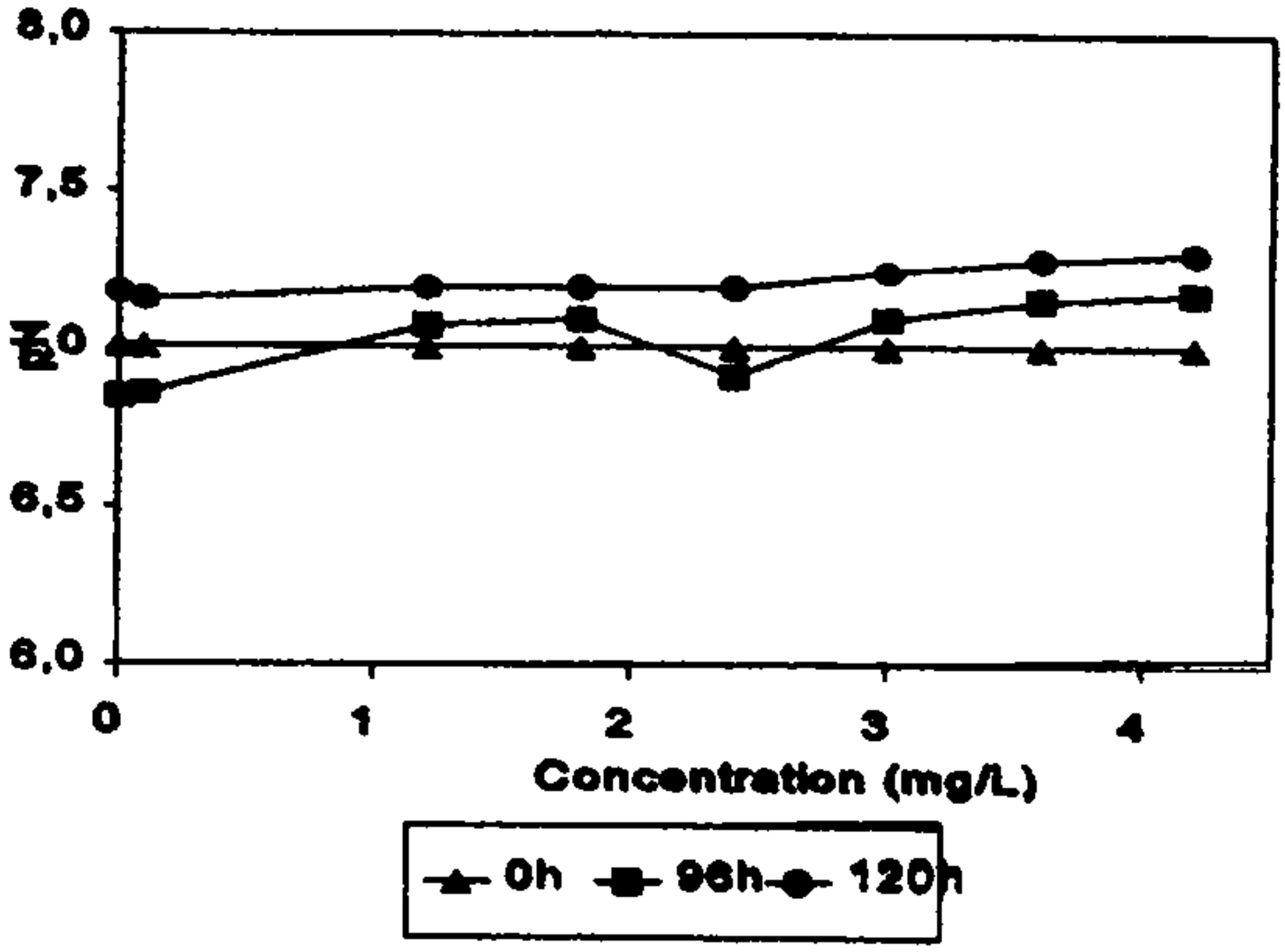

Figure 2: pH measured on different times and in all concentrations, during one test (ANMT-6) with the fungus *Metarhizium flavoviride* and the species *Anisops sardeus*.

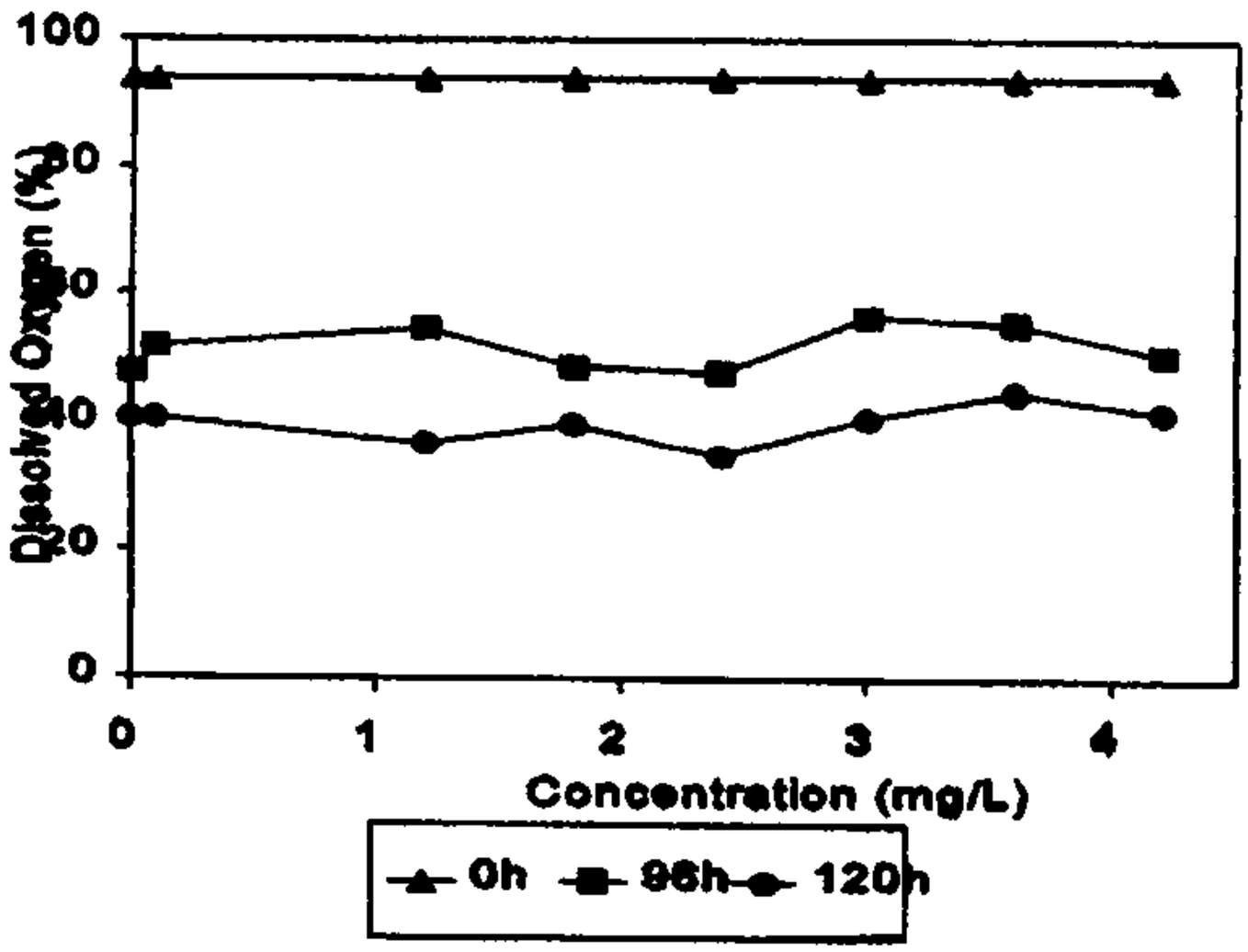

Figure 3: DO measured on different times and in all concentrations, during one test (ANMT-6) with the fungus *Metarhizium anisopliae* and the species *Anisops sardeus*.

DISCUSSION

S. sudanicus and *A. sardeus* were highly sensitive to the four synthetic insecticides used in this study. The much higher sensitivity of *S. sudanicus* to teflubenzuron and triflumuron is explained by the toxic mechanism of these substances. Both insecticides are insect growth regulators and inhibit chitine synthesis in moulting stages of arthropods. Because the imagos of *A. sardeus* do not moult, these insecticides have less impact on this species. *S. sudanicus* on the contrary, keeps moulting continuously during its life-cycle. Each individual moults once every four to five days. They are affected as soon as moulting occurs. These results are in agreement with those from tests with an other benzoyl-urea, diflubenzuron, tested previously (Lahr *et al.* 1996).

For triflumuron and *S. sudanicus* the EC_{50} values found were below the maximum solubility of the insecticide in water (which is 25 µg/L) (Tomlin, 1994), but for *A. sardeus* the values were approximately ten times higher than the solubility. For teflubenzuron and *S. sudanicus* only the 24h value exceeded the solubility of the compound (19 µg/L) (Tomlin, 1994), but for *A. sardeus* both LC_{50} values exceeded it. A distinct dose-effect relationship was neverthless obtained on all occasions in which concentrations passed the solubility of a compound. The toxic effects still increase when water is charged above the solubility.

Although a clear dose-response relationship was found between the suspensions of the spores of *M. anisopliae* and the effects on both species, it could not be established if these effects were caused by growth of *M. anisopliae* in the organisms. Dead specimens of *S. sudanicus* were on many occasions covered with green spots or a green film, but these did not look like ordinary *Metarhizium* growth.

A possible explanation for the impact of the fungus may be the effect of secondary, toxic by-products which are produced by *M. anisopliae* . These destruxins can be produced and excreted during growth of the mycelium into the test organisms, and before sporulation takes place. Such substances may cause lethal effects while no signs of sporulation of the fungus are visible (H. van der Valk, pers. comm.).

It may also be possible that the fungus acts through indirect action, for instance by provoking changes in water quality. The spores, which obviously consist of organic matter, may for example cause growth of bacteria that alter the composition of the test water or excrete toxins. In one test with *A. sardeus*, pH and DO were measured in all concentrations. These parameters can be indicators of bacterial growth. The data showed that there was hardly any difference in pH between separate concentrations, nor was there an effect in time. DO obviously decreased during the test (Fig 3), but there was no additional effect of the concentration of the spores. If there was any indirect effect of the fungus on water quality, it seems therefore unlikely that this involved changes in these two parameters.

The interaction between *S. sudanicus* and *M. anisopliae* may also be mechanical. In tests with high concentrations of spores it was sometimes observed that the thoracic appendages of the animals became green. Spores can probably become trapped in these fine structures. In the case of severe clogging this may interfere with swimming and sieving of food particles, effectively starving the organisms.

The exposure of the two aquatic species in tests to *M. flavoviride* represented a worst case scenario because a detergent was used. Given the effort that was needed to disperse the highly hydrophobic spores, it is unlikely that operational applications with the fungus would result in equally high concentrations of spores in surface water. When the spores are applied in oil-based formulations it is to be expected that the spores will have a tendency to remain in the formulation. This will probably form a film on the water. The film itself may pose a greater threat to aquatic organisms than the fungus itself, especially to surface breathing hemipterans whose respiratory tubes can become clogged. Seye (1994) showed that a 70/30 v/v mixture of kerosene and peanut oil used to formulate *M. anisopliae* caused rapid mortality (in a matter of minutes) among *Anisops spp.* in acute toxicity tests. The 48h-LC_{50} of the formulation corresponded to an application rate of 12 L/ha (since the film is lying on the water, only the surface cover matters and not the depth). This is equal to six times the recommended application rate of 2L/ha.

CONCLUSIONS

The standard methods for both tests gave good results when applied to four novel synthetic insecticides.

Under these test conditions:

- propoxur/phoxim, beta-cyfluthrin, teflubenzuron and triflumuron are highly toxic to the fairy shrimp *Streptocephalus sudanicus* and to the backswimmer *Anisops sardeus*, and
- *Metarhizium anisopliae* causes lethal effects in both aquatic test species.

The exact cause of death of each of the species exposed to *M. anisopliae* could not be established. Many of the *S. sudanicus* that died in the tests were covered with green spots or a green film. These sometimes increased during incubation in sealed and humidified petri-dishes. The spots or film were not observed with dead *A. sardeus*. If the mechanism would indeed be toxicity, the fungus can be considered slightly toxic to *A. sardeus* and moderately toxic to *S. sudanicus*.

RECOMMENDATIONS

Contamination of temporary ponds by any of the four formulated synthetic insecticides (or mixtures) tested here should be avoided.

Further research will be necessary to verify whether the death of the organisms in tests with *M. anisopliae* was caused by growth of the fungus, indirect effects or something else. Affected *S. sudanicus* and *A. sardeus* should be observed with stronger microscopes to determine whether the fungus is growing in the organisms.

M. anisopliae may also be tested in its formulated form.

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CHAPTER 5:

Acute toxicity tests with *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and *Anisops sardeus* (Hemiptera, Notonectidae): effects of synthetic pyrethroids and methodological aspects

Egbertha Schuiling, Simone Marquenie, Aliou Badji & Joost Lahr

SUMMARY

During Desert Locust and grasshopper invasions in West-Africa large quantities of synthetic insecticides are applied during control measures. One of the activities of the FAO-LOCUSTOX Project in Senegal is to conduct research on environmental effects of these applications. Tests with important indigenous species from different habitats are currently developed for routine screening of the ecotoxicity of different compounds. This report deals with several aspects of acute toxicity tests in which two aquatic species from temporary ponds are used.

The two test species, the fairy shrimp *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and the backswimmer *Anisops sardeus* (Hemiptera, Notonectidae), were captured in natural temporary ponds. Both species are pelagic macroinvertebrates.

Part 1: Effects of pyrethroids on *Streptocephalus sudanicus*

Three different pyrethroids were tested on *S. sudanicus* to investigate the effects of these compounds on this species in more detail. All three were synthetic, Type II pyrethroids: beta-cyfluthrin, deltamethrin and lambda-cyhalothrin. The tests lasted 96 hours and 13-14 different concentrations were used (between 1 and 5000 ng/L) for each test. For all three pyrethroids, the observed dose-response curves were roughly the same. They did not have the shape of the classical S-curve. After 72-96 hours the curve showed two peaks because organisms died more slowly at intermediate than at lower or higher concentrations. An adequate explanation for this phenomenon could not be found, but one possible way of looking at the problem is discussed and involves different metabolic rates.

Part 2: Assessment of immobility in *Streptocephalus sudanicus* using its phototactic response

Several experiments were conducted to investigate an objective method to assess immobility in *S. sudanicus* after exposure to insecticides based on the phototactic reaction of the organism.

A first test was carried out to observe the normal behavior of *S. sudanicus*. In a second experiment, the reaction of healthy organisms to different light sources was investigated under dark and light conditions by assessing the distribution of the organisms in a beaker shortly after switching on different light sources. On another occasion immobility in several acute toxicity tests with different insecticides was measured, both with the standard method and using light sources of different colors. The EC_{50} values estimated with both methods were then compared.

During the first tests it was found that short and infrequent lapses into immobile behavior are normal for *S. sudanicus* unexposed to insecticides. When lights were used, they reacted most strongly to white, blue and green bulbs and a fluorescent lamp. In general this reaction was stronger in a dark room, but even in the dark, only some of the animals showed a response.

No significant differences were found between the results of toxicity tests in which immobilization was assessed with lamps or by ordinary observation of the organisms. It is concluded that using the

phototactic reaction of *S. sudanicus* to distinguish immobile from healthy individuals does not improve the reliability or sensitivity of acute toxicity tests with this species.

Part 3: Modeling toxicity and degradation of fenitrothion in tests with *Anisops sardeus*

A statistical model for the analysis of data of static toxicity tests with non-persistent toxicants has recently been developed by Widianarko and Van Straalen (1996). In traditional bioassays it is assumed that no degradation of the compounds takes place. When non-persistent substances are used, exposure may decrease in time because the toxicant is degraded. In the model of Widianarko and Van Straalen, an explicit expression for time- and concentration-dependent survival is derived. The model predicts that, with increasing exposure time, survival will approach a nonzero baseline value for certain initial concentrations.

Two replicate 168h toxicity tests were carried out with the insecticide fenitrothion and the species *A. sardeus*. Observations were carried out every 12 hours. From the results, an estimation of the degradation rate (half-life), elimination rate and the initial concentrations causing an ultimate mortality of 50% (μ or ultimate LC_{50}) was made.

One of the most important assumptions in the model of Widianarko and Van Straalen, the principle that in the end survival in bioassays will approach a nonzero baseline value for certain initial concentrations, could be validated. After 132-144h the number of survivors in the initial concentration of 5 µg/L remained unchanged. The LC_{50} values remained stable from this moment until the end of the tests. The estimated average half-life for fenitrothion in the tests was of the same order of magnitude as those observed in river water and Sahelian temporary ponds.

It is concluded that the theoretical model of Widianarko and Van Straalen can in principle be used for further analysis of the static aquatic toxicity tests used by the LOCUSTOX Project.

GENERAL INTRODUCTION

In various parts of the world plagues of locusts and grasshoppers are being treated with insecticides to protect crops. The insecticides not only have an impact on locusts and grasshoppers, but may also have a detrimental effect on other, non-target, organisms in contaminated ecosystems. In West-Africa, the target species are the Desert Locust, *Schistocerca gregaria*, and grasshoppers like *Oedaleus senegalensis*. Control of grasshoppers is more common and regular, especially during the rainy season. Both species can be treated with the same insecticides, but dose rates are usually higher in locust than in grasshopper control. Although large outbreaks occur irregularly, the areas treated and the amount of insecticides applied are considerable (Everts, 1990a).

This report contains the results of acute aquatic toxicity tests, conducted with indigenous aquatic organisms from temporary ponds in Senegal. These ponds are used by local people and their livestock, but are also important natural resources. They exist during and shortly after the annual rainy season, which is also the period of the year during which most of the treatments against locusts and grasshoppers are carried out.

Two species of test organisms were collected from temporary ponds around Nioro du Rip: the fairy shrimp *Streptocephalus sudanicus* (Branchiopoda, Anostraca) and the backswimmer *Anisops sardeus* (Hemiptera, Notonectidae) (Lahr and Diallo 1993; Lahr *et al.*, 1995). These two species were chosen because they are distributed widely throughout the Sahel, which means that the results of these tests apply to the whole region. The methods for acute toxicity tests with both species were developed by Lahr *et al.* (1996).

The different studies described here were part of further efforts to optimize and standardize the two tests. The report is divided in three parts. In Part 1 the effects of three pyrethroids on *S. sudanicus* were further investigated because the dose-response relationships for these compounds found in previous studies were different from those usually found for other (traditional) insecticides (Lahr *et al.* 1996; Marquenie *et al.* 1997). *S. sudanicus* was also used in Part 2 in which the possibilities to develop a less arbitrary method to assess immobility of this organism using their phototactic reaction were surveyed. *A. sardeus* was used in Part 3 for modeling toxicity and degradation of the insecticide fenitrothion using a new theoretical model for static aquatic toxicity tests.

PART 1

EFFECTS OF PYRETHROIDS ON *STREPTOCEPHALUS SUDANICUS*

INTRODUCTION

Synthetic pyrethroids have been introduced as agricultural insecticides because of their excellent activity against a wide range of insect pests and their non-persistence in the environment (WHO 1990). During previous tests with three of these compounds it was observed that the dose-response relationship for *S. sudanicus* did not have the shape of the classical S-curve (Lahr *et al.* 1996; Marquenie *et al.* 1997). In fact the curves seemed to show an unexplained dip at intermediate concentrations.

Tests were carried out with *S. sudanicus* and three pyrethroids, deltamethrin, beta-cyfluthrin and lambda-cyhalothrin, to find out if all three pyrethroids show the same dose-response curves.

METHODS AND MATERIALS

Each insecticide was tested once according to the protocol described by Lahr *et al.* (1996), but using a wider range of concentrations than in previous tests. The tests lasted 96 hours; every 24 hours observations were made. For all three insecticides, the minimum and maximum concentrations were 0 and 5000 ng/L respectively. Characteristics of the three insecticides are given in Table 1.

Table 1: Characteristics of three tested pyrethroids used in Desert Locust control.

Active ingredient	Commercial name	Conc. (g a.i./L)	Solubility in water (ng/L)	Formu-lation	Manufacturer	Year pur-chased	Batch number
beta-cyfluthrin	Bulldock©	12	2000	ULV	Bayer, Germany	1996	AB0101527 FL0212
deltamethrin	Decis©	7.5	< 200	ULV	AGREVO, France	1994	1.7x0206
lambda-cyhalothrin	Karate©	40	5000	ULV	Zeneca, Côte d'Ivoire	1995	F95/-/128MB

RESULTS

For each insecticide, the effects are shown separately for each observation (24, 48, 72 and 96 hours) and for each category of effects ("fit", immobile, debilitated and dead; Lahr *et al.* 1996). The results are shown in Figs. 1a-d.

The dose-response curves were roughly the same for each of the three pyrethroids. After 24 hours (Fig. 1a) most animals in the lower part of the concentration range were "fit", while the animals in the intermediate concentrations were mostly debilitated. In the highest concentrations almost all organisms were immobile or debilitated. Dead organisms were not found in the highest concentrations, only a few in lower ones. After 48 hours (Fig. 1b) death started to occur, however most of it in the lowest concentrations. The number of debilitated animals decreased in almost all concentrations. Immobility increased.

After 72 hours death occurred in the majority of the higher and in many lower concentrations, but its frequency was surprisingly low in the intermediate range. The dose-response curve for death for each of the three compounds therefore displays a dip (Fig. 1c). Debilitation at this time had almost vanished and immobilization in the tests had further decreased. At the end of the tests (96h) most of the organisms were either alive or death. The "dip" for dead animals was still observed for deltamethrin and lambda-cyhalothrin (Fig. 1d). A few fit *S. sudanicus* in these two tests were still found in the middle range of the concentration series.

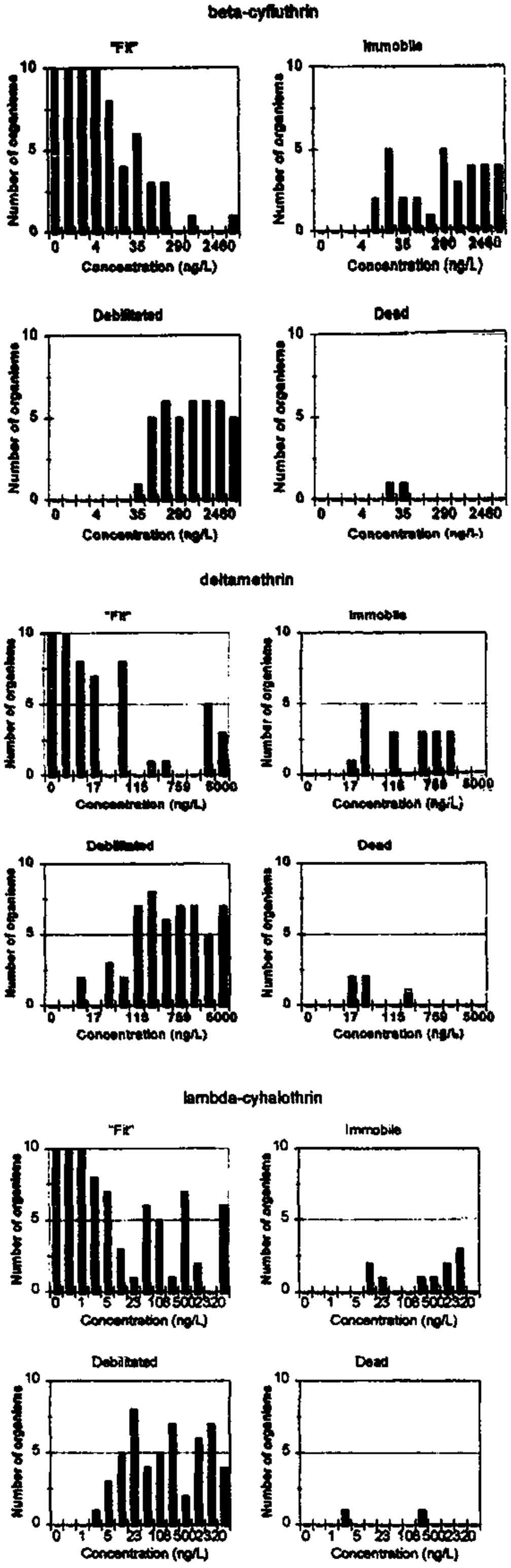

Figure 1a: Effects of three pyrethroids on *S. sudanicus* after 24 hours.

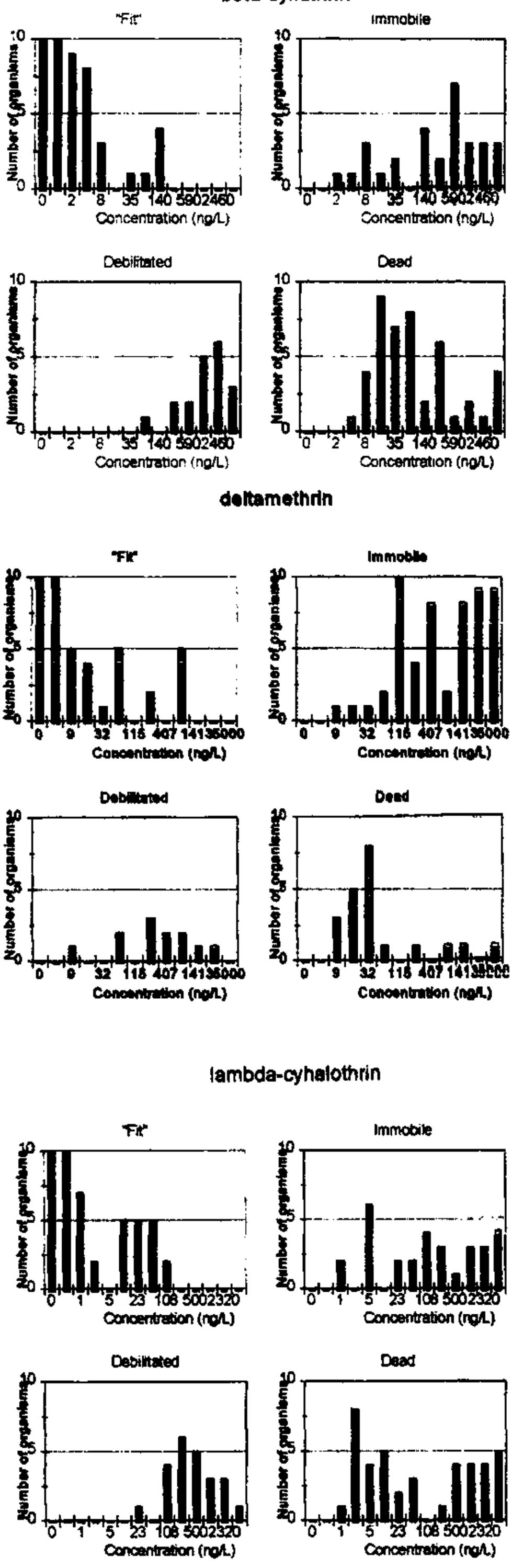

Figure 1b: Effects of three pyrethroids on *S. sudanicus* after 48 hours

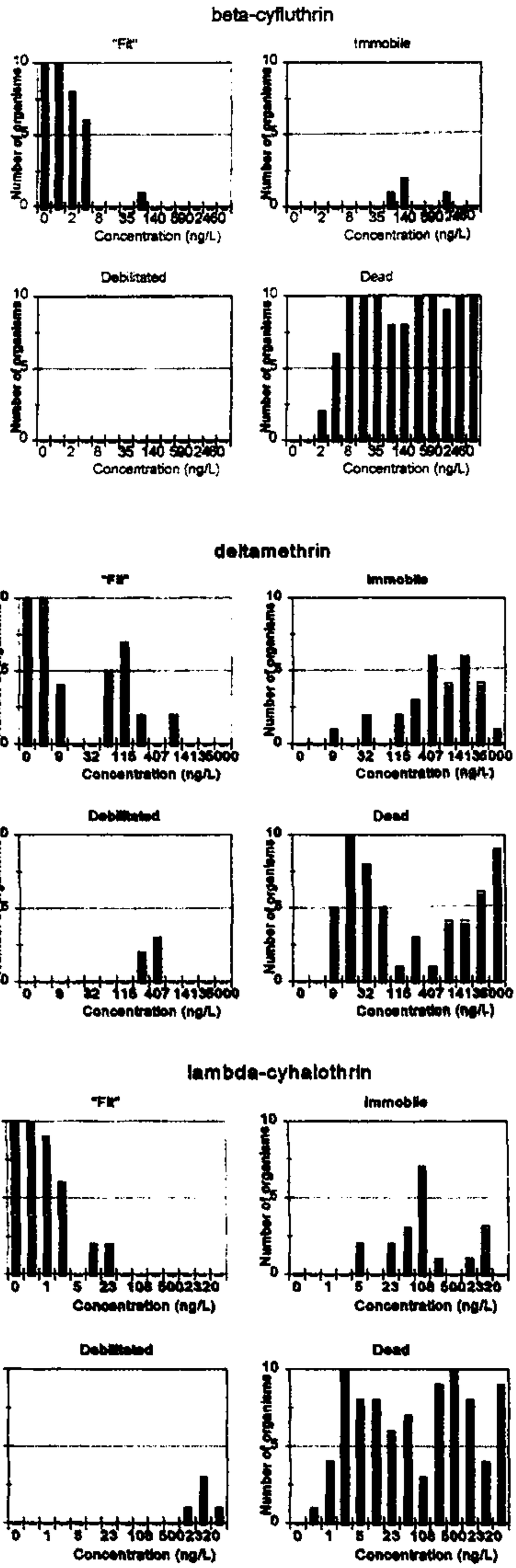

Figure 1c: Effects of three pyrethroids on *S. sudanicus* after 72 hours

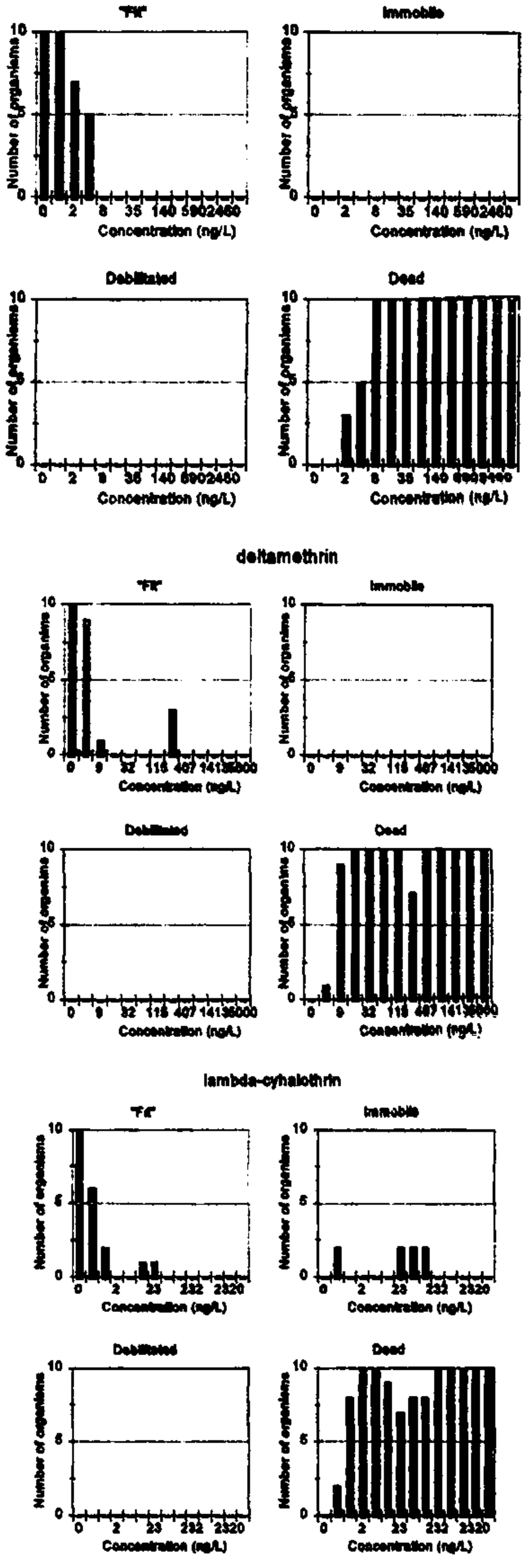

Figure 1d: Effects of three pyrethroids on *S. sudanicus* after 96 hours

DISCUSSION

Synthetic pyrethroids are neurotoxins acting on the axons in the peripheral and central nervous system in organisms through interaction with sodium channels. A single dose produces toxic signs, such as tremors, hyperexcitability and paralysis. In some cases these signs may be reversed and the animals recover. At near-lethal dose levels, synthetic pyrethroids cause transient changes in the nervous system, such as axonal swelling and/or breaks in myelin degeneration in the sciatic nerves (WHO 1990).

A possible explanation for the delayed occurrence of death in intermediate concentrations may be the behavioral response of *S. sudanicus* to the pyrethroids in connection with their metabolic rates. Hyperactivity (debilitation) most likely leads to higher metabolic rates which may enable the organisms, at least temporarily, to detoxify or depurate the compounds more quickly. At intermediate concentrations, where debilitation is strongest in the beginning of the tests, some of the effects of the insecticides may be delayed or even temporarily reversed through this mechanism. At still higher concentrations, the uptake is probably too high to be undone. Low metabolic rates at the lowest concentrations may contribute to a rapid lethal effect because these concentrations are too low to provoke debilitation (Fig. 1b). Eventually all organisms exposed to intermediate and high concentrations will die, but at different rates.

Some pyrethroids like deltamethrin are, apart from their effect on the nervous system, also known to cause desiccation. Everts (1990b) found proof for this effect in a group of terrestrial arthropods, the lyniphiid spiders. It can not be excluded that such an effect also occurs in aquatic invertebrates, but to our knowledge this has never been studied.

Immobilization and debilitation of *A. sardeus* by insecticides has never been observed with any of the insecticides tested in the past (Lahr *et al.* 1996; Marquenie *et al.* 1997). Insecticides only provoke lethal effects in this organism and dose-response relationships were always of the classical type. The three pyrethroids were no exception to this. The nervous system of these backswimmers is probably different from that of fairy shrimps.

Synthetic pyrethroids can be distinguished into two classes according to their toxicity mechanism: Type I (T-syndrome) and Type II (CS-syndrome). Type II pyrethroids have an α-cyano group, which provokes prolonged increases in permeability of the sodium channels in nerve cells. Type I pyrethroids do not possess an α-cyano group. The latter decrease the length of the nerve impulse trains in sensory organs. All three compounds tested here, were Type II pyrethroids. The described two-peak phenomenon for lethality in *S. sudanicus* after 72-96h was observed for each of them. We do not know if the same would be observed with *S. sudanicus* and Type I pyrethroids.

CONCLUSION

- Deltamethrin, lambda-cyhalothrin and beta-cyfluthrin caused different effects in *S. sudanicus* over a wide range of concentrations (from 1 to 5000 ng/L). For each of them death occurred less rapidly at intermediate concentrations. This phenomenon may be connected to a high instant occurrence of debilitation at the beginning of the tests.

RECOMMENDATIONS

- Further (physiological and biochemical) research into the toxic mechanisms of synthetic pyrethroids in *S. sudanicus* would be necessary to explain our observations.

- Additional and more detailed bioassays may also reveal more about the exact effects of these insecticides in *S. sudanicus*. One could think of exposing individual animals to similar concentrations in separate small beakers instead of in one to investigate in which order and at how fast they develop different symptoms of intoxication.

PART 2

ASSESSMENT OF IMMOBILITY IN *STREPTOCEPHALUS SUDANICUS* USING ITS PHOTOTACTIC RESPONSE

INTRODUCTION

For *S. sudanicus* it can sometimes be delicate to classify its exact reaction after exposure to insecticides. For non-pyrethroid insecticides there are three different categories of behavior that can be distinguished in these bioassays:

1 "fit": the organisms swim actively and freely in all directions; they do not show any signs of intoxication,

2 "immobile": the organisms lie on the bottom of the beakers and do not or hardly swim; their thoracic appendages do still move (in routine tests with *S. sudanicus* individuals are called immobile when they do not swim during at least one minute of observation), and

3 dead: the fairy shrimps lie on the bottom of the beakers and their thoracic appendages have stopped moving; their color turns from transparent to white.

Sometimes an apparently immobile organism may suddenly swim or jerk a little when it is observed for several minutes. The label "immobile" may therefore be somewhat arbitrary. A more objective method to assess immobility of *S. sudanicus* would be desirable. In an attempt to develop a quick, reliable and objective method to judge immobility of this species, several experiments with light sources were conducted.

When *S. sudanicus* are transferred from the field to the laboratory, it was observed that they reacted strongly to the light source above the aquariums. The aquariums were illuminated by fluorescent lamps. The animals would swim up and down in vertically orientated circles, all of them together forming a doughnut-shaped pattern. When the light is switched off, the organisms abruptly and randomly scatter through the aquarium. Given this phototactic response, we figured it would be possible to develop a method to assess the immobility of *S. sudanicus* using a light source. It was hypothesized that organisms which are still fit would react more strongly to light than the ones that are immobilized by their exposure to insecticides.

Some preliminary observations were carried out to see if immobile behavior also occurred in healthy organisms. Besides, the reliability of the reaction was tested, *i.e.* it was established if all *S. sudanicus* reacted to the light-source. Four different properties of phototactic behavior were investigated: to which color of light they would react most strongly, when this reaction would occur, how long it would last, and if the reaction would be stronger in a well lighted or in a dark room. At the same time light sources were also applied during some of the toxicity tests described by Marquenie *et al* . (1997) to find out if the criterium of the phototactic response would modify the EC_{50} values.

METHODS AND MATERIALS

The tests were carried out in the same test water and under the same conditions as described by Lahr *et al.* (1996).

General observations on behavior

To study the normal behavior of the organisms, two glass beakers were filled with 2L of test water. No insecticides were added, only 1000 µl acetone assure that circumstances in this test and in routine toxicity tests with *S. sudanicus* would be similar. Five healthy organisms were put in each beaker. This relatively small number was used because it was not possible to observe the organisms individually in beakers containing more specimens. During a period of two hours every aspect of the behavior that the animals displayed was observed. Extra attention was paid to any form of behavior similar to immobility. The 2h observations were carried out 24 and 48 hours after the organisms were put into the beakers. The timing of these observations corresponds with those in the routine tests. Tests were repeated two times with new organisms.

Natural reaction to different colors of light

The natural phototactic reaction of *S. sudanicus* was investigated in two separate experiments. In a first experiment the control beakers from the routine toxicity tests carried out by Marquenie *et al.* (1997) were used to investigate the animals' reaction to the sudden presence of light of different colors. On each occasion the organisms were observed for a couple of minutes. Six different light sources were used:

- a white fluorescent lamp,
- a blue bulb (40 Watt),
- a red bulb (40 Watt),
- a yellow bulb (40 Watt),
- a green bulb (40 Watt),
- a white bulb (60 Watt).

In a second experiment their reaction was assessed under different background light conditions. Four glass beakers were filled with 2L test water and 10 organisms. No insecticides were added, only 1000 µl acetone. Two glass beakers were placed in a dark room and two in front of a window. The beakers were placed on a sheet of white paper with a line, which divided the glass beakers into two equal compartments for observation. One by one the six light sources were held close to the wall of the beaker: the side where the bulb was held was called the proximate side and the other side the far side. The light sources were only switched on when there would be five organisms in each compartment. The numbers of fairy shrimps at each side of the line were counted after 10, 30 and 60 seconds. The tests were replicated several times. Any reactions beyond one minute were not investigated because longer observation times would not have been useful for the quick assessment of any response needed in the toxicity tests. The average percentage of organisms in the proximate side after 10, 30 and 60 seconds were tested for significance against those at the start of the tests (50%) using a two-tailed t-test for the comparison of two sample means (Sokal and Rohlf 1995).

Application of light sources to toxicity tests

The numbers of immobilized animals in the routine tests described by Lahr *et al.* (1996) and Marquenie *et al.* (1997) were counted by ordinary observation of swimming behavior. In several of these tests these numbers were also assessed using some of the light sources described above. The lamps were held next to the beakers during one minute and only individuals that swam freely during this time were called "fit".

Four different light-sources were used. Three of the light sources used were those which gave the

strongest reactions during the observations of natural behavior: the white bulb, the blue bulb and the fluorescent lamp (see sections 3.2.1 and 3.2.2). In addition, the red bulb which had no effect whatsoever during the previous observations, was used on a few occasions as a control.

The difference between the 48h-EC_{50} values calculated with both methods of observation were compared using the corresponding 95% confidence intervals. When the limits overlapped it was concluded that the EC_{50} values did not differ significantly. This method is not strictly valid, but it gives a rough indication (Robertson and Preisler, 1992).

RESULTS

General observations on behavior

During the observations without insecticides or light sources it was found that normal, undisturbed behavior of *S. sudanicus* in test beakers shows the following characteristics:

- *S. sudanicus* usually swims with its dorsal side down,
- it swims near the bottom of the test beakers most of the time,
- it displays more and less active swimming periods,
- it will very occasionally lie on the bottom of the beaker for a period of 30 seconds to 2 minutes, only moving its thoracic appendages (thus, it looks immobile),
- when actively swimming, it can flip over (sometimes very quickly) or spin around its axe,
- it sometimes shakes for some time while swimming in jerks,
- when it lies on the bottom of the beaker, it does so either on its back or on its side, but always next to the wall,
- when it is lying on its side, its thoracic appendages always point towards the wall of the beaker.

After 48 hours in the beakers (without food) active swimming behavior became less frequent. Inactive periods, when the organisms were lying on the bottom, also lasted longer (sometimes > 2 minutes). In general, they also swam more slowly. The pace of the movements varied: sometimes they all lied down on the bottom, but on other occasions they all suddenly started swimming together and spinning around their axes. These crude observations indicate that the organisms become less active after a few days in the beakers and that immobile behavior is observed, although infrequently, in *S. sudanicus* which are not exposed to insecticides.

Natural reaction to different colors of light

General observations

A summary of the observations in these tests are given below.

-White and blue bulbs:	Some of the animals swam away from the light, but after a few minutes they were dispersed normally through the beaker again.
-Fluorescent lamp:	Some of the organisms reacted on both switching the lamp on and off with a strong and sudden movement. When the lamp was turned on they usually swam away from it. Not all the organisms reacted similarly however, some did not even react at all.
-Green and yellow bulbs:	Some of the animals swam away from this light as well, but dispersed normally through the beaker after a short delay.
-Red bulb:	They did not react at all.

In brief, the most promising light sources to distinguish between fit and immobile individuals of *S. sudanicus* were the white and blue bulbs and the fluorescent lamp.

Phototactic reaction in a light and a dark room

The results of the tests are given in Fig. 2. The strongest and most significant reactions were observed 10 and 30 seconds after switching on the bulbs in the dark room. The sources that provoked these reactions were the green and white bulbs and the fluorescent lamp. However, to none of these light sources the reaction was very strong. At most, only half of the organisms in the proximate side of the beakers would swim to the far side.

Application of light sources to toxicity tests

Eleven standard toxicity tests were used. For eight of them observations were made using one single light source, but in three tests with beta-cyfluthrin four different light sources were used.

Among tests in which one single light source was used, none revealed a significant difference with the EC_{50} values calculated from the observations made in the usual way, *i.e.* without light (Table 2).

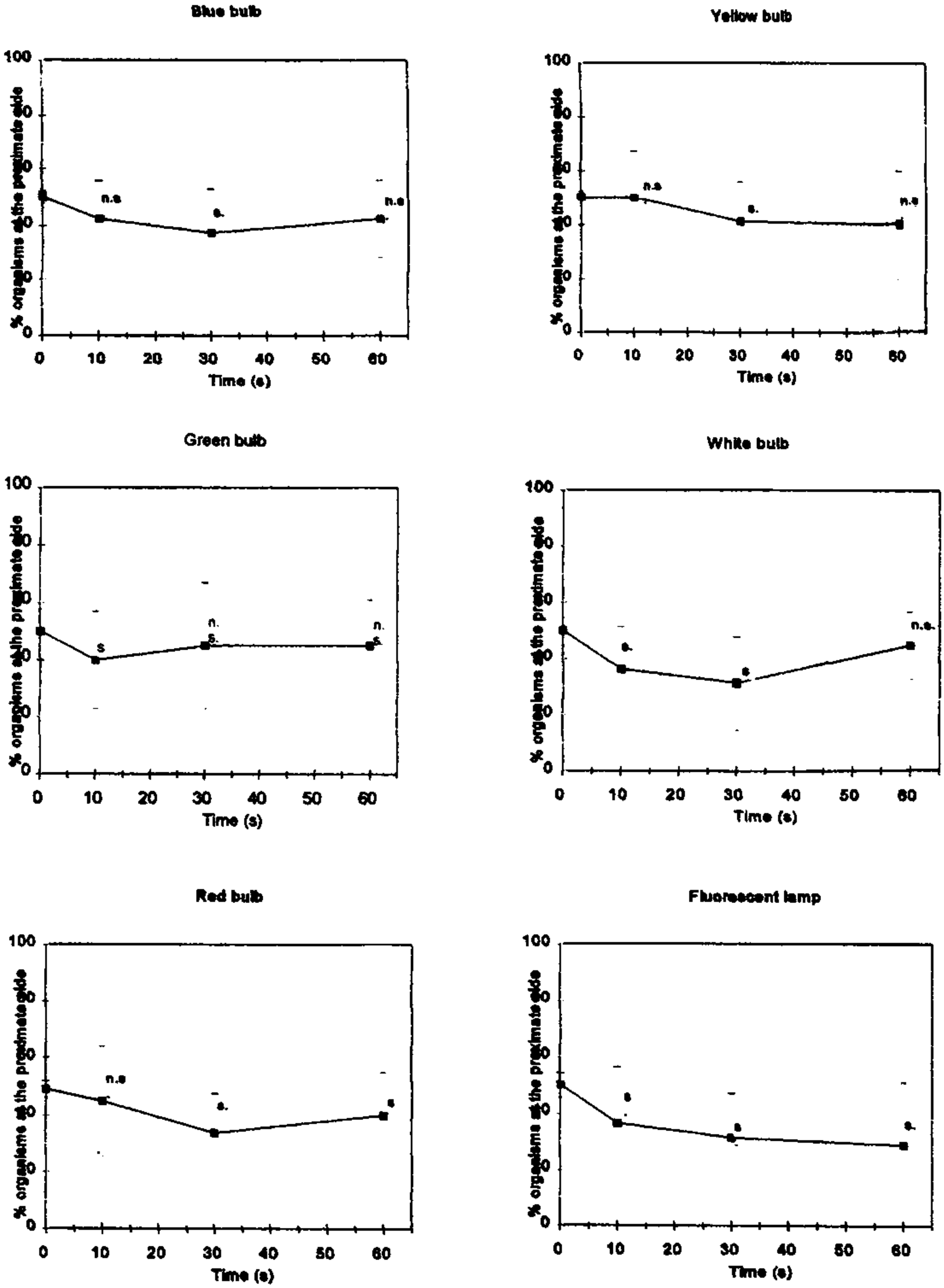

Figure 2a: Phototactic response of *Streptocephalus sudanicus* to different light sources in an illuminated room. The graph shows the average percentages (±s.e.) with their significance (s.=significant, n.s.=not significant) present in the proximate side of 2L glass beakers at different time intervals after switching on the light sources. Average values are from eight tests.

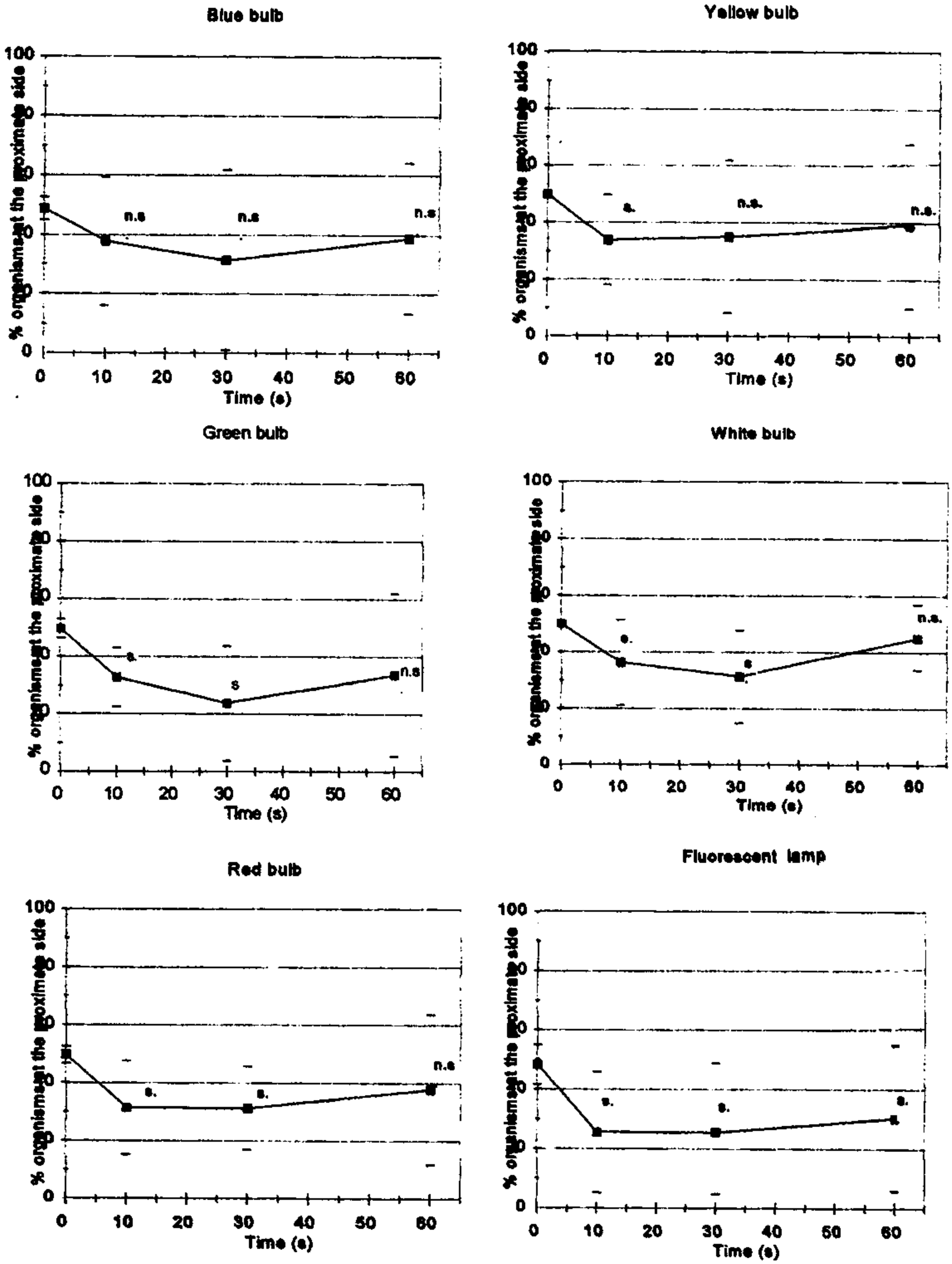

Figure 2b: Phototactic response of *Streptocephalus sudanicus* to different light sources in a dark room. The graph shows the average percentages (±s.e.) with their significance (s.=significant, n.s.=not significant) present in the proximate side of 2L glass beakers at different time intervals after switching on the light sources. Average values are from eight tests.

Table 2: 48h-EC_{50} values (µg/L) for tests in which immobilization was assessed with and without a light source.

Insecticide	Light source	Without light source		With light source	
		EC_{50}	95% CI	EC_{50}	95% CI
beta-cyfluthrin	blue	0.0046	0.0021-0.0103	0.0068	0.0029-0.0157
propoxur/phoxim	white	0.91	0.60-1.36	0.91	0.60-1.36
propoxur/phoxim	white	0.90	0.69-1.17	0.99	0.76-1.32
teflubenzuron	blue	1.66	0.91-3.03	1.65	0.92-2.98
teflubenzuron	white	0.44	0.32-0.60	0.44	0.32-0.60
triflumuron	white	0.141	0.071-0.280	0.141	0.071-0.280
triflumuron	white	0.261	0.133-0.511	0.261	0.133-0.511
triflumuron	fluorescent	0.261	0.132-0.517	0.260	0.130-0.520

Also, in the three tests which were counted using several light sources none of the results differed significantly from those obtained without a light source (Table 3).

Table 3: 48h-EC_{50} values (µg/L) for tests in which immobilization was assessed with and without different light sources.

Insecticide	Light source	48h-EC_{50}	95% CI
beta-cyfluthrin	none	0.00572	0.00291-0.01126
beta-cyfluthrin	red	0.00493	0.00273-0.00890
beta-cyfluthrin	blue	0.00493	0.00273-0.00890
beta-cyfluthrin	white	0.00572	0.00291-0.01126
beta-cyfluthrin	fluorescent	0.00493	0.00273-0.00890
beta-cyfluthrin	none	0.00304	0.00128-0.00724
beta-cyfluthrin	red	0.00692	0.00294-0.01625
beta-cyfluthrin	blue	0.00365	0.00159-0.00840
beta-cyfluthrin	white	0.00365	0.00168-0.00794
beta-cyfluthrin	fluorescent	0.00454	0.00218-0.00946
teflubenzuron	none	0.24	0.12-0.45
teflubenzuron	red	0.24	0.12-0.45
teflubenzuron	blue	0.24	0.12-0.45
teflubenzuron	white	0.24	0.12-0.45
teflubenzuron	fluorescent	0.27	0.14-0.52

DISCUSSION

Despite the strong phototactic reactions observed in *S. sudanicus* that arrived in the laboratory from the field and the experiences of several authors with fairy shrimps (*e.g.* Bernice 1972; Brendonck *et al.* 1995) responses to different sources of light observed in the beakers which are used for standard tests with *S. sudanicus* by the LOCUSTOX Project were not very strong. Even the strongest responses observed were rather feeble and the organisms would adapt to the light sources after a short time. Maybe the light sources used in this study were not strong enough. Brendonck *et al.* (1995) for instance used a 2000 Watt source.

CONCLUSION

- Lapses of immobile behavior do naturally occur in *S. sudanicus* in glass beakers used in acute toxicity tests. In unexposed organisms however, these lapses are much less frequent than in animals that are exposed to insecticides.

- The blue, white, green and fluorescent light sources used in these experiments all provoked a feeble photonegative response in *S. sudanicus*, but adaptation to these light sources would start after one minute and the animals would quickly relapse to normal behavior.

- When light sources were applied to distinguish immobile from unaffected organisms in acute toxicity tests, the EC_{50} values calculated from these observations were not significantly different from those based on the observations made without applying a light source.

RECOMMENDATIONS

The standard method of assessing immobilization of *S. sudanicus* in the standard acute toxicity tests of the LOCUSTOX-Project can not be improved using the phototactic responses to any of the light sources tested during the experiments described here.

PART 3

MODELING TOXICITY AND DEGRADATION OF FENITROTHION IN TESTS WITH *ANISOPS SARDEUS*

INTRODUCTION

Widianarko and Van Straalen (1996) recently developed a statistical model for the analysis of data from static toxicity tests with non-persistent chemicals. In traditional bioassays it is always assumed that no degradation of the compounds takes place, but when non-persistent substances are used, exposure may decrease in time because the toxicant disappears.

The new model was adapted from methods described by Kooijman *et al.* (1996). Widianarko and Van Straalen introduced a new toxicity parameter, the "initial median lethal concentration" (ILC_{50}). This is defined as "the external initial concentration that will cause 50% mortality after a defined exposure time".

For non-persistent chemicals such as insecticides, the degradation rate, characterized by the half-life, is a very important variable in determining ecological effects and it may affect LC_{50} values. The probability of dying of test organisms depends on the internal concentration of a toxicant. This concentration in turn depends on the external concentration in the test medium (which decreases in static tests with non-persistent chemicals) and on the elimination rate. The model assumes linear one-compartment toxicokinetics with exponentially decreasing input (first order degradation of the toxicant).

The external concentration at time t can be described as

$$C(t) = C_0 \exp(-k_0 t)$$

where

C_0 = external initial concentration (e.g. µg/g or µg/L)
k_0 = rate constant for degradation of the chemical in the medium (d^{-1} or h^{-1})

The half-life of the chemical is expressed as

$$t_{½} = \frac{\ln 2}{k_0}$$

An explicit expression for time- and concentration-dependent survival was derived by Widianarko and Van Straalen. The model predicts that, with increasing exposure time, survival will approach a nonzero baseline value for certain initial concentrations.

$$S(t) = \exp \frac{C_0 \ln 2}{\mu(k_2 - k_0)} [k_e(1 - \exp(-k_2 t)) - k_2(1 - \exp(-k_0 t))]$$

where

S(t) = survival rate at time t
µ = ultimate ILC_{50} reached when survival remains unchanged
k_2 = rate constant for elimination from the organisms

This formula can be used for the analysis of experimental data. Widianarko and Van Straalen (1996) did this for a terrestrial isopod exposed to soil containing the insecticide diazinon.

In this part of the report, it was investigated if the theoretical model could also be applied to the static aquatic toxicity tests used by the LOCUSTOX Project. To this extent long-term tests were carried out with the insecticide fenitrothion and *A. sardeus*. This species was used because the species can survive for longer periods in the test beakers without food. It was also tried to establish the half-life of the compound in test water through residue analysis for a comparison with the half-life derived from the toxicokinetic model.

METHODS AND MATERIALS

Tests

Two replicate tests were carried out according to the methods for *A. sardeus* described by Lahr *et al.* (1996). The tests lasted 168 hours instead of the ususal 48h. Six concentration levels of fenitrothion were applied: 0 (control), and 5.0, 7.5, 11.25, 16.75 and 25.0 µg/L. Each beaker contained 3L test water instead of the ususal 2L. Test 1 was executed with 20 organisms per beaker, Test 2 with 15.

Observations were carried out after 2, 4, 8, 12 hours, and for the remaining period every 12 hours. Once every 24 hours, the dead organisms were removed from the beakers to avoid bacterial growth.

Test 2 was carried out in duplicate. One series was used for the assessment of survival and measurement of chemical parameters. From the duplicate series, every 24 hours 300 mL water per beaker was collected for residue analyses.

Statistical analyses

The data from the tests were used to estimate the degradation rate constant k_0, the elimination rate constant k_2 and the ultimate ILC_{50} (μ) by applying the equation for S(t) to an iterative subroutine for non-linear regression from the statistical software package SYSTAT®.

RESULTS

Fig. 3 shows the survival of the organisms during both replicate tests. Indeed survival approached a nonzero baseline in the initial concentration of 5 µg/L after 132 hours in Test 1 and after 144 hours in Test 2. This is illustrated in Fig. 4 with the ILC_{50} values calculated with the RIZA-LC_{50} programme based on the method of Kooijman (1981). The calculated ultimate ILC_{50} values (μ) were 4.9 and 4.8 µg/L for the first and second test respectively.

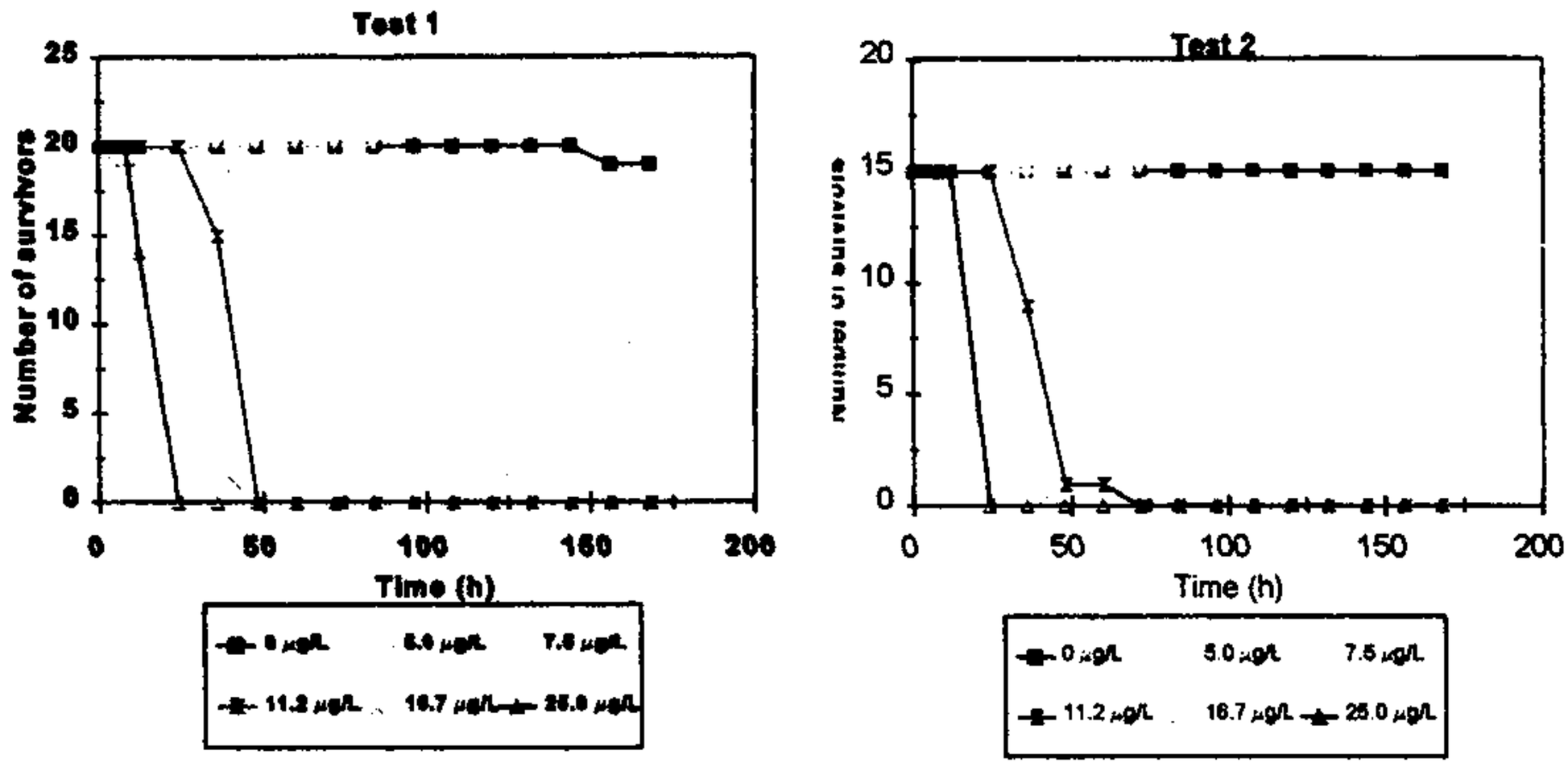

Figure 3: Survival of *Anisops sardeus* in two long-term toxicity tests with the insecticide fenitrothion.

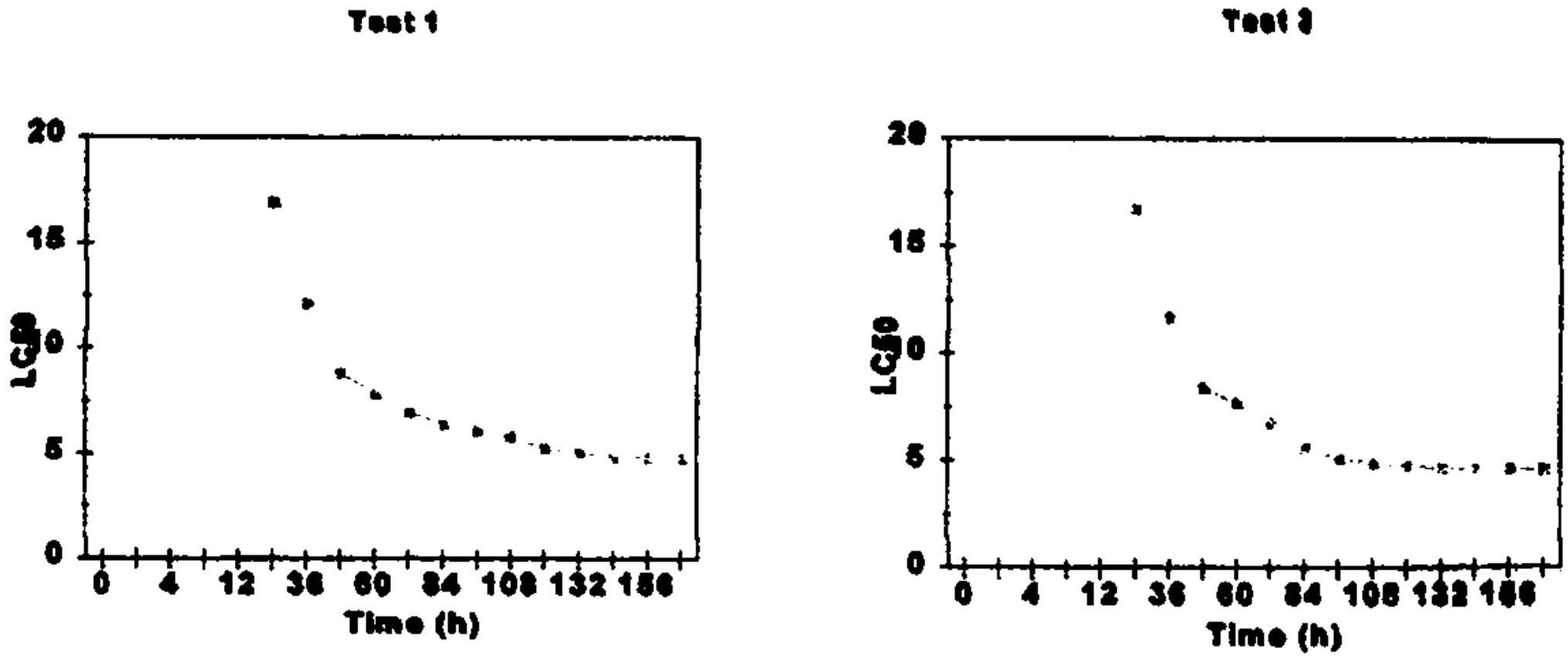

Figure 4: ILC_{50} values (µg/L) for *Anisops sardeus* in two long-term toxicity tests with the insecticide fenitrothion.

The estimated parameters k_0, k_2 and µ for both tests are given in Table 4.

With the degradation rate constant, k_0, the half-lives of fenitrothion in the tests were estimated: 99h for Test 1 and 33h for Test 2 (average = 66h).

Table 4: Parameter estimates for the toxicokinetics-based survival model for two replicate tests with *Anisops sardeus* and fenitrothion. k_0=degradation rate constant; k_2=elimination rate constant; µ=initial concentration causing an ulimate mortality of 50%; a.s.e.=asymptotic standard error of the estimate; C.V.=coefficient of variance.

		Test 1			Test 2		
Parameter	Unit	Estimate	a.s.e.	C.V. (%)	Estimate	a.s.e.	C.V. (%)
k_0	h^{-1}	0.007	0.002	28	0.021	0.017	81
k_2	h^{-1}	0.075	0.038	51	0.021	0.017	81
µ	µg/L	1.9	0.7	36	2.1	0.5	24

DISCUSSION

The µ-values obtained with the model were of the same order of magnitude, but more than two times lower than those calculated with the RIZA-LC_{50} programme. Also, the estimates obtained for k_0 and k_2 obtained for both tests were very different, despite the similarity of their µ-values.

The average estimated half-life of fenitrothion was 66h. The half-life of fenitrothion in river water is 50h (brochure of manufacturer Sumitomo). Lahr and Diallo (1993) reported a half-life of 43h in temporary ponds in Senegal. These values are in good agreement with the average half-life estimated by the model.

The values of k_0 were supposed to be validated by residue analysis. Unfortunately the residue analyses carried out during the second test were unsuccessful for unknown reasons (B. Gadji, pers. comm.). Therefore no comparison could be made between the estimated and measured k_0-values for the test itself.

CONCLUSIONS

- It was found that one of the most important assumptions in the model of Widianarko and Van Straalen, the principle that in the end survival in bioassays will approach a nonzero baseline value for certain initial concentrations, was true for tests with the insecticide fenitrotion and the species *A. sardeus*. After 132-144h the number of survivors in the initial concentration of 5 µg/L remained unchanged. The LC_{50} values remained stable from this moment until the end of the tests.

- The estimated average half-life for fenitrothion in the tests was in good agreement with those observed in river water and Sahelian temporary ponds.

- It is concluded that the theoretical model of Widianarko and Van Straalen can in principal be applied for further analysis of the static aquatic toxicity tests used by the LOCUSTOX Project.

RECOMMENDATIONS

Further tests need to be carried out to verify if the the theoretical model of Widianarko and Van Straalen can also be applied to other insecticides than fenitrothion and to tests with *Streptocephalus sudanicus*. But for this purpose the survival of *S. sudanicus* after 48 hours using the current test methods would need to be improved considerably.

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CHAPTER 6:

The impact of locust control insecticides on termites and ants in the arid zone of northern Senegal: A first assessment.

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SUMMARY

The effects of three insecticides used in Desert Locust control were assessed on non-target ants and termites in a semi-arid savanna grassland in northern Senegal. The study was executed during the rainy season of 1992, when the treatments were carried out, as well as for seveal weeks in same period of 1993, one year after treatment. The insecticides applied were fenitrothion (at an average rate of 775 g ai/ha), bendiocarb (at an average rate of 170 g ai/ha) and diflubenzuron (at an average rate of 105 g ai/ha). These dose rates were approximately 1.7x the rates recommended against the Desert Locust, to simulate a "worst case scenario". Each insecticide was sprayed on 4 plots of 1.6 ha, while 4 other plots were left as untreated controls. Termite actvity was assessed with baited traps and transect counts of foraging patches; ant activity by transect observations of nest entrances.

Treatments with fenitrothion affected catches of *Psammotermes hybostoma*, a dominant termite species in the semi-arid savanna of northern Senegal. A relative reduction of 70% was observed for a period of four weeks after treatment. One year after spraying, no effect could be discerned any more. Trap catches of all termites combined showed a relative reduction of 30%, one year after the application of diflubenzuron. There were indications that *Microcerotermes* sp., a wood termite, was affected by this chitin synthesis inhibitor, but the data were not fully conclusive. Bendiocarb did not have a significant impact on termite catches.

All three insecticides caused a relative increase in termite foraging patches, on the soil surface, between the 2nd and the 5th week after treatment. The most common species in the patches was *Odontotermes nilensis*. An explanation for this effect could not be given.

Ant activity was significantly affected after treatments with fenitrothion during the whole study period, including the year after treatment. A relative reduction of 50%, on average, was observed during the 5 weeks after spraying, while activity was still reduced with 35% one year later. Bendiocarb and diflubenzuron did not cause ecologically significant effects on ant activity in this study.

With respect to the assessment methods it was found that Pearce-traps provide a simple means for the assessment of insecticide impact on on termite activity. The technique may be optimised by determining the minimum periods the traps need to be in the ground so that, in addition to termite abundance, bait consumption can be measured reliably. The traps only caught two species in sufficient numbers, so additional assessment methods are required to draw more general conclusions about insecticide effects on non-target termites. Similarly, the results with respect to ant activity are limited to species with are either abundant or have clearly visible nest entrances. Other techniques, such as the use of pitfall traps or baits, will need to be used as well to get a more complete idea about insecticide impact on the ant fauna.

As a result of this study, it can be concluded that locust control with certain insecticides may have long term negative side-effects on both ant and termite activity. Therefore, given the fact that only a limited number of taxa was assessed, and the preliminary status of the study, this subject merits further attention.

INTRODUCTION

The role of termites and ants in arid and semi-arid ecosystems

Most Desert Locust control occurs in the arid and semi-arid parts of Africa, the Middle East and South-West Asia. Among the ecosystems concerned, deserts and semi-arid savanna grasslands are predominant. Termites (Isoptera) and ants (Hymenoptera, Formicidae) are among the most numerous macro-arthropods in these ecosystems (Le Houerou 1986, MacKay 1991, Polis and Yamashita 1991). Because of their number, their biomass, and their activity, termites and ants have a major impact on the structure and functioning of the ecosystems concerned.

Termites

Termites are detritivorous and/or phytophagous insects (a small number of species are pests of crops or timber). Most species live in symbiosis with cellulase producing micro-organisms in their gut, while the Macrotermitinae tend so-called fungus gardens in their nests. Both digestive strategies allow termites to break down organic matter almost completely, at assimilation efficiencies exceeding 60% (Wood and Sands 1978). Termites can regulate the environmental conditions within their nests, and to a more limited extent while foraging, which means that they can be active year-round, largely independent of the weather. Despite seasonal variations in above ground foraging, termite activity in deserts is more stable and continuous than climate-driven models of decomposition would suggest (Jones 1990).

Social insects, such as termites and ants, have a great impact on the physical structure of their environment. They construct extensive nest systems, and as a result they have a profound effect on redistribution of soil particles, on physical and chemical properties of soils, and consequently on vegetation (Wood and Sands 1978). Despite numerous studies of termites in Africa, their effects on the processes of soil formation and nutrient cycling have not been quantified in much detail. Few of the studies to date have been process oriented (Jones 1990).

There are two contrasting theories on the effects of termites on aeration and water infiltration in the soil. Termites either repack the soil so that it forms a compact structure reducing water infiltration, or they increase aeration and infiltration by incorporating organic matter into the soil and constructing galleries (Lobry de Bruyn and Conacher 1990). Studies in the Chihuahuan desert have shown that the latter process dominates (Jones 1990), although this may be different in other deserts.

There seems to be little doubt that termites generally reduce the organic matter content of the soils in which they are active. A large fraction of the organic matter which potentially could be incorporated into the soil, is consumed by termites. Up to 15% of litter production in a Sahel savanna was estimated to be consumed by termites (Wood and Sands 1978); this can amount to 100% in the Chihuahuan Desert (Jones 1990, Nash and Whitford 1995).

The impact of termites on the nutrient status of the soil is less clear. Jones (1990) argues that termite activity greatly reduces nutrients available to plants, and that these insects may be a major reason for the low productivity of African arid and semi-arid ecosystems. Lobry de Bruyn and Conacher (1990) suggest that in environments where the soil is inherently low in nutrients, termites may be an important contributing factors to nutrient enrichment. In this respect, it should be noted that in the absence of termites, hardly any nutrient enrichment will take place during the long dry periods in deserts and semi-deserts, when microbial breakdown is greatly reduced. On the contrary, high temperatures and wind will cause weathering and subsequent losses of organic matter and certain nutrients to the atmosphere (Noy Meir 1985).

Organic matter and nutrients sequestered by termites may return to the ecosystem in several ways. Termite faeces have generally low organic matter content. Faeces are used in the construction of galleries and fungus combs. For many subterranean species, these are relatively deep down in the soil, and this seems to contribute little to improving the plant root zone. Both faeces and constructional saliva are used for the construction of sheetings, galleries and runways by species which forage on the soil surface. Since these structures are generally very fragile, they erode rapidly and are constantly rebuilt. They may thus provide an more continuous input of organic matter and nutrients into the topsoil. It was

estimated that up to 50% of the soil turnover by *Macrotermes subhyalinus* in northern Senegal (of a total of 2000 kg/ha/year) was linked to the construction of sheetings (Wood and Sands 1978). This pathway has been little studied, and may well be underestimated as a contributing factor to soil fertility in arid zones.

A third pathway in which organic matter will return to the ecosystem is through termite tissue itself. Many organisms feed on termites, although often opportunistically. Ants are among the most important termite predators, but birds, mammals and reptiles all may feed on termites (Wood and Sands 1978). In deserts and semi-deserts, relatively more vertebrate predators appear to specialise on termite prey (Abensperg-Traun 1994). It has been suggested that the abundance of termites in arid regions of Australia has led to a remarkable development of the number of lizard species (Morton and James, cited in MacKay 1991). Similar developments may have occured as a result of the high abundance of social insects in other deserts. Macroarthropod detritivores, such as termites, are a much more reliable food source for organisms at higher trophic levels than herbivore arthropods, which depend on rainfall for vegetation growth. Therefore, much of the desert ecosystem appears to be structured around the detritivore part of the food web. Biodiversity and productivity of deserts and semi-deserts is likely to depend to a large extent on the existence of a properly functioning detritivore food web (Van der Valk 1997).

It is not clear what impact a reduction in termite activity in (semi-)arid ecosystems will have on the on the overall structure of the ecosystem. However, in spite of the uncertainties of the exact influences termites may exert, it is clear that they are extremely important organisms in arid ecosystems. For this reason, they have been called the "keystone" species of deserts (Jones 1990, MacKay 1991, Nash and Whitford 1995).

Ants

Ants are highly succesfull organisms in almost all ecosystems of the world (Hölldobler and Wilson 1990). Biomass and abundance of ants are also high in deserts and semi-arid ecosystems (MacKay 1991, Heatwole and Muir 1991). Yet most information on their importance in deserts is purely descriptive (MacKay 1991). Desert ants may be obligate predators on other arthropods or granivorous harvesters. Many, though, are omnivores that can adapt their feeding strategies to the varying abundance of different types of food.

As is the case with termites, ants tend to have a profound impact on soil structure and chemical composition. However, their is much less work on the relationship between ants and soil than on termites and soil (Lobry de Bruyn and Conacher 1990). Soil turnover rates have been reported to vary worldwide from 0.05 to 11.4 tons/ha/year. Contrary to what was the case for termites, there seems to be more agreement about the positive effect ants have on soil infiltration and aeration. Also, nutrient enrichment is generally observed in and around ant nests, although this may vary according to location and soil type (Lobry de Bruyn and Conacher 1990, Mackay 1991, Whitford and DiMarco 1995). The latter authors observed 2-5 fold increases in annual plant cover around harvester ant nests, in sub-shrub grassland in a Chihuahuan Desert watershed. Le Houérou (1986) cites studies that show a tenfold increase in productivity of annual plants around ant nests in the Algerian Sahara.

The relationships between plants and ants are numerous and variable. Several forms of symbiosis are mutualistic, benefitting both plant and ant. They include processes such as protection of the plant against herbivores, seed dispersal and "pruning and weeding" (Hölldobler and Wilson 1990). Seed harvesting ants can have large effects on the composition of desert plant communities. They remove seeds for consumption, thus affecting the potential competition between plants. They also assure dispersion of seeds (myrmecochory), and thus may change the spatial distribution of desert plants. In Australia, for instance, ants are responsible for the distribution of seeds from *Acacia* trees (MacKay 1991), and this may be the case in semi-arid Africa as well.

As was the true for termites, evidence shows that ants are very important components of arid ecosystems (MacKay 1991).

Termites, ants, and pesticides

Since certain species of termites may also be important agricultural pests or attack timber, their control has received wide attention for decades. Termite control with insecticides has turned out to be notoriousy difficult. Often, highly persistent insecticides are required for effective control, although more recent developments with pathogens and insect growth regulators show some promise. It is therefore tempting to conclude that locust control, with insecticides of generally low persistence, will probably not have much impact on termites in the ecosystems concerned. However, this need not necessarily be so. It should be borne in mind that the termites attacking timber and, especially, root crops tend to lead an almost entirely below-ground life, thus effectively avoiding exposure to insecticide sprays. This is not the case for species which forage on the soil surface, such as harvester termites and sheeting/runway builders. Large parts of their worker caste may be exposed to insecticides, and contaminated material may be taken into the nest where it is fed to the larvae.

Gueye and Everts (1990) observed a 60% reduction in the activity of sheeting building termites, 4 weeks after an aerial treatment with fenitrothion (825 g ai/ha) in northern Senegal. Diflubenzuron, applied at 83 g ai/ha, did not appear to affect activity. They caution that their study should be considered as very preliminary, given the limited number of observations carried out.

Side effects of insecticides on ants have been observed more often, also in Africa. In reviews of the impact of insecticide use in tsetse fly control in Africa, ants are mentioned to be among the first affected arthropods (Everts and Koeman 1987, Van der Valk and Koeman 1988). Tingle (1993) observed a short term effect on one ant species after tsetse fly control with deltamethrin, and provides indications that DDT applications may have affected colony health of a number of species in the longer term.

Large numbers of dead ants have also been observed after locust and grasshopper control (Van der Valk 1990, Balança and de Visscher 1996). It is, however, not always clear to what extent such high casualty counts also have consequences at population level. As Tingle (1993) points out, due to the colonial habit of ants, killing large numbers of workers away from the nest may have little impact on the survival of the colony as a whole. Van der Valk (1990) showed that a relatively high dose of fenitrothion (825 g ai/ha) reduced ant activity with 80% for at least 2 weeks, in a semi-arid savanna. Johannessen (1991) evaluated fenitrothion applications against grasshoppers (76 and 158 g ai/ha) and found reductions in pitfall catches of 50-60% up to 6 days after treatment. Keith (1992) observed a initial 75% reduction in pitfall catches and bait station visits after aerial malathion treatments at 750 g ai/ha. Balança and de Visscher (1994) showed reductions in ant catches of more than 80% after treatments with pyridaphenthion (250 and 500 g ai/ha) and lambdacyhalothrin (20 g ai/ha). Effects lasted a minimum of 4 weeks in certain cases. The same authors studied applications of fipronil (1 and 2 g ai/ha) in Niger (Balança and de Visscher 1996). They observed a 100% reduction in activity on ant trails directly after treatment, and 20-70% less visits to bait stations. These actvity parameters had recovered to control levels by day 10 after treatment, in most cases.

Some of these studies suggest that locust control, even with relatively non-persistent insecticides, may reduce ant activity for relatively long periods. This could have consequences for colony health, but observations are generally not carried long enough to substantiate this. Further evidence for population level effects comes from studies in agro-ecosystems. It has been observed regularly that insecticide use may reduce predatory ants populations, with pest insects subsequently becoming a problem where they weren't one before (Perfecto 1990, Way and Khoo 1992).

The study below attempts to make a preliminary assessment of the impact of locust control on termites and ants in a semi-arid savanna ecosystem in northern Senegal. In addition to fenitrothion, an often studied insecticide, the carbamate bendiocarb and the chitin synthesis inhibitor diflubenzuron have also been included in the study. Apart from being the first study to look at termites, this work distinguishes itself from the previous studies in the duration of the observations, which were carried out up to one year after the treatments. Its objectives are both to evaluate insecticide impact, as well as to get a better idea about the applicability and validity of the methods that may be used in such assessments.

METHODOLOGY

Study area

The study was carried out in northern Senegal in a hot arid savanna ecosystem. The experimental plots were located 17 km south-west of the town of Richard Toll, approximately 2 km from the village of Thiago (15"43'W, 16"20'N). The site is characterized by sandy soils, poor in organic matter. The herb layer of the vegetation is dominated by *Cenchrus biflorus*, *Tribulus terrestris* and *Cassia* spp. Tree and shrub cover is low and consists mostly of *Balanites aegyptiaca*, *Acacia senegal*, *Acacia raddiana*, and *Boscia senegalensis*. Small depressions scatterred through the zone have slightly heavier soils, and a denser vegetation. Annual rainfall in the area normally varies between 100 and 300 mm and falls from July to September. In 1992 and 1993 it was 140 and 270 mm respectively, at the Richard Toll meteorology station.

Study design

The study was designed according to the Before-After Control-Impact (BACI) principle described by Stewart-Oaten *et al* (1986). In the design, paired samples are collected several times before and after the insecticide application, simultaneously (or nearly so) in both the Control (untreated) and Impact ([to be] treated) plots. The basic idea behind this design is that there can be natural differences between Control and Impact sites, and temporal variability operating on a large spatial scale influencing both sites similarly. By sampling in both treated and untreated plots repeatedly during Before and After periods, the design "controls" for such natural variation (Stewart-Oaten *et al* 1986, Bence *et al* 1996). Treatments were replicated in space to allow for stronger inferences about the general validity of treatment effects (Eberhardt and Thomas 1991).

Sixteen experimental plots were layed out in the area. Each plot measured 125 by 130m (1.63 ha). Interplot distance was at least 150m, and often more. Plots were located mostly in the depressions in the area, where vegetation was slightly denser than in the grassy plains in between, and would stay green longer.

The plots were blocked in 4 groups of 4 plots each. This was done to allow for a light gradient in the study site. Plots were blocked so that vegetation and topography appeared more homogeneous within the blocks than between them. Each block received treatments with the 3 different insecticides while the remaining plot was left untreated as a control.

Sampling started in 1992 on 17 August and lasted until 16 October. Insecticide treatments were carried out from 23 to 26 September. In 1993 the plots were resampled from 7 to 10 September and again from 12 to 26 October to assess if any longer term effects had occurred.

Insect assessments

Termite activity

Termites were caught using a trap adapted from the one described by Pearce (1990) (referred to as "Pearce-trap" in the rest of this report). It consists of two cardboard disks (diameter 10cm, 2mm thick) sandwiched between two glass plates (10 x 20cm). The cardboard disks had been soaked for several hours in a 1:3 solution of molasses and water [1)]. Nine traps were horizontally placed, 5 meters apart, in the soil at the centre of each plot at a depth of 2-4cm. At this depth, the traps were expected to attract termites species which are active on or just below the soil surface. After placement, the traps and the soil around them were humidified using a watering-can.

[1)] In preceding tests, the addition of molasses had proved highly effective in increasing the catches.

Traps were placed once a week, always exactly on the same spot. After 4 days in the ground (except for the first two trapping sessions which were left longer), the traps were removed and immediately put in plastic bags to avoid the termites from escaping. Sampling was as a rule done between 8:30h and 11h, before the soil had heated up too much and the insects would move deeper down.

The termites were collected from the traps within 48 hours after sampling, killed, and stored in 70% ethanol until further identification and counting. The bait disks were labelled and stored as well. At the end of the study the surface area of the disks was measured using an optical leaf area meter. Surface area loss was used as an indication of termite feeding activity.

Termite activity was also assessed directly along two permanent transects which had been marked out in each plot. The transect was 75m long and 2m wide. Once a week the number of active termite foraging patches (sheetings and galleries on the soil and dead vegetation) were counted in the transect. A foraging patch was considered active if live termites were observed, or if freshly constructed galleries existed within the patch. Care was taken to minimize disturbance to the foraging patches during the weekly counts. Assessments were carried out from 8h to 11h, before the temperature would be too high and insect activity would diminish.

Traps were generally placed and collected the same day on all plots, or when this was not possible on consecutive days. The same was the case for transect observations. Completely simultaneous sampling was not possible, however, immediately after treatment since spraying was done over a period of 4 days.

Ant activity

Ant activity was assessed weekly during the same transect counts as for termites. The number of active ant nest entrances was noted. A nest entrance was considered active if ants were observed entering or leaving, or if nest debris had been freshly deposited around it. The latter case could easily be distinguished from old nest debris because the higher humidity content of the fresh material, which resulted in a deeper red-brown colour.

Insecticide treatments

The following three insecticides were applied: fenitrothion (Sumithion® 500 g a.i./L UL), an organophosphate; bendiocarb (Ficam® 200 g a.i./L UL) a carbamate; and diflubenzuron (Dimilin® 450 g a.i./L ODC), a benzoyl-urea chitin synthesis inhibitor. The latter insecticide was diluted down to 60 g a.i./L with diesel oil. All three insecticides were to be applied at approximately double the dose rate considered effective against the Desert Locust (FAO 1996), to simulate a worst case scenario (i.e.: effective rates are: fenitrothion at 450 g a.i./ha; bendiocarb at 100 g a.i./ha; and diflubenzuron at 60 g a.i./ha).

A spinning disk sprayer (MicroULVA®) was used, powered with five 1.5V dry batteries. According to the manufacturer's information this was expected to generate a droplet diameter (VMD) averaging 60-80µm. Treatments were carried out in a crosswind, using a track spacing of 10m. Flagmen marked the start and finish of each spray run. The walking speed of the spray operator had been calibrated prior to the treatments, and additionally every spray run was timed to allow for corrections during spraying. Windspeed and temperature were measured at the start and at the end of the application.

Residue analysis

Vegetation and soil samples were taken at different times after treatment for residue analysis. *Tribulus terrestris* leaves and stems were sampled along a diagonal in each plot. This species was chosen since it was the most abundant herb in the area. Additionally, its low-lying morphology increases the probability that ants and termites come into contact with the plant. Soil cores, to a depth of 4 cm, were taken along a diagonal in each plot.

Samples treated with bendiocarb and fenitrothion were stored in a freezer, until analysis at the Crop Protection Directorate's residue laboratory in Dakar. Those treated with diflubenzuron were air-dried and

then stored in the dark. Bendiocarb and fenitrothion residues were analysed using gas chromatography; diflubenzuron using high performance liquid chromatography. Details about the sampling, handling, extraction and analysis procedures are described by Gadji (1993b).

Data analysis

The effect parameter used in all analyses was the (log-transformed) difference between the treatment value (count or measurement) for a given sampling date, and its paired control value within the same block (Stewart-Oaten *et al*, 1986). This pairing of treated and control values within each block was done under the assumption that variability within blocks was generally less than among blocks (see 2.2). The effect parameter was calculated as "ln(treated count + 1) - ln(control count + 1)". Percentages were transformed using an arcsine transformation (Sokal and Rohlf 1981), resulting in an effect parameter calculated as "treated arcsin$\sqrt{}$(%/100) - control arcsin$\sqrt{}$(%/100)".

One or more values of the effect parameter after treatment were then tested against the average effect parameter before treatment, using sampling dates as (pseudo)replicates. Otherwise said, one tests if the difference of counts between the treated and control plots has significantly changed after treatment when compared with the average difference before the treatment.

Since each block contained all treatments and a control plot, the study resembles a randomised complete block (RCB) design. However, a RCB analysis of variance (ANOVA) assumes that no interaction exists between blocks and treatments (Sokal and Rohlf 1981, Dutilleul 1993). This could not be excluded here *a priori*. Therefore, a two-way ANOVA, with explicit assessment of the interaction term, was applied instead.

The corresponding ANOVA table is given below (Table 1). This is a mixed model ANOVA with blocks considered a random factor and treatment as fixed (Sokal and Rohlf 1981, Bennington and Thayne 1994). The effect of blocks is not formally tested. This is because one is not free to randomly assign a plot to any one block, as the three plots within any one block are limited to that block. As a result, there is a high probability that a "restriction error" has to be taken into account, which means that there is no suitable denominator mean square (MS) over which to test the block MS (Sokal and Rohlf 1981). Anyway, one is not interested in block effects, since they are expected to exist and were the reason why the treatments were blocked in the first place.

The effect of the treatment ($MS_{\text{before vs. after}}$), which is our main interest in this study, is tested over the interaction term if the latter is significant. A significant interaction means that the effect of treatment is not generalised over all blocks. In such a case, the treatment effect will only be considered "ecotoxicologically significant", if it is significant over the interaction. When the interaction is not significant, one may decide to pool the interaction and error mean squares. There does not seem to be general agreement among statisticians if this should be done (Sokal and Rohlf 1981). Therefore, conservatively, MSs were not pooled if the F-ratio based on the error mean square was already significant. Only if this was not the case, and after verifying Bancroft's rules for pooling (Sokal and Rohlf 1981), were the interaction and error MS pooled and used as the denominator over which to test the treatment effect.

Table 1: Description of the analysis of variance used in the study to assess the effect of insecticide treatment on ant and termite catches and activity.

Source of variation	degrees of freedom	F-ratio	Conditions
Blocks	3	not tested	
Before vs. After (=effect of treatment)	1	1] $MS_{bef.\ vs.\ aft.} / MS_{interaction}$	if interaction is significant
		or 2] $MS_{bef.\ vs.\ aft.} / MS_{error}$	If interaction is not significant
		or 3] $MS_{bef.\ vs.\ aft.} / MS_{pooled\ error}$	if interaction is not significant, AND 2] is not significant, AND pooling of interaction + error terms is allowed
Interaction	3	$MS_{interaction} / MS_{error}$	
Error	4(t1-1)(t2-1)		
Total	4(t1+t2)-1		

t1 = number of dates (replicates) before treatment; t2 = number of dates (replicates) after treatment; MS = mean square

The size of the effect of treatment is expressed as the percentage reduction or increase in trap catches, compared to the 4 weeks before treatment, and corrected for any control variation. Effect size was calulated as:

$$\%\ relative\ effect = \left[1 - \frac{avg.\ TR\ after\ treat.}{avg.\ TE\ after\ treat.} \times \frac{avg.\ TE\ before\ treat.}{avg.\ TR\ before\ treat.}\right] \qquad (1)$$

with:
- *avg. TR* = *average abundance in the treated plots*
- *avg. TE* = *average abundance in the control plots*
- *before treat.* = *the 4 trapping weeks before treatment*
- *after treat.* = *one or more trapping weeks after treatment*

RESULTS AND DISCUSSION

Insecticide applications

The results of the insecticide applications are given in Table 2. The actually applied dose rates of the three insecticides were all approximately 1.7x the rates that are listed for Desert Locust control, slightly less than originally intended. Variation in application rates among the different plots was low, the maximum standard error being 5% (for bendiocarb). This suggests that the applications were carried out in a consistent manner. Conditions for ultra low volume drift spraying were acceptable, with wind speeds averaging above 1 m/s on most occasions (FAO 1992). Temperatures were relatively high (varying from 26 to 32 °C), but no treatments were carried out under convective circumstances.

As a result, initial insecticide deposition on vegetation was good (Table 2), and corresponds with residue levels measured in similar studies of ULV applications in the Sahel (Gadji 1993a, 1996, 1997). Intial residues in the top soil were much lower than those observed on vegetation. This is to be expected, given that drift spraying generates a mostly horizontal displacement of spray droplets, which will impinge primarily on vertical surfaces such as vegetation.The relatively high diflubenzuron residues on soil, when compared to the two other insecticides, was not proportional to its respective application rate. No obvious explanation could be found for this (Gadji 1993b).

Termite activity

Taxonomic distribution

Almost 370 000 termites were caught in the Pearce-traps during the study, belonging to three species: *Microcerotermes* sp., *Psammotermes hybostoma* and *Odontotermes nilensis*. Overall, the most abundant species was *Psammotermes hybostoma* (Desneux) (Rhinotermitidae, Psammotermitinae), a sand termite. It represented 51% of the total catch in the control plots. It was especially abundant in 1992 (Figure 1). The genus *Psammotermes* is typical of arid zones, and is present in the Palearctic, Afrotropical, Malagasy and Indomalayan biogeographical regions (Pearce and Waite 1994). *P. hybostoma* occurs from Senegal in the west, all along the Sahara and Arabian deserts to (at least) Kuwait in the east. (Lepage 1972, Pearce *et al.* 1986, Ayyad and Ghabbour 1986,

Table 2: Treatment parameters of the plots sprayed in 1992. All plots measured 130x125m (1.63 ha). Applications were done with a hand-held MicroULVA spinning disk sprayer, with a red restrictor. Temperature and humidity were determined at the start of treatment; windspeed is the range over the treatment period. Insecticide residues are according to Gadji (1993b); soil residues were measured in top 4 cm.

Plot number and insecticide	Date	Hour		volume applied (L)	Dose applied (g a.i./ha) (rounded off)	Temperature (°C)	Relative humidity (%)	Wind speed (m/s)	Initial insecticide deposit mg a.i. / kg fresh weight	
		start	end						vegetation	soil
fenitrothion										
1	23 Sept.	8 : 05	8 : 55	2,59	795	28	85	1.1 - 2.2	87	n.a.
4	23 Sept.	8 : 00	8 : 40	2,31	710	27	92	2.0 - 3.2	86	0,25
8	23 Sept.	9 : 00	10 : 30	2,6	800	31	67	0.5 - 2.5	62	0,13
11	23 Sept.	9 : 35	10 : 25	2,57	790	31	73	0.8 - 1.9	95	n.a.
bendiocarb										
2	25 Sept.	8 : 05	9 : 15	1,37	170	26	71	1.0 - 2.5	27	0,03
5	25 Sept.	9 : 50	10 : 40	1,58	195	31	63	1.0 - 2.5	51	0,03
10	25 Sept.	9 : 50	10 : 25	1,36	165	32	n.a.	0.8 - 2.2	39	0,03
14	26 Sept.	8 : 30	9 : 10	1,24	155	28	71	1.0 - 4.0	n.a.	n.a.
diflubenzuron										
3	24 Sept.	7 : 55	9 : 25	3	110	28	80	1.0 - 3.0	48	3,24
7	24 Sept.	9 : 30	10 : 35	2,85	105	30	79	0.5 - 1.4	n.a.	n.a.
12	25 Sept.	7 : 55	9 : 00	2,76	100	27	71	1.4 - 2.5	n.a.	n.a.
16	24 Sept.	8 : 10	9 : 15	2,81	105	27	92	1.1 - 1.9	34	2,39

n.a. : not available

Abushama and Al-Houty 1988). In an earlier study in northern Senegal (Lepage 1972), it was also the most abundant termite species encountered, during several years of observations. It builds subsoil nests. *P. hybostoma* feeds mainly on dead vegetation, but has been observed on living *Acacia* trees and *Calotropis* plants as well (Lepage 1972, Pearce *et al.* 1986, Ayyad and Ghabbour 1986). It is not considered an important pest species in Africa (Pearce *et al.*, undated).

Microcerotermes sp. (Termitidae, Termitinae) represented 46% of the total catch in the control plots. This species was abundant in 1993, when it accounted for 85% of the catches (Figure 1). *Microcerotermes*, a small-sized wood termite, feeds mainly on dead wood and plant residues, but may attack live trees as well (Lepage 1972, Harris 1971). Species in this genus build nests either in the subsoil or located in trees. The genus *Microcerotermes* is widely distributed, and is represented in all biogeographical regions except the Nearctic (Pearce and Waite 1994). It is considered a potentially important pest species in Africa (Pearce *et al.*, undated).

The least abundant species in the Pearce-traps was *Odontotermes nilensis* (Emerson) (Termitidae, Macrotermitinae), representing only 3% of the trap catches in the control plots. The fungus growing genus *Odontotermes* occurs in the Afrotropical and Indomalayan regions (Pearce and Waite 1994). It feeds on dead wood and vegetation debris, but may also attack live trees and crops. It is considered a major pest genus in Africa (Pearce *et al.*, undated). *O. nilensis* is becoming a problem in the Senegal River valley where it attacks irrigated crops, especially in the dry season. Although certain species of *Odontotermes* are mound-building, *O. nilensis* does not appear to be so in northern Senegal, and has subsoil nests (Lepage 1972).

During the transect counts of foraging patches and sheetings, no distinction was made between species. However, from incidental sampling it was established that most patches belonged to *Odontotermes nilensis* and to a lesser extent *Psammotermes hybostoma*. *Microcerotermes* sp. was not observed in these patches, but low numbers of *Macrotermes subhyalinus* (Termitidae, Macrotermitinae), a mount building species, were sometimes encountered.

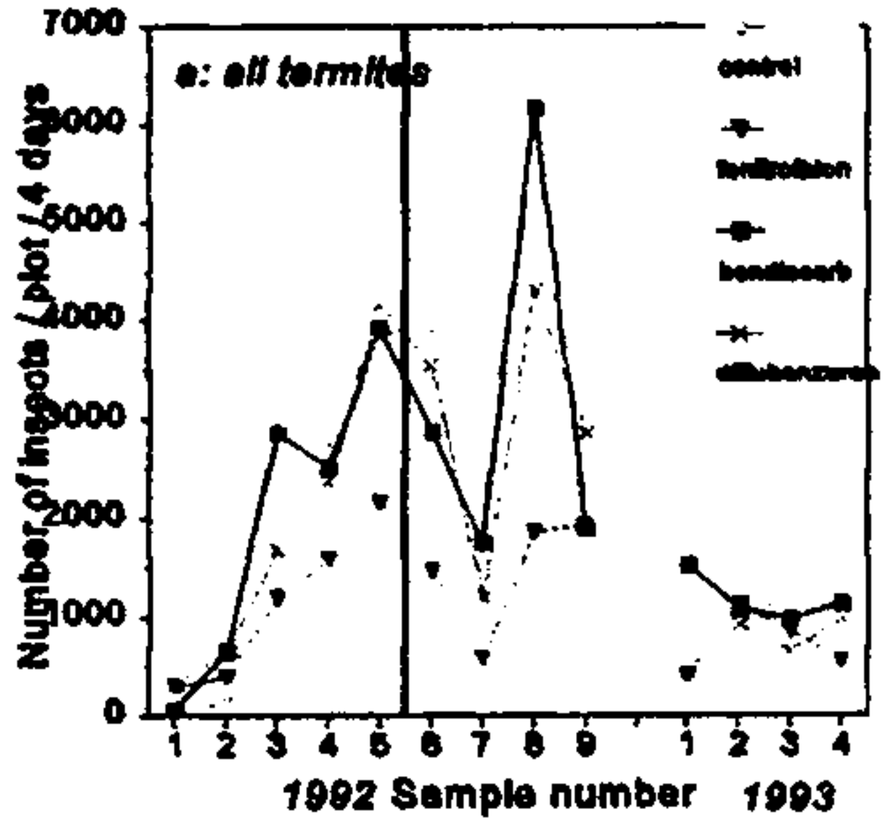
Pearce-traps

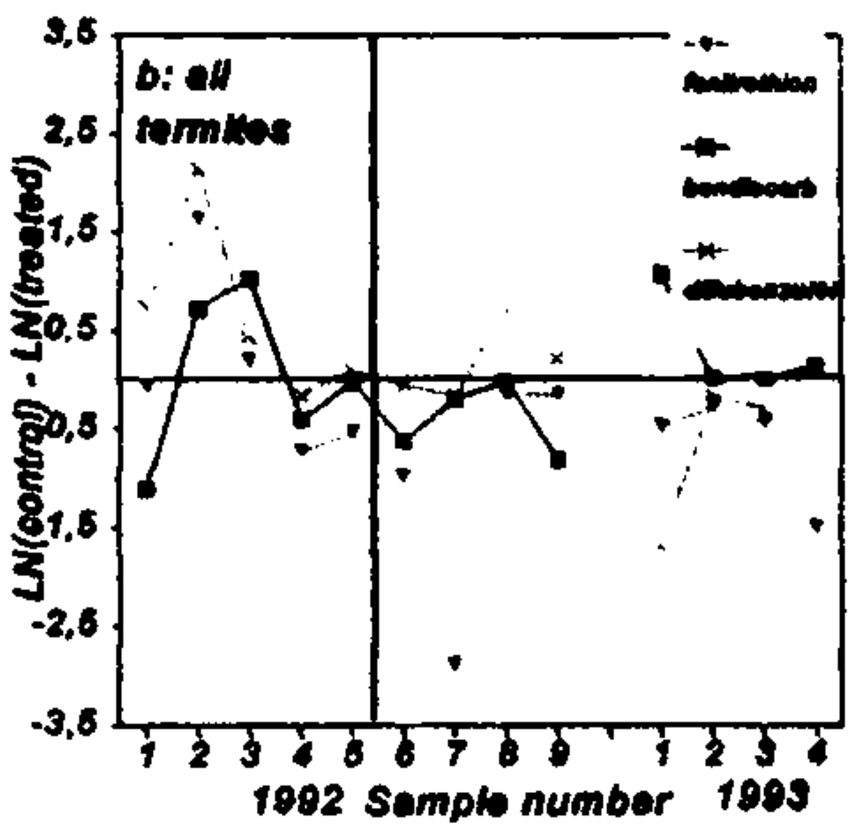
In 1992, Pearce-traps were placed at weekly intervals, 5 weeks before and 4 weeks after the insecticide treatments. In 1993, on the other hand, one sampling session took place during the second week of September (sampling date 1/1993 in the graphs and tables), and three others, at 4 day intervals, in October (sampling dates 2 to 4/1993). For the statistical analysis, effect parameters for one or several weeks after treatement (be that in 1992 or in 1993) were compared with the average effect parameter of the 4 weeks before the treatment. The very first week of sampling was omitted from the analysis, to minimize the effect of disturbance by the initial trap placement. These data are, however, shown in the graphs. Results of the ANOVAs are summarized in Table 3.

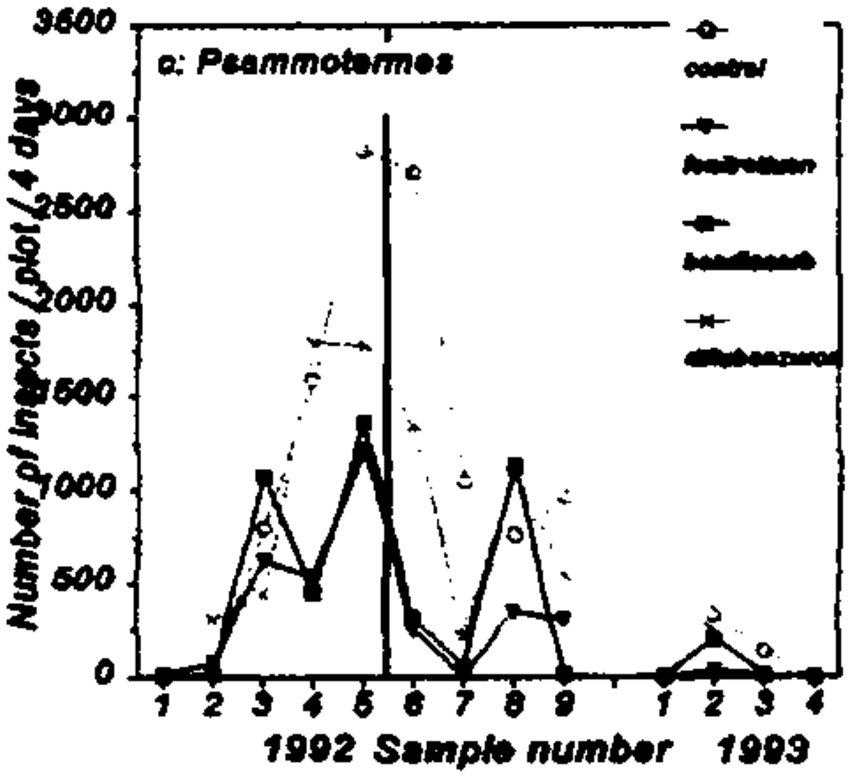
Figure 4 shows the average Pearce-trap catches for each of the insecticides and the untreated controls, as well the log-transfomed differences between treated and control plots, used in the statistical tests. Table 4 summarizes the effects observed.

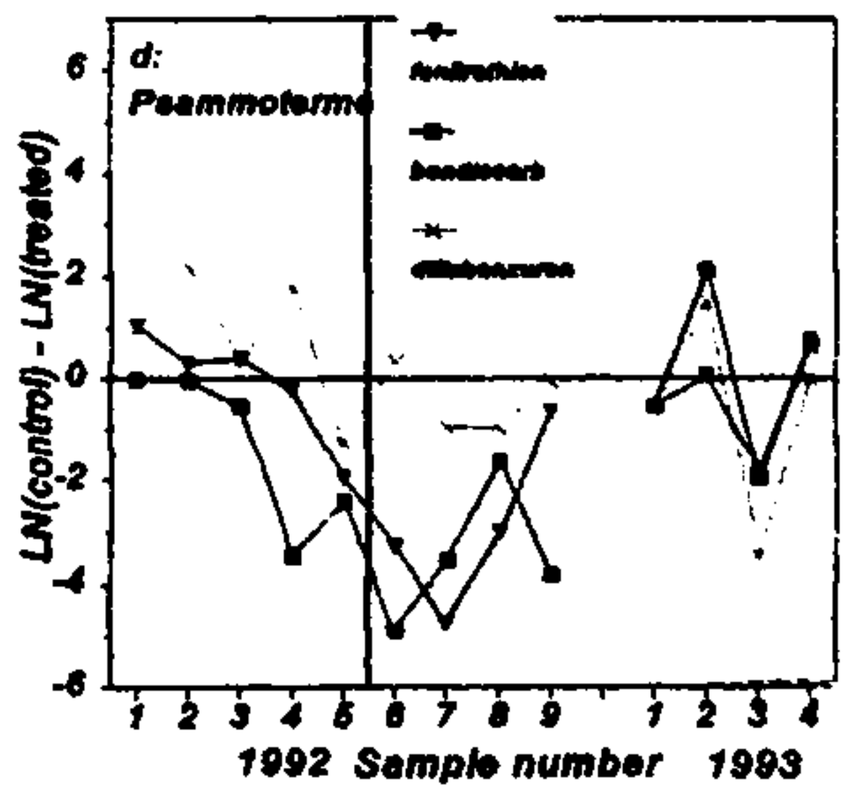
Fenitrothion applications caused a short term reduction of ±45% in the total termite catches. Two weeks after treatment, this effect is not statistically significant anymore. The effect of fenitrothion shortly after treatment appears mostly due to a reduction in catches of the sand termite, *Psammotermes hybostoma* (Figure 4c,d). An average reduction of 70% is observed in this species during the 4 weeks following treatment (Table 4). The apparent reductions in *Microcerotermes* catches were not statistically significant for this insecticide.

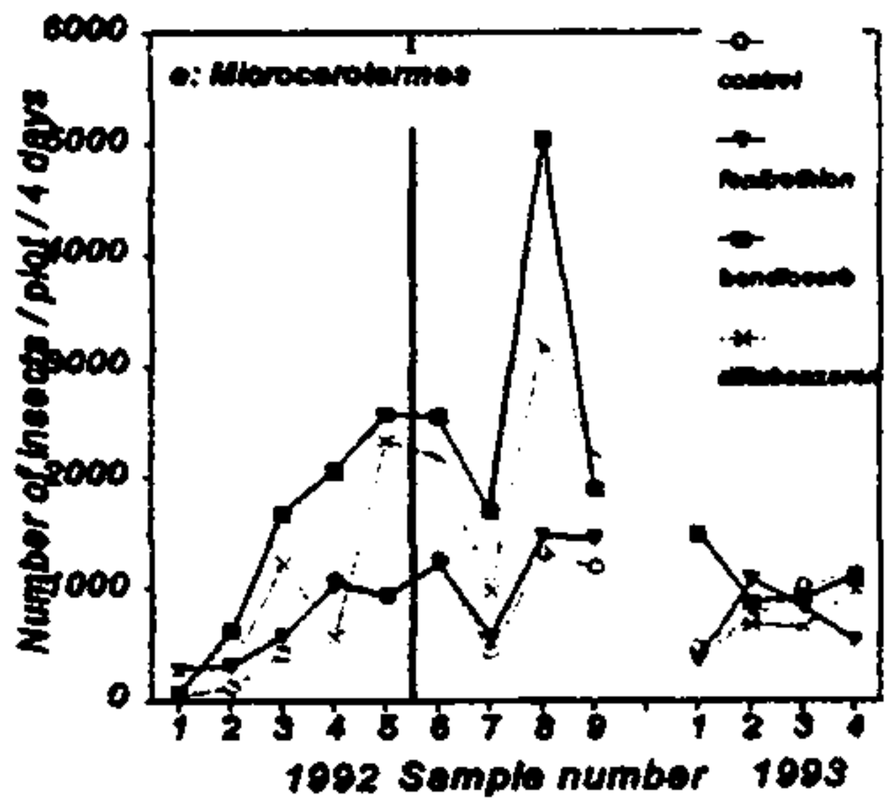
A 30% relative reduction in total catches was observed in the diflubenzuron plots in 1993, one year after treatment. This was statistically significant for the first sampling week in September, as well as for the whole September-October period combined. This effect cannot be attributed to just to one species. A significant reduction of *Psammotermes* was seen during one sampling week only, in mid-October 1993. *Psammotermes* catches were generally low during 1993, however, even in the untreated plots. On the other hand, statistically significant effects in the diflubenzuron plots were observed in 1993 for *Microcerotermes*. But since *block x treatment* interactions were also highly significant during this period, the observed effects can not be considered generalised over the experimental area.











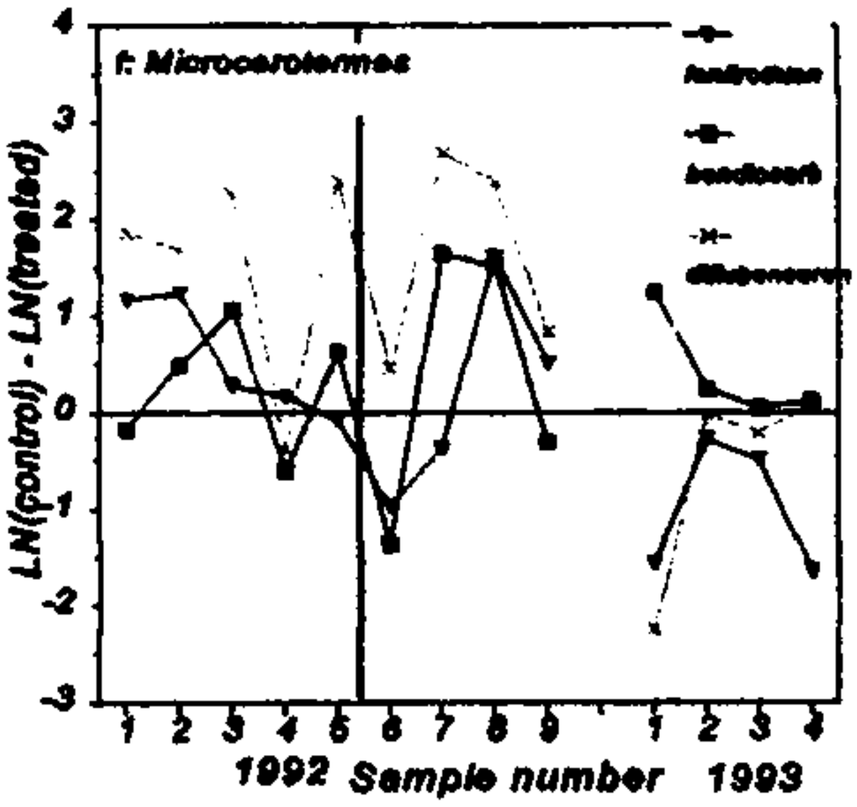


Figure 2: Average Pearce-trap catches (of 4 plots) of the total termite population, *Psammotermes hybostoma* and *Microcerotermes* sp. (left graphs), and the log-transformed differences between treated and control plots (right graphs). The vertical lign marks the moment of treatment. Results of the statistical comparisons are given in Table 3.

Table 3: Summary of the results of the analyses of variance carried out for the different termite activity parameters evaluated during the study. The probabilities (p) of a type I error for a treatment effect are shown for all $p \leq 0.05$; if no probability is mentioned, the effect of the treatment was not significant at this level. In all cases, except when specifically mentioned, $MS_{before\ vs.\ after}$ was tested over MS_{error}.

Parameter	Insecticide	Sampling dates 1992 : Weeks after treatment					Sampling dates 1993 : Week numbers			
		1	2	3	4	5	1	2	3	4
Total catch	fenitrothion	0,007								
	bendiocarb									
	diflubenzuron						0			
							0,026			
Psammotermes catch	fenitrothion	0	0	0						
		0.016 [1]								
	bendiocarb									
	diflubenzuron						0			
Microcerotermes catch	fenitrothion									
	bendiocarb									
	diflubenzuron									
bait consumption	fenitrothion									
	bendiocarb									
	diflubenzuron									
total foraging patches (transects)	fenitrothion					0,037				
						0,012				
	bendiocarb					0,043				
						0,011				
	diflubenzuron					0	0,017			
						0,023				
% active foraging patches (transects)	fenitrothion									
	bendiocarb									
	diflubenzuron									

[1]: effect of treatment was significant when data were analysed for the boxed sampling dates together.
[2]: effect tested over $MS_{pooled\ error}$.

Ahmad *et al* (1986) showed that diflubenzuron, albeit at much higher residue concentrations than measured here, caused 100% mortality and complete inhibition of egg hatching in 3 species of termites. Su et Scheffrahn (1993), more recently, found that species of Rhinotermitidae were affected by concentrations of diflubenzuron as low as 0.25-1.0 mg/kg, well in the range observed in our study. Given the relatively long persistence of diflubenzuron, it is not excluded that contaminated food taken into the nest may have affected termite brood, or even egg producing imagos. Since such an effect would take some time before showing, the reduced catches did not become apparent in the month after treatment.

Treatments with bendiocarb did not cause any statistically significant deviations in Pearce-trap catches.

Bait consumption was not significantly affected by any of the insecticides (Figure 3). This may seem contradictory to the effects observed on trap catches. However, this is probably a methodological problem, linked to the limited time periods that the traps/baits were left in the field. During a sampling round (4 days) only between 15 and 30% of the baits was consumed. This low consumption, together with the inherent variabilty among plots, may be the reason that any effects of treatment, if they occurred, could not be confirmed statistically. It is suggested that in future studies baits are left sufficiently long in the soil so that on average at least 50-75% is consumed in the control plots. This will "stretch out" any differences between treated and control plots, if they occur, and increase the probability

of finding statististically significant effects.

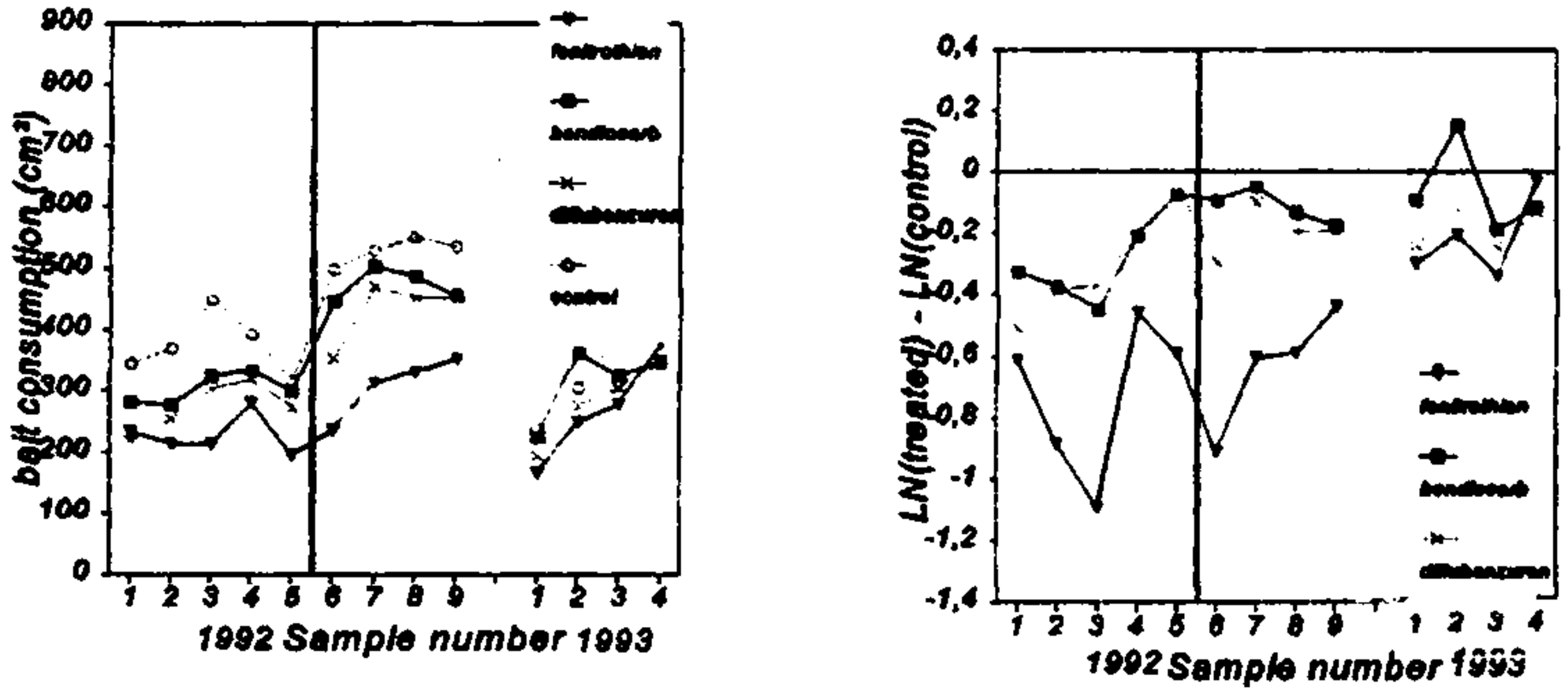


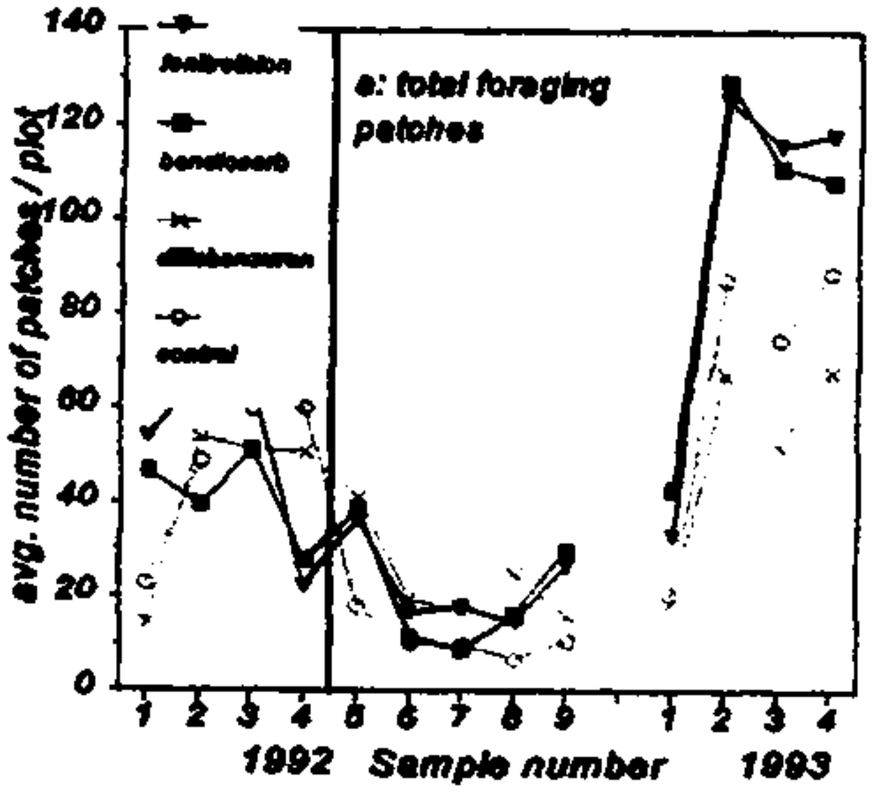
Figure 3: Average bait consumption (of 4 plots) in Pearce-traps (left graph), and the log-transformed differences between treated and control plots (right graph). The vertical lign marks the moment of treatment. Results of the statistical comparisons are given in Table 3.

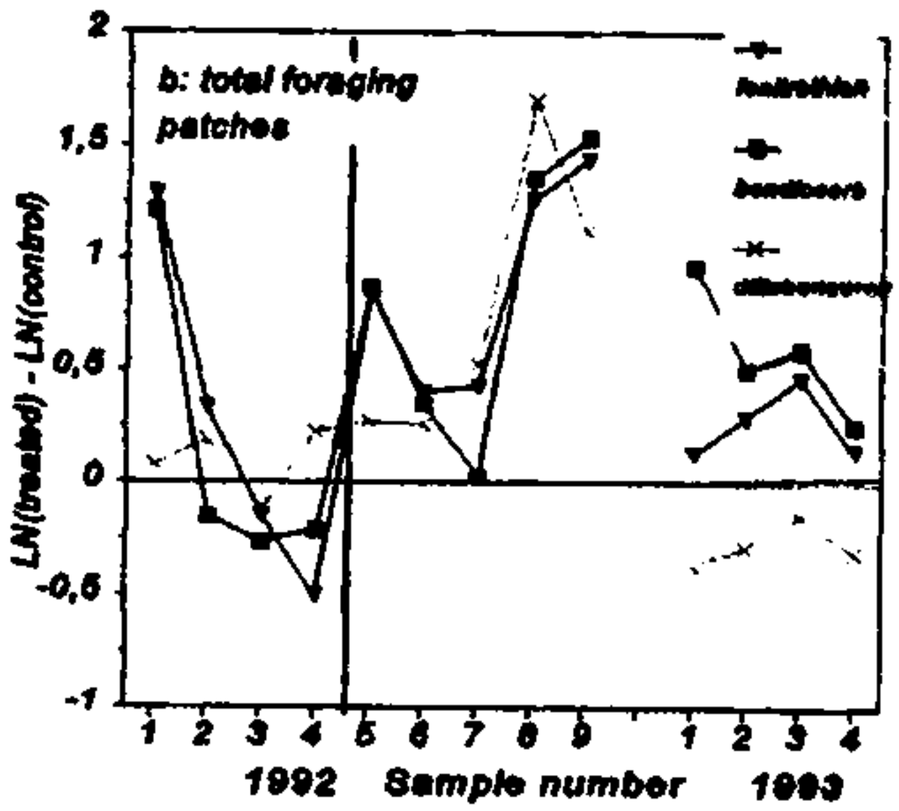
Transect counts

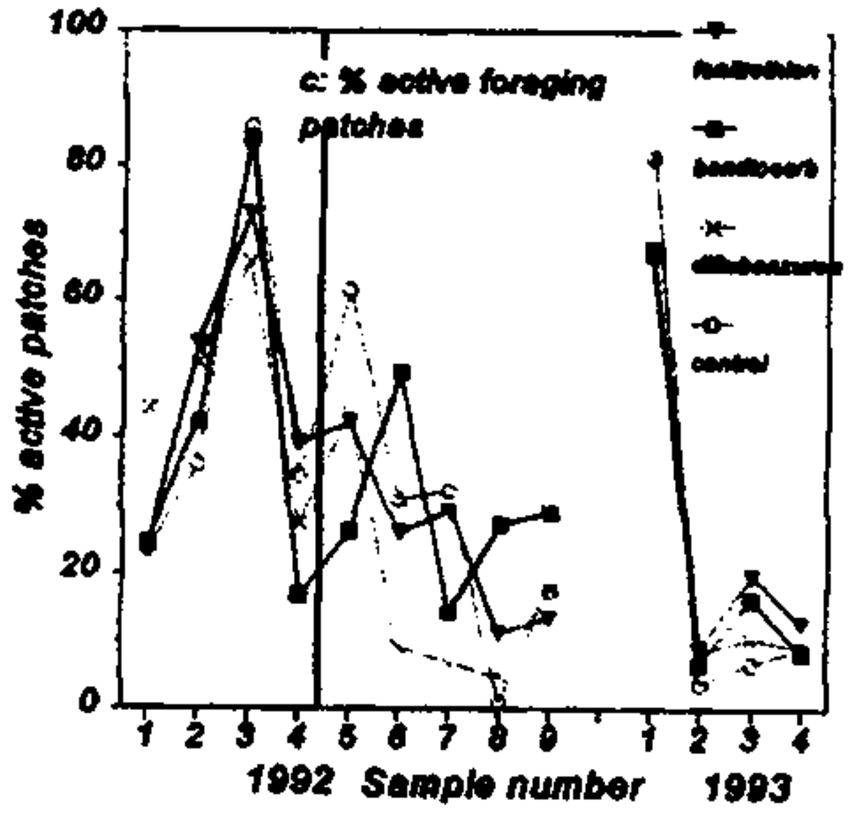
Transects counts were carried out with the same frequency as the Pearce-traps. However, only 4 counts were carried out before treatment, while 5 were done after the insecticide applications (one more than the Pearce-traps). For the statistical analysis, effect parameters for one or several weeks after treatement (be that in 1992 or in 1993) were compared with the average effect parameter of the 4 weeks before the treatments. No pre-treatment week was omitted since there was no reason to expect that much disturbance due to the initial laying-out of the transects had taken place. Results of the ANOVAs are summarized in Table 3.

An relative increase in foraging activity is observed in the transect counts of termite foraging patches for all three insecticides, mostly towards the end of the sampling period in 1992 (Figure 4a,b). Increases varied from 100 to 300%, when compared with the period before treatment, and after correction for variations in the control plots (table 4). It should be noted, though, that these effects occurred in a period of relatively low activity with respect to sheeting and gallery construction.

The predominant species encountered in the transect counts was *Odontotermes nilensi*, which was very poorly represented in the Pearce-traps. It may be that after the observed reductions in the populations of *Psammotermes hybostoma*, another sheeting/gallery constructing species, *O. nilensis* encountered less competition, allowing it to increase its foraging activity. However, given the difference in size, *O. nilensis* is considerably larger than *P. hybostoma*, it is difficult to imagine that the latter is able to win any direct interference competition. But more subtle competition processes may have been at play. Another possibility is a temporary release from predator pressure, resulting in an increase in foraging activity. One of the most important group of termite predators, ants, were clearly affected by fenitrothion, but the other insecticides did so much less (see below). This can thus only explain in part the increase in termite foraging patches. A third possible explanation is that the increase in total foraging patches, which can only be due to new construction activity, occurred to compensate for reduced foraging activity in the previous period (for instance, due to a repulsive effect of the insecticides). But no significant reductions in the percentage active patches were seen directly after treatment (Figure 4c,d), so this hypothesis does not seem to hold either. A good explanation for these observations can therefore presently not be given.







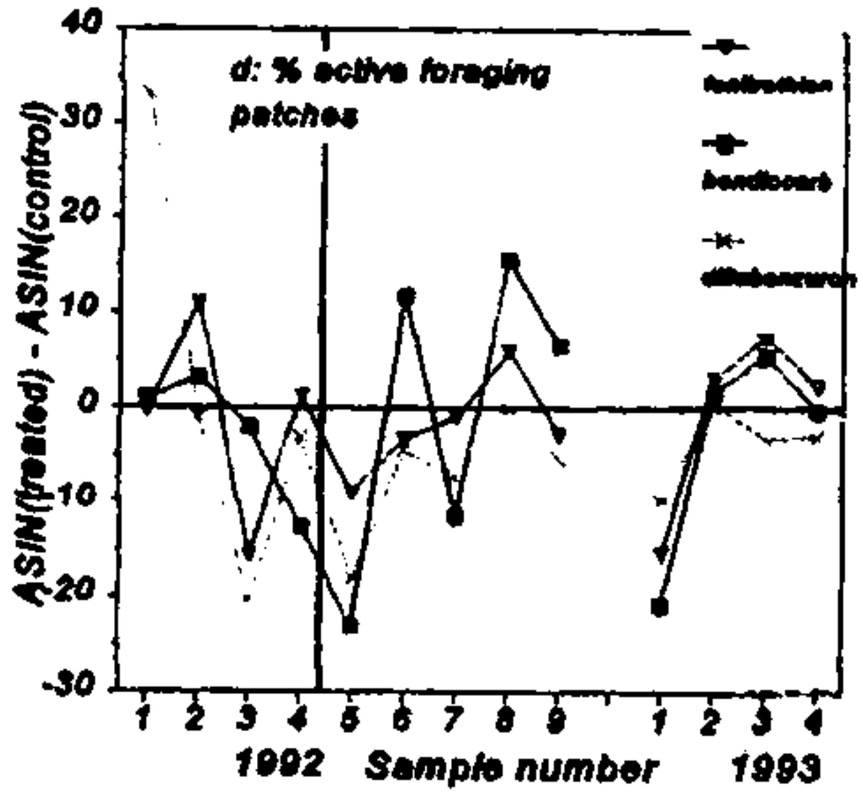


Figure 4: Average number of foraging patches (of 4 plots) per 150m transect, and the fraction of these patches which were active (left graphs), and the log-transformed differences between treated and control plots (right graphs). The vertical lign marks the moment of treatment. Results of the statistical comparisons are given in Table 3.

Table 4: Summary of the effects observed on the the different termite activity parameters. Effect size is expressed at the percentage change in the parameter when compared to the average of the 4 weeks before treatment, and corrected for any control variation (§ 2.6).

Parameter	Insecticide	Significant effect observed?	Effect size	Effect duration	Remarks
Total trap catches	fenitrothion	yes	45% reduction	2 weeks AT [1]	
	bendiocarb	no	--	--	
	diflubenzuron	yes	30% reduction	4 weeks at 1 year AT	
Psammotermes trap catches	fenitrothion	yes	70% reduction	4 weeks AT	
	bendiocarb	no	--	--	
	diflubenzuron	yes	100% reduction	3rd week at 1 year AT	
Microcerotermes trap catches	fenitrothion	no	--	--	
	bendiocarb	no	--	--	
	diflubenzuron	no	--	--	absence of significance in 1993 due to interactions
bait consumption	fenitrothion	no	--	--	absence of effects due to methodological weakness?
	bendiocarb	no	--	--	
	diflubenzuron	no	--	--	
total foraging patches (transects)	fenitrothion	yes	120% increase	weeks 4-5 AT	
	bendiocarb	yes	200% increase	weeks 4-5 AT	
	diflubenzuron	yes	130% increase	weeks 2-5 AT	
% active foraging patches (transects)	fenitrothion	no	--	--	
	bendiocarb	no	--	--	
	diflubenzuron	no	--	--	

[1]: AT = after treatment

Ant activity

Taxonomic distribution

No quantitative data were collected on the different ant species present in the study area. The nests which were monitored belonged mostly to two species of *Monomorium* (Formicidae, Myrmicinae). These appear generally to be omnivorous ants (Hölldobler and Wilson 1990). We have observed *Monomorium* in the field attacking foraging termites, other insects (especially Lepidoptera larvae), as well as collecting seeds. The Myrmicinae are the largest ant subfamily, both in Africa as well as worldwide. The genus *Monomorium* is cosmopolitan and extremely widely distributed (Bolton 1994). More detailed studies on a similar semi-arid savanna site, approximately 60 km east of the present location, have recently been carried out (Diedhiou Diatta 1996, L. Bâ - Locustox project - pers. comm.). They showed that the ant community (as monitored with baited pitfall traps) appears to be largely dominated by one species of *Monomorium*, the same which was also observed in this study.

Transect counts

Transects counts of ant nest entrances were carried out simultaneously with those of the termite foraging patches. They were statistically treated in the same way (§ 3.2). Results of these ANOVAs are summarized in Table 5.

No effects were observed on the total density of nest entrances for any of the insecticides (Figure 5a,b). The fraction of these entrances which were active, on the other hand, was consistently reduced by the fenitrothion treatments (Figure 5c,d). Ant activity was significantly reduced for only one week, when weeks were tested individually. But activity during both the whole 1992 sampling period, as well as the 1993 sampling period, was significantly affected by fenitrothion. Average relative reductions of 50% and 35%, respectively, were noted (Table 6). The relative reduction in ant activity in the fenitrothion plots in 1993 is caused by the fact that the fenitrohion plots before treatment showed more activity than the

control plots. It should be noted that absolute differences between treated and control plots in 1993 were small (Figure 5c). While these results corroborate previous studies of locust control with organophosphates (see §1.2), it is, to our knowledge, the first time that an effect on ant activity has been observed which appears to last until the year after treatment.

A short term reduction in ant activity (70% during the 3rd week after treatment) was also observed in the bendiocarb plots. Such a short term effect will quite probably have little ecological significance. No further data were found in the literature describing the impact of bendiocarb on ants.

Diflubenzuron did not seem to affect ant activity. This corresponds with results from Catangui *et al* (1996) in North Dakota rangelands. In their study, diflubenzuron applied at rates ranging from 8 to 34 g ai/ha, did not significantly reduce pitfall catches.

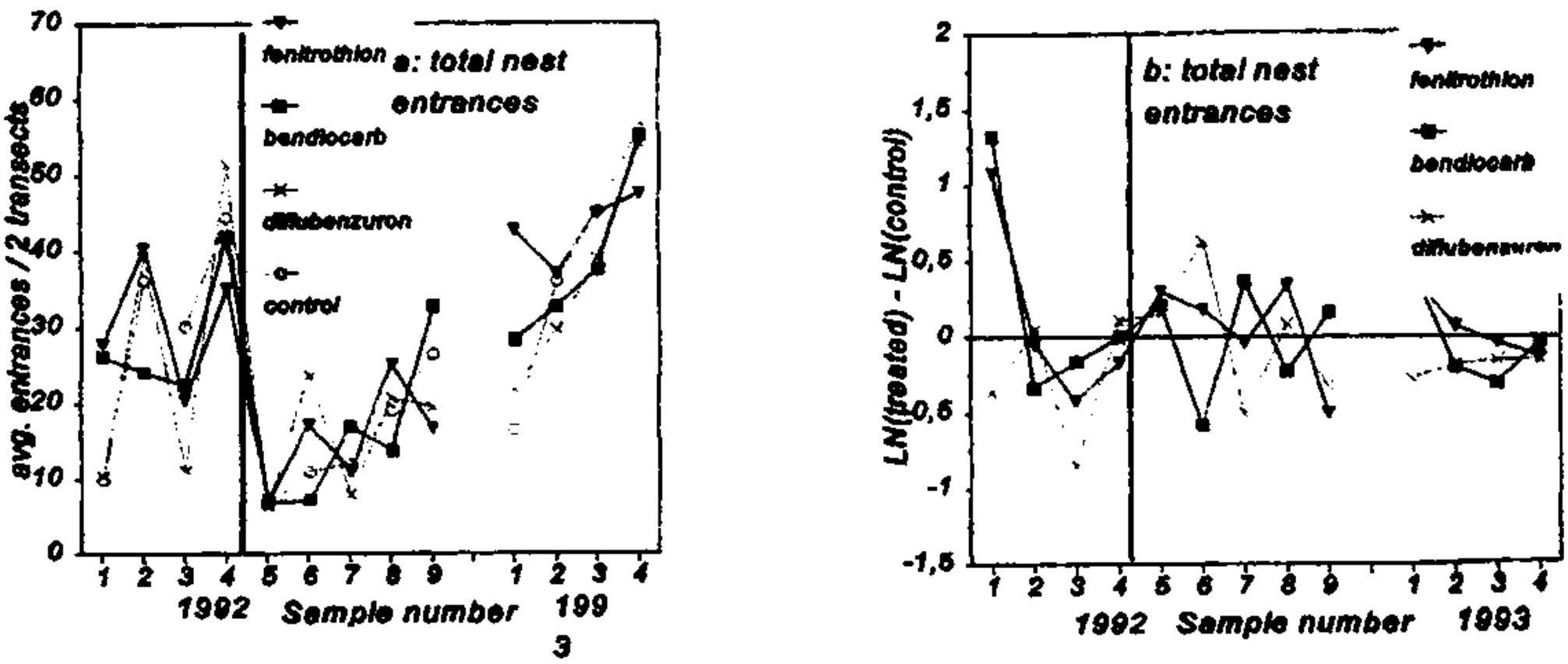


Figure 5: Average number ant nest entrances (of 4 plots) per 150m transect, and the fraction of these which were active (left graphs), and the log-transformed differences between treated and control plots (right graphs). The vertical lign marks the moment of treatment. Results of the statistical comparisons are given in Table 5.

Table 5: Summary of the results of the analyses of variance carried out for the different ant activity parameters evaluated during the study. The probabilities (p) of a type I error for a treatment effect are shown for all $p \leq 0.05$; if no probability is mentioned, the effect of the treatment was not significant at this level. In all cases, except when specifically mentioned, $MS_{before\ vs.\ after}$ was tested over MS_{error}.

Parameter	Insecticide	Sampling dates1992 : Weeks after treatment					Sampling dates 1993 : Week numbers			
		1	2	3	4	5	1	2	3	4
total nest entrances (transects)	fenitrothion									
	bendiocarb									
	diflubenzuron									
% active nest entrances (transects)	fenitrothion	0.015								
		0,006					0,023			
	bendiocarb		0.039 [2]							
	diflubenzuron									

[1]: effect of treatment was significant when data were analysed for the boxed sampling dates together.
[2]: effect tested over $MS_{pooled\ error}$

Table 6: Summary of the effects observed on the the different ant activity parameters. Effect size is expressed at the percentage change in the parameter when compared to the average of the 4 weeks before treatment, and corrected for any control variation (§ 2.6).

Parameter	Insecticide	Significant effect observed?	Effect size	Effect duration	Remarks
Total nest entrances	fenitrothion	no	--	--	
	bendiocarb	no	--	--	
	diflubenzuron	no	--	--	
% active nest entrances	fenitrothion	yes	50% reduction; 35% reduction	5 weeks AT [1]; 4 weeks at 1 year AT	activity depressed for the entire period after treatment
	bendiocarb	yes	70% reduction	3rd week AT	
	diflubenzuron	no	--	--	

[1]: AT = after treatment

3.4 Assessment methods

The Pearce-traps used in the termite assessments were easy to use, and yielded large numbers of insects rapidly after installation of the trapping grids. The glass plates are rather fragile though, especially given the rough terrain over which they are transported every week. It is suggested that in future studies plexiglass is used instead. The number of insects caught in the traps is obviously influenced by their location with respect to termite nests in the area and to potential food sources. This is no real problem as far as insecticide assessments are concerned, as long as the traps are always placed on the same spot, and the analysis compares "after vs. before" data on a plot per plot basis (as in the BACI design used in this study). Catches are also influenced by the time of the day of trap collection. Since the traps are placed just under the soil surface, increasing temperatures during the day will result in overheating of the traps after a certain hour. This has not been explicitly studied, and it was assumed that collection before 11:00h would not be influenced much by this phenomenon. One may be able to further reduce variability by identifying "treshold temperatures" above which termite foraging inside the traps ceases, and thus trap collection should be avoided.

The use of bait consumption as an effect parameter is a potentially more precise solution. Because bait consumption is a form of integrating the activity of the termites over the period that the baits were placed, this is much less influenced by the moment of the day at which the traps are collected. Using an optical scanner to quantify bait consumption is fast and precise, as long as the initial bait disks were cut to the same size. In our study, bait disks had to be manually cut, which introduced some minor variability in initial disk sizes. It is suggested that machine-cut bait disks are used in future studies (eg. unprinted beer mats, as suggested by Pearce [1990]), to further reduce variability in this parameter, and to avoid wasting time cutting the disks. As was discussed above (§ 3.2), the duration of bait placement in the field needs to be adapted to the speed of consumption by the termites. It certainly needs to be longer than the 4 days used to assess activity in this study.

Transect counts for termite foraging patches are rather laborious, but other species of termites are sampled than with the Pearce traps. It therefore remains a usefull complementary technique. In this study, the total number of foraging patches appeared to be more sensitive to insecticide perturbation than the percentage active patches. This may be because, like with the Pearce traps, percentage activity is dependent on ambient temperature and other meteorological factors. Density of total foraging patches is much less sensitive to such factors, while still being sufficiently transient to show insecticide effects relatively rapidly.

Other traps have been used in the past to assess termite activity that may prove useful for ecotoxicological side-effect studies. The use of capture-recapture methods, using (fluorescent) dyes, as was recently proposed by Miller (1993) and Haagsma and Rust (1993), could be a way of estimating densities rather than activity. This may provide more precise as well as more ecologically meaningfull parameters for ecotoxicological studies.

Ant activity was only assessed in the transect by counting active nest entrances. Similar to the termite foraging patches, this method is partly dependent on daily activity cycles of the ants. Inclusion of parameters such as the presence of fresh chaf or sand granules around the entrance, reduces the need to observe the ants physically moving around the nest. As with termites, a reduction of activity may be due either to toxic or to repulsive effects of the insecticide. Other techniques which have been tried to assess insecticide disturbance on ants include the use of baits (Tingle 1993), pitfall traps (baited or not) (Catangui *et al* 1996), or the quantification of sorties by workers along ant trails (Balança and de Visscher 1996). Each of these methods has its advantages and disadvantages, and a combination of several will probably be needed to assess insecticide perturbations on a representative part of the ant fauna.

3.5 Study design

As was discussed above, the study setup resembles a randomised complete bock design, with all treatments carried out in each block of plots. If indeed variability among blocks is greater than among plots within the blocks, as was implied during plot lay-out, such a design should allow for a more powerful statistical analysis than a completely randomised, one-way ANOVA, design. In total, 62 different ANOVAs were calculated for this study. In 80% of the cases was the *Block* Mean Square larger than the *Error* Mean Square. This suggests that variability among blocks was generally greater than among plots within the blocks. It justifies the choice which was made at the start of the study to block the treatments according to the perceived vegetation gradient.

Very rarely did significant *block x treatment* interactions occur which prevented treatment effects from being considered statistically significant. This was only the case for the effect of diflubenzuron on *Microcerotermes* trap catches in 1993 (Table 4). Apparently, diflubenzuron only had a significant effect on a few plots, but this was not generalised. In all other cases were treatment effects undoubtedly generalised. The fact that significant interactions between the treatment effect and blocks did occur, even though rarely, underlines the importance of not blindly applying a RCB ANOVA, in which it is assumed that no interactions exist. A two-way ANOVA with explicit consideration of interactions seems to be more appropriate for this type of, relatively large scale, field study.

CONCLUSIONS

1. Treatments with fenitrothion, at a rate of ±775 g ai/ha, affected Pearce-trap catches of *Psammotermes hybostoma*, a dominant termite species in the semi-arid savanna of northern Senegal. A relative reduction of 70% was observed for a period of four weeks after treatment. One year after spraying, no effect could be discerned any more.

   Pearce-trap catches of all termites combined showed a relative reduction of 30%, one year after the application of diflubenzuron (±105 g ai/ha). There were indications that *Microcerotermes* sp., a wood termite, was affected, but this could not be conclusively shown.

   Bendiocarb, sprayed at ± 170 g ai/ha, did not have a significant impact on termite catches.

   All three insecticides caused a relative increase in termite foraging patches, predominantly of *Odontotermes nilensis*, between the 2nd and the 5th week after treatment. An explanation for this effect could not be given.

2. Ant activity was significantly affected after treatments with fenitrothion during the whole study period, also the year after treatment. A relative reduction of 50%, on average, was observed during the 5 weeks after spraying, while activity was still reduced with 35% one year later.

   Bendiocarb and diflubenzuron did not cause ecologically significant effects on ant activity in this study.

3. Pearce-traps provide a simple means for the assessment of insecticide impact on of termite activity. The technique may be optimised by determining the minimum periods the traps need to be in the ground so that, in addition to termite abundance, bait consumption can be measured reliably. The traps only caught two species in sufficient numbers, so additional assessment methods are required to draw more general conclusions about insecticide effects on non-target termites.

   Similarly, the results with respect to ant activity are limited to species with are either abundant or have clearly visible nest entrances. Additional techniques, such as the use of pitfall traps or baits, will need to be used to get a more complete idea about insecticide impact on the ant fauna.

4. Because of the limited number of taxa that were followed in this study, general conclusions about the side-effects of locust control on ants and termites cannot yet be drawn. However, the study provided strong indications that certain insecticides may affect termite and ant activity, with side-effects still being discernible one year after treatment. It is therefore suggested that further work is done to clarify the risk of locust control on these important groups of arthropods of semi-arid and arid ecosystems.

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CHAPTER 7:

Further toxicity tests with locust control insecticides on *Pimelia senegalensis* and *Trachyderma hispida* (Coleoptera, Tenebrionidae).

Harold VAN DER VALK & Hamidou DIAKHATE

SUMMARY

Toxicity tests were carried out on the tenebrionid beetles *Pimelia senegalensis* and *Trachyderma hispida* with a number of insecticides used for Desert Locust control. These tests complemented a study previously reported by Van der Valk et al. (1996a). Dietary and topical toxicity data for both species are now available of the following insecticides: bendiocarb, chlorpyrifos, deltamethrin, fenitrothion, fipronil, lambda-cyhalothrin and malathion.

Topical 96h-ED_{50} values for *Pimelia senegalensis* varied from 0.018 µg/g insect for deltamethrin to 53.6 µg/g insect for chlorpyrifos. They ranged from 0.10 µg g insect for fipronil to 66.8 for chlorpyrifos in the case of *Trachyderma hispida*.

Fipronil was the most toxic insecticide for *P. senegalensis*, when administered in the diet, and chlorpyrifos the least. Their average 96h - EC_{50} values were 0.26 mg/kg diet (millet bran) and 641 mg/kg respectively. This was also the case for *T. hispida*, with average 96h - EC_{50} values of 0.45 mg/kg for fipronil and 223 mg/kg for chlorpyrifos.

P. senegalensis was often slightly more susceptible than *T. hispida*, but this was not the case for all insecticides.

It was concluded that the test system with field-collected tenebrionid beetles can be considered fairly robust. The variability in acute toxicity data was of a similar order of magnitude as observed with other laboratory reared insects.

An assessment of the hazard of the insecticides was carried out, based on a comparison between dietary toxicity data and initial residues found on vegetation after locust control. Initial residues similar to or above 96h-EC_{50} values were considered to pose a high risk of acute effects on tenebrionid populations in the field. Initial residues of a factor 5 or more below 96h-EC_{50} values were not considered to pose a risk of large acute population reductions.

These predictions generally agreed well with results from semi-field bio-assays and full-scale field studies reported in the literature. A possible exception were chlorpyrifos treatments, which in a number of field studies resulted in more impact than predicted from the hazard analysis.

An assessment was made of the acute risk for tenebrionid beetles of these 7 insecticides, when applied at effective rates against the Desert Locust. The assessment was based on all available results from laboratory toxicity tests, field bio-assays, full-scale field experiments and data on initial insecticide residues. It was concluded that treatments with fenitrothion and fipronil are likely to result in severe population reductions of tenebrionid beetles. There is also a high possibility that malathion will cause population reductions, but of a moderate size. Effects of treatments with chlorpyrifos, deltamethrin and lambda-cyhalothrin may be moderate; both chlorpyrifos and deltamethrin will probably only have a small effect. It is unlikely that bendiocarb will cause acute population reductions of tenebrionid beetles.

INTRODUCTION

Van der Valk *et al.* (1996a) (Locustox report 96/6) published results of a number of toxicity tests on tenebriond beetles with insecticides used for Desert Locust control. This study was carried out to evaluate the impact such insecticides may have on non-target Tenebrionidae, which perform important functions as detritivores and predators of orthopteran egg pods in arid and semi-arid ecosystems.

Since that publication, one additional insecticide has been tested, and a number of gaps in the data set were filled. This report provides an update to Van der Valk et al. (1996a). It was kept as concise as possible, and the reader is referred to the previous publication for more background information.

As previously, two species of tenebrionid beetles were used in the tests, *Pimelia senegalensis* and *Trachyderma hispida* Forskål. Two types of laboratory toxicity tests were carried out, representing different ways of exposure to the insecticides. A topical exposure test, in which the insecticide is directly applied onto the insect cuticle; and a dietary test, in which the insecticide is mixed with millet bran and then fed to the beetles.

Six insecticides were studied this time: chlorpyrifos, malathion (organophosphates), deltamethrin, lambda-cyhalothrin (pyrethroids), bendiocarb (carbamate) and fipronil (phenyl-pyrazole). Lambdacyhalothrin had not yet been tested previously; data on all other insecticides either existed only for one species, or were otherwise considered incomplete (Van der Valk et al. 1996a). No new data on fenitrothion were added.

STUDY METHODS

A detailed description of the testing methods was given in Van der Valk et al. (1996a). No changes in these procedures were made [1].

Preparation of the insecticide solutions

All test solutions were prepared from parent stock of commercial ULV formuations (table 4.1 and 4.2.). Dilutions were made with analytical grade acetone, using volumetric flasks and automatic , and volumetric pipettes. Doses to be applied were established according to series of logarithms of 10 (^{10} log).

All glassware used was first washed in acetone and in concentrated soap, then rinsed out with tap water and distilled water before use. Precautions were taken to avoid any cross-contamination between the different solutions. Acetone was used as a control in all tests.

Topical exposure test

Insecticide solutions were applied with a manual micro-applicator (Burkhard ®) equipped with a micro-syringe. A 1 µl droplet was deposited at the joint between the thorax and the abdomen, a relatively supple part of the insect where the droplet can quickly penetrate. No anaesthesia was used during the treatments. Treatments were conducted using increasing concentrations, starting with the control solution (acetone). Between two concentrations the syringe was rinsed with the next solution to be tested.

Ten of fifteen insects were treated per concentration and placed in a plastic tray (30 cm diameter x 12 cm high) covered with a cardboard sheet. They were not fed during the observation period. Mortality observations were made 24, 48, 72 and 96 hours after treatment, and sometimes over a longer period. The insects were considered "dead" if their legs or antennae did not react any more to a slight stimulation with a pair of tweezers. Insects were considered "moribund" if they had completely lost locomotory control and became immobilized (they often ended up on their back without being able to turn themselves any more).

Dietary exposure test

The insects were fed on millet contaning different quantities of the test compound. Ten ml of a test solution of the insecticide dissolved in acetone were mixed with 10 grammes of millet bran in a glass petri dish. The bran was then air-dried for thirty to forty five minutes until the complete evaporation fo the acetone. A control treated with acetone only was included in all tests. Each petri dish was subsequently placed in a plastic tray (20 cm diameter x 12 cm high) containing ten of fifteen insects, which was covered with a cardboard sheet during the whole test period.

The insecticide treated food was renewed every 24 hours and effects evaluated, over a 96-hour period. In a number of cases, after these 96 hours of exposure, the insects were given uncontamined millet bran and observed for several additional days to assess any subacute effects. Mortality and immobility were evaluated as in the topical exposure tests. Individual food uptake (and thus individual exposure to the insecticide) was not measured.

Tables 1 and 2 list the different tests which were carried out, the insecticides evaluated, and the collection periods of the beetles.

Table 1 : Date on the insecticides and insects used in the toxicity tests executed with Pimelia senegalensis (Col. Tenebrionidae) since publication of report 96/6. All insects were collecte in a radius of 25 km around Linguère (15°23'N - 15°07'W), in Northern Senegal.

Insecticide (brand name)	Formulation	Batch number	Collection period of the insects	Type of test	Date of test	Test no. [1]
bendiocarb (Ficam)	200 g a.i./l ULV	6.1. A 2992	07/96	topical	16/08/96	8
			07/96	dietary	08/08/96	5
deltamethrin (Decis)	5 g a.i./l ULV	LEPRI 21293	07/96	topical	25/08/96	5
			07/96		04/10/96	7
fipronil (Regent)	7.5 g a.i./l ULV	OP 950443/ CDE 5076	07/96	topical	08/10/96 22/10/96	2 4
			11/96	dietary	07/11/96	2
			11/96		20/11/96	4
lambda-cyalothrin (Karate)	40 g a.i./l ULV	F 95/-/128 HB	07/96	topical	03/09/96	3
			07/96		10/09/96	4
			07/96		20/09/96	6
malathion (Fyfanon)	1230 g a.i./l ULV	not indicated	07/96	dietary	13/10/96	2
			07/96		22/10/96	3
			11/96		07/11/96	4
			07/96	topical	19/07/96	2
			07/96		19/08/96	5
			07/96		27/08/96	6
			07/96	dietary	10/09/96	2
			07/96		20/09/96	4
			07/96		30/09/96	5

[1] Test numbers refer to the toxicity test archived at the Locustox project in Dakar; tests which did not comply with the ASTM criteria are not listed in this table (see Van der Valk et al. 1996a).

[1] Standard Operation Procedures n° ENTO/LAB/02 (topical test) and ENTO/LAB/03 (dietary test).

Table 2: Data on the insecticides and insects used in the toxicity tests executed with *Trachyderma hispida* (Col., Tenebrionidae) since publication of report 96/6. All insects were collected in a radius of 25 km around Linguère (15°23'N - 15°07'W), in Northern Senegal.

Insecticide (brand name)	Formulation	Batch number	Collection period of the insects	Type of test	Date of test	Test no.[1]
bendiocarb (Ficam)	200 g a.i./l ULV	6.1.A 2992	11/96 11/96	dietary	28/11/96 08/12/96	3 4
chlorpyrifos (Dursban)	240 g a.i./l ULV	JA 12272009	07/96	topical	30/10/96	4
lambda-cyhalothrin (Karate)	40 g a.i./l ULV	F95/-/128 HB	11/96 11/96 11/96	topical	11/11/96 27/11/96 03/12/96	1 4 5
			11/96 11/96	dietary	27/11/96 04/12/96	2 3
malathion (Fyfanon)	1230 g. a.i./l ULV	not indicated	11/96 11/96	dietary	03/12/96 06/12/96	4 5

[1]: Test numbers refer to the toxicity test archive at the Locustox project in Dakar; tests which did not comply with the ASTM criteria are not listed in this table (see Van der Valk *et al.* 1996a).

RESULTS

As in the previous report, we used the combined dead and moribund fraction as the toxicological endpoint in the analysis. The insects that recovered from immobilization during the observation period were considered unaffected in the toxicity analysis. This occurred almost exclusively with the pyrethroids. Such a classification may underestimate the lethal effect of a compound in the field to some extent since laboratory conditions were relatively lenient. Immobilized beetles would quite certainly suffer more from predation or dehydration under field conditions than they did in the laboratory.

Topical exposure tests

For the comparison of toxicity of the insecticides, 96h-ED_{50} values were calculated (ED_{50} = effective dose for 50% of the population of beetles). These are listed in Table 3, together with their 95% confidence intervals. Table 3 does not only provide the results of the toxicity tests described in Tables 1 and 2, but also lists the previously calculated 96h-ED_{50} values. It can thus be considered an update of the results published by Van der Valk *et al.* (1996a).

The exhaustive list of ED_{50} values at all observation times of the present study is given in Appendix 1.

Table 3: Complete overview of the results of the topical toxicity tests with *Pimelia senegalensis* and *Trachyderma hispida*. Shown are the 96h-ED_{50} values (µg a.i./g insect) and their 95% confidence intervals. ED_{50} values for each insecticide (both species combined) followed by the same letter are considered highly similar. In bold are the tests carried out since publication of report 96/6.

Insecticide	Test no.	*Pimelia senegalensis*			Test no.	*Trachyderma hispida*		
		ED_{50}	95% CI			ED_{50}	95% CI	
bendiocarb	3	2.61	1.27 - 5.34	ab	2	16.3	12.1 - 22.0	c
	4	2.18	1.49 - 3.19	a	3	12.4	9.13 - 16.8	bc
	5	2.04	1.24 - 3.36	a	4	10.3	7.34 - 14.5	bc
	8	**6.35**	**3.75 - 10.8**	b				
chlorpyrifos	2	53.6	45.4 - 63.3	a	1	66.8	48.2 - 90.5	a
	3	52.3	44.5 - 61.4	a	**4**	**40.7**	**32.6 - 50.8**	a
	4	49.7	41.2 - 60.0	a				
deltamethrin	2	0.15	0.10 - 0.22	a	1	0.41	0.23 - 0.71	bc
	3	0.24	0.23 - 0.26	b	2	0.30	0.21 - 0.43	abc
	6	**0.022**	**0.013 - 0.036**	d	3	0.32	0.25 - 0.41	bc
	7	**0.018**	**0.011 - 0.028**	d	4	0.29	0.22 - 0.37	abc
					5	0.35	0.28 - 0.45	c
fenitrothion	2	5.29	3.56 - 7.85	a	1	1.05	0.57 - 1.95	b
	3	4.80	3.17 - 7.27	a	2	2.68	2.14 - 3.88	a
					3	2.71	1.68 - 4.36	ab
fipronil	2	**0.082**	**0.070 - 0.095**	a	2	0.22	0.19 - 0.25	b
	4	**0.076**	**0.069 - 0.085**	a	4	0.10	0.09 - 0.11	c
lambda-cyhalothrin	**3**	**0.055**	**0.038 - 0.080**	a	**1**	**0.47**	**0.34 - 0.65**	c
	4	**0.134**	**0.087 - 0.208**	b	**4**	**0.26**	**0.19 - 0.37**	bc
	6	**0.173**	**0.097 - 0.308**	b	**5**	**0.24**	**0.17 - 0.35**	bc
malathion	**2**	**21.3**	**15.8 - 28.9**	ab	2	14.1	10.5 - 18.7	a
	5	**18.0**	**13.6 - 23.9**	a	3	12.4	8.50 - 18.0	a
	6	**33.2**	**25 - 44.2**	b				

The results of the additional topical toxicity tests carried out with bendiocarb (*P. senegalensis*) and chlorpyrifos (*T. hispida*) fall within the ranges already established, and do not change any of the previous conclusions. The ED_{50} values obtained for deltamethrin (*P. senegalensis*), on the other hand, are approximately a factor 10 lower than previous ones. It did not become clear why this was the case. Further assays may be required with this insecticide.

The results of the toxicity tests with fipronil and malathion on *P. senegalensis* are of a similar order of magnitude as those already obtained for *T. hispida*. As was observed previously, the 96h- ED_{50} of fipronil underestimates the toxicity of the compound; the final ED_{50} (144 h) appearing to be approximately 0.05 µg/g insect (Appendix 1). Mortality had almost stabilised at 96 hours for malathion.

The toxicity of lambda-cyhalothrin is very similar to deltamethrin, the other pyrethroid tested. Mortality had stabilised at 96 hours, except in test no. 6 (*P. senegalensis*), where the ED_{50} arrived at 0.09 µg/g insect after 8 days. This was closer to the 96h values of the other two tests.

Fipronil was the most toxic insecticide to both tenebrionid species after topical exposure, but was closely followed by deltamethrin and lambda-cyhalothrin. Chlorpyrifos had the lowest acute toxicity.

Dietary exposure tests

Table 4 lists the 96h-EC_{50} values calculated for each of the tests (EC_{50}= effective concentration in the food for 50% of the population of beetles). Again, the table provides a complete update of the data provided in Van der Valk *et al.* (1996a). A list of EC_{50} values at all observation times is given in Appendix 1 for the new tests only.

The supplementary data for bendiocarb (*P. senegalensis*) and malathion (*T. hispida*) confirm earlier results. The EC_{50} values for bendiocarb on *T. hispida*, however, are significantly lower than those previously recorded. It is not clear why this should be so, and additional tests with this compound may be recommended.

The new data on *P. senegalensis* for chlorpyrifos, fipronil and malathion are of the same order of magnitude as of *T. hispida*. The EC_{50} values of chlorpyrifos and malathion had almost stabilised after 96 hours of exposure. This was not the case for fipronil. The 144h-EC_{50} of fipronil varied from 0.07 to 0.10 mg/kg bran, which is approximately 70% lower than the values listed after 96 hours (Table 4 and Appendix 1).

The toxicity of lambda-cyhalothrin to *P. senegalensis* was very similar to deltamethrin. But this was not the case for *T. hispida*. Lambda-cyhalothrin was an order of magnitude more toxic to this species than deltamethrin. Since the topical (intrinsic) toxicities of the two insecticides are very similar for both species, deltamethrin may be transferred from the gut of *T. hispida* to its site of action less effectively then lambda-cyhalothrin. Another possibility is a higher repulsiveness of deltamethrin as compared to lambda-cyhalothrin, resulting in lower consumption. However, why this should be so for one species of tenebrionid and not for the other is not clear.

Fipronil had clearly the highest acute dietary toxicity, especially when one takes into account the relatively slow speed of action. Lambda-cyhalothrin had the next lowest ED_{50} values. Chlorpyrifos was the least toxic insecticide after dietary exposure.

Table 4: Complete overview of the results of the dietary toxicity tests with *Pimelia senegalensis* and *Trachyderma hispida*. Shown are the 96h-EC_{50} values (mg a.i./kg millet bran) and their 95% confidence intervals. EC_{50} values for each insecticide (both species combined) followed by the same letter are considered highly similar. In bold are the tests carried out since publication of report 96/6.

Insecticide	Test no.	*Pimelia senegalensis*			Test no.	*Trachyderma hispida*		
		EC_{50}	95% CI			EC_{50}	95% CI	
bendiocarb	3	63.8	38.6 - 105	ab	1	292	207 - 411	c
	5	**49.3**	**34.6 - 70.2**	a	2	392	271 - 567	c
					3	**124**	**80 - 191**	b
					4	**85**	**63 - 116**	ab
chlorpyrifos	1	516	273 - 975	ab	1	142	89.5 - 226	c
	2	653	481 - 886	a	2	260	203 - 332	bc
	3	648	475 - 884	a	3	301	217 - 419	bc
	4	773	656 - 912	a				
deltamethrin	1	7.22	3.29 - 15.9	a	1	48.5	25.4 - 92.5	bc
	2	6.96	5.60 - 8.65	a	2	72.9	51.3 - 104	c
	3	7.10	4.91 - 10.3	a	3	28.9	21.1 - 39.7	b
	4	4.48	2.74 - 7.31	a				
fenitrothion	1	8.51	3.33 - 21.8	b	1	368	166 - 816	f
	2	32.2	19.5 - 53.3	cd	2	82.3	44.7 - 152	de
	3	1.87	1.40 - 2.50	a	3	262	150 - 457	ef
	4	8.55	7.45 - 9.81	b				
	5	17.2	13.1 - 22.6	c				
fipronil	**2**	**0.26**	**0.14 - 0.48**	ab	2	0.63	0.37 - 1.07	b
	4	**0.27**	**0.15 - 0.49**	ab	3	0.22	0.13 - 0.36	a
					4	0.65	0.48 - 0.88	b
lambda-cyhalothrin	**2**	**2.48**	**1.25 - 4.92**	a	**2**	**3.29**	**1.88 - 5.77**	a
	3	**3.48**	**2.18 - 5.53**	a	**3**	**2.26**	**1.60 - 3.21**	a
	4	**1.57**	**0.51 - 4.83**	a				
malathion	**2**	**60.8**	**36.0 - 103**	a	3	73.8	56.3 - 96.7	a
	4	**116**	**104 - 131**	b	**4**	**73.0**	**48.0 - 111**	ab
	5	**86.5**	**53.0 - 141**	ab	**5**	**88.1**	**71.7 - 108**	ab

DISCUSSION

Species sensitivity

Van der Valk *et al.* (1996a) concluded that it was impossible to identify which of the two species was more susceptible to insecticides, but that this might have been due to incompleteness of the data set. Perusal of tables 3 and 4 shows that *Pimelia senegalensis* is often the more susceptible species, but not always. If a choice has to be made between the two species, it is probably best, from a ecotoxicological point of view, to continue testing with *P. senegalensis*. On the other hand, since *Trachyderma hispida* occurs in a much larger number of countries affected by the Desert Locust, this latter species may be preferred for biogeographical reasons.

Robustness of the test system

The variability among the results from tests carried out with the same species and using the same mode of exposure is a measure of the robustness of the test system. In our case, such variability can be caused by genetic or phenological variability in the test organisms, differences in environmental conditions in which the tests were carried out, or variation in the execution of the tests due to different technicians being involved.

Genetic (as well as phenological) variability among the test organisms will definitely exist since the animals are field-collected, sometimes at different locations and different times of the year. It will be difficult to reduce such variability given the problems associated with rearing this type of tenebrionid beetles. Also, the longer insects are reared in a laboratory, the larger is the chance that their susceptibility to insecticides to deviates from field populations.

The toxicity tests have so far been carried out under ambient laboratory conditions. Average temperatures may differ from one test to another by up to 10 °C, although often much less. Relative humidity tends to vary more, and tests have been carried out in humidities ranging from 30% to 85%. Increased standardisation of the testing conditions may well reduce variability in the results. However, it should be kept in mind that ambient test conditions were chosen so as to minimize the costs and the need for specialised climate chambers. If the environmental conditions for these tests are more standardised, there is a risk that considerably fewer laboratories in Africa will be able to carry them out. Probably, defining a range of conditions which are close to the ones encountered in the field, but relatively easy to reproduce in a laboratory, would provide a sensible compromise.

Robertson *et al.* (1995) studied the importance of natural variation in toxicity tests on insects. They tested *Bacillus thuringiensis* (*B.t.*) on cohorts of Colorado potato beetle (*Leptinotarsa decemlineata*) and of diamondback moth (*Plutella xylostella*) during 83 and 37 consecutive weeks respectively. Insects came from the same continuous laboratory rearing colony. Furthermore, they tested DDT and pyrethrins on more than 90 consecutive generations of the same strain of western spruce budworm (*Choristoneura occidentalis*). As a measure of natural variability the ratio between the highest and the lowest LD_{50} were used (among other measures). They found response ratios of 12.8 for *B.t.* on Colorado potato beetle and 3.7 on diamondback moth, all tests carried out with insects coming from the same laboratory colony. Response ratios of 12.7 for DDT and 4.3 for pyrethrins were encountered among tests carried out on different generations of the same genetic strain of western spruce budworm. They argue that this variability is almost solely due to natural variation among the insects, and that scientists should be aware that such natural variability exists and should be taken into account.

We carried out a similar analysis in table 5. For every pesticide, mode of exposure, and species the ratio between the highest and lowest $ED(C)_{50}$ was calculated. These response ratios varied from 1.1 to 13.3 for the topical exposure tests, and from 1.0 to 17.2 for the dietary tests. Twenty-four out of 28 ratios were less than 3.5, and considerably lower than the values obtained by Robertson *et al.* (1995). Clearly, one should be cautious in comparing these two data sets, since Robertson and colleagues used a large number of repeated tests for only a few insecticides, while we used a limited number of repetitions for each insecticide, albeit with a larger number of compounds. The analysis does show, however, that the ranges of $ED(C)_{50}$ values observed in the tests with tenebrionid beetles are not much larger than those obtained with closely controlled laboratory populations. This in spite of the fact that the beetles were collected in the field from different populations, and tests were carried out under varying environmental

conditions, and by different technicians. Therefore, the results suggest that the tenebrionid test system, as it is being used at the moment by CERES-LOCUSTOX, can be considered fairly robust. Although more constant test conditions would be desirable, the use of field-collected insects seems to be appropriate for insecticide risk assessment.

Table 5: Variability in the results from the toxicity tests. Calculated are the ratios between the highest and lowest $ED(C)_{50}$ measured for each pesticide and species.

Insecticide	Ratio of highest and lowest $ED(C)_{50}$			
	Topical exposure		Dietary exposure	
	Pimelia senegalensis	*Trachyderma hispida*	*Pimelia senegalensis*	*Trachyderma hispida*
bendiocarb	3.1	1.6	1.3	4.6
chlorpyrifos	1.1	1.6	1.5	2.1
deltamethrin	13.3	1.4	1.6	1.5
fenitrothion	1.1	2.7	17.2	4.5
fipronil	1.1	2.2	1.0	3.0
lambda-cyhalothrin	3.1	2.0	2.2	1.5
malathion	1.8	1.1	1.9	1.2

Hazard assessment

A provisional hazard assessment was carried out in the previous report which was based on a comparison between initial insecticide residues on vegetation and the dietary EC_{50} values. This assessment was then validated with data from field studies, as far as these were available (Van der Valk *et al.* 1996a). Below we extend this analysis to include the additional toxicity data which are now available (Table 6).

All data on initial vegetation residues were obtained by LOCUSTOX and refer to (semi-)arid grassland vegetation or, if such data were not available, to millet leaves. Results from the semi-field bio-assays have previously been described by Van der Valk *et al.* (1996a). Initial residues similar to or above 96h-EC_{50} values were considered to pose a high risk of acute effects on tenebrionid populations in the field. Initial residues of a factor 5 or more below 96h-EC_{50} values were not considered to pose the risk of large acute population reductions.

It appears that a risk of effects of bendiocarb is present, but the initial vegetation residues provided in Table 6 are probably higher than the ones which will be encountered after regular Desert Locust control. The recommended rate of bendiocarb (100 g a.i./ha) is lower than the ones for which residue data were available, and the risk of acute effects of this insecticide on tenebrionid beetles is probably low. No results from field studies are available at present to validate this prediction.

A comparison of vegetation residues with the dietary EC_{50} of chlorpyrifos suggests that acute effects of this insecticide are improbable. This was confirmed in a semi-field bio-assay, as well as by the field study of Van der Valk (1990). Both Rachadi *et al.* (1995) and Balança and de Visscher (1997a), though, observed reductions in pitfall trap catches of one or more species of tenebrionids after treatments with chlorpyrifos.

These reductions were low, not amounting to more than 30% when compared to controls. It should be

noted, in this respect, that the International Organization of Biological Control (IOBC) considers mortalities of <25% in field studies as "harmless", and of 25-50% as "slightly harmful" (Hassan *et al.* 1994). Although this classification can be questioned, the effects of chlorpyrifos in the field are probably limited.

Deltamethrin applications could result in limited mortality in the field, a prediction which is confirmed by results from the semi-field bio-assays. No reliable data from field studies are actually available.

The results from the toxicity tests suggest that large mortality in field populations of certain tenebrionid beetles is likely with fenitrothion. This was indeed confirmed by a field study which showed on average 60% reduction in pitfall catches over a 4-week period of a major species (Van der Valk 1990).

A similar prediction can be made for fipronil, with the distinction that both species in the laboratory tests had ED_{50} values well below initial fipronil residues on vegetation. Large reductions of tenebrionid catches were observed by Rachadi *et al.* (1995) and Balança and de Visscher (1997a) at dose rates down to 4.2 g a.i./ha, while application rates of 1 and 2 g a.i./ha still caused an impact on certain species (Balança and de Visscher 1997b).

Dietary ED_{50} values of lambda-cyhalothrin are close to initial residues observed on vegetation after Desert Locust control, which indicates that at least some acute effects of this insecticide are likely to occur. Results of a study by Balança and de Visscher (1995) indeed show a significant reduction in pitfall catches in 1 out of 3 different treatments.

Acute mortality of tenebrionid beetles can also be expected after malathion treatments. Limited field data are available from Keith *et al.* (1995), who had indications of effects on one species of tenebrionid, but did not report significant reductions in pitfall trap catches. This may be partly due to the fact that malathion was underdosed on their study plots. Effects can be expected to be larger after correct application.

Based on the above, we conclude that a hazard assessment based on dietary toxicity tests with *P. senegalensis* and *T. hispida* together with data on initial residues on vegetation, generally appears to provide valid results. For those insecticides for which field data were available, only in the case of chlorpyrifos did some impact in the field occur while this was not predicted by the hazard assessment. Therefore, in the absence of field data, results from the dietary toxicity test when compared to a measure (or estimate) of initial vegetation residues, can provide an appropriate preliminary assessment of insecticide hazard to tenebrionid beetles in Sahelian grassland ecosystems.

Table 6: Hazard assessment for non-target Tenebrionidae of the insecticides tested. Initial field residues and corresponding field rates are those measured by the Locustox Project on (semi-)arid grassland or on millet leaves. Observed effects in field trials refer to experiments in (semi-)arid ecosystems showing impact on tenebrionid beetles in general after treatments at approximately recommended rates against the Desert Locust.

Insecticide	Initial vegetation residues (mg/kg fresh weight)	Corresponding field dose rates (g a.i. /ha)	Species	dietary EC_{50} (mg/kg millet bran)[1]	Effect in the field expected [2]	Effect in bioassay observed	Population reduction in field observed	Duration before recovery
bendiocarb	27 - 51 [b]	165 - 195	*P.s.*[3]	56	possibly	–[4]	–	–
			T.h.	186	no	--		
chlorpyrifos	19 - 35 [c]	200 - 230	*P.s.*	641	no	no [e]	no [g], yes [h], yes [i]	≥4 weeks at up to 30% reduction [h, i]
			T.h.	223	no	--		
deltamethrin	1.2 - 2.2 [c]	15 - 18	*P.s.*	6.3	possibly	yes [e]	--	–
			T.h.	47	no	--		
fenitrothion	39 - 92 [a]	440 - 455	*P.s.*	9.5	yes	--	yes [g]	≥4 weeks at 60% reduction
			T.h.	199	no	--		
fipronil	2.7 - 5.6 [m]	9 - 12.5	*P.s.*	0.26	yes	--	yes [h,i]	≥4 weeks at >90% reduction [h, i]
			T.h.	0.45	yes	yes [e]		
lambda-cyhalothrin	1.5 [f]	19	*P.s.*	2.4	possibly	--	sometimes [k]	10 days at 50% reduction (1 plot);
			T.h.	2.7	possibly	--		other plots no sign. effect
malathion	50 - 95 [d]	925	*P.s.*	85	yes	--	indications for	no details
			T.h.	78	yes	--	reduction [j]	

1: Geometric means of 96h-EC_{50}'s listed in table 4; 2: at Desert Locust control rate; 3: *P.s.=Pimelia senegalensis*, *T.h.=Trachyderma hispida*; 4: – : no data available

Sources : a= Gadji 1993a, b= Gadji 1993b, c= Gadji 1996, d= Gadji unpublished results, e=Van der Valk *et al.* 1996a, f= Van der Valk *et al.* 1996b, g= Van der Valk 1990, h= Rachadi *et al.* 1995, i=Balança and de Visscher 1997a, j= Keith *et al.* 1995, k= Balança and de Visscher 1995, m=Gadji *et al.*

Table 7: Risk assessment of 7 insecticides used in Desert Locust control for non-target tenebrionid beetles. The assessment is based on effective field dose rates for Desert Locust control[1]. See text for further explanation.

Insecticide	Effective dose rate (g a.i./ha) [1]	Likelihood and predicted size of impact on tenebrionid beetles in the field
bendiocarb	100	possible, small effect [3]
chlorpyrifos	240	possible, small effect
deltamethrin	12.5	possible, small effect
fenitrothion	450	likely, large effect
fipronil	6.25 (12.5 [2])	likely, large effect
lambda-cyhalothrin	20	possible, moderate effect
malathion	925	likely, moderate effect

1: FAO 1997, 2: 12.5 g a.i./ha inside the barrier in case of barrier treatment, 3: "small effect" = <25% population reduction, "moderate effect" = 25-50% population reduction, "large effect" = >50% population reduction.

An assessment of the acute risk of these 7 insecticides to tenebrionid beetles can now be made (Table 7). It is based on all available results from laboratory toxicity tests, field bio-assays, full scale field experiments and data on initial insecticide residues. The risk of population reduction is assessed for the effective application rates as reported by FAO (1997). We classified the occurrence of an effect as "likely" if both laboratory and field studies indicated that impact would take place. If either laboratory or field experiments (but not both) indicated that population effects would (sometimes) occur, we classified an effect as "possible". When both the laboratory tests as well as the field experiments resulted in no impact at Desert Locust control rates, an effect would be classified as being "unlikely". Note that chronic effects of the insecticides, such as an impact on reproduction, cannot be predicted from these data.

The classification of effect size used in this table is more strict than the one applied by the IOBC. This was done because tenebrionid beetles in Sahelian ecosystems tend to have more limited dispersal capacities and lower population reproduction rates than most of the beneficial arthropods generally assessed by IOBC (Hassan *et al.* 1994). Therefore we consider a reduction in the field of more than 50% as an ecologically important effect, while IOBC sets its criteria "harmful" in excess of 75% effect.

We conclude that treatments with fenitrothion and fipronil are likely to result in severe population reductions of tenebrionid beetles. There is also a high possibility that malathion will cause population reductions, but of a moderate size. Treatments with bendiocarb, chlorpyrifos, deltamethrin and lambda-cyhalothrin could possibly result in reductions of some tenebrionid populations. The impact of lambda-cyhalothrin may be moderate; bendiocarb, chlorpyrifos and deltamethrin will probably have only a small effect.

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APPENDIX 1

Topical ED_{50} values and dietary EC_{50} values for *A: Pimelia senegalensis* and *B: Trachyderma hispida* at different observation times. Test numbers refer to tables 3 and 4 in the text.

A: Pimelia senegalensis

bendiocarb (topical : µg/g insect)				
No.	*Time (h)*	*ED50*	*95% confid.*	Interval
8	24	21	9,63	45,9
	48	16,5	8,17	33,4
	72	7,72	4,46	13,4
	96	6,35	3,75	10,8
	120	6,35	3,75	10,8

bendiocarb (dietary : mg/kg millet bran)				
No.	*Time (h)*	*ED50*	*95% confid.*	Interval
5	24	130,1	86	196,7
	48	109	73,6	161,4
	72	63	44,1	90
	96	49,3	34,6	70,2
	120	36,8	25,7	52,8
	144	29,1	20	42,2
	168	299,1	20	42,2

deltamethrin (topical : µg/g insect)				
No.	*Time (h)*	*ED50*	*95% confid.*	Interval
5	24	0,056	0,034	0,093
	48	0,056	0,034	0,093
	72	0,036	0,022	0,058
	96	0,022	0,013	0,036
	120	0,015	0,009	0,025
	148	0,014	0,009	0,023
7	24	0,024	0,015	0,037
	48	0,019	0,012	0,03
	72	0,019	0,012	0,03
	96	0,018	0,011	0,028
	120	0,018	0,011	0,028
	144	0,018	0,011	0,028
	168	0,018	0,011	0,028
	192	0,012	0,008	0,019

fipronil (topical : ng/g insect)				
No.	*Time (h)*	*ED50*	*95% confid.*	Interval
2	24	933	552	1578
	48	220	149	326
	72	96,3	76,7	121
	96	81,9	70,3	95,4
	120	76,2	64,1	90,6
	144	49,3	34,6	70,2
	168	49,3	34,6	70,2
4	24	326	234	454
	48	225	166	304
	72	95,3	74,1	123
	96	76,2	68,8	84,5
	120	75,4	67,7	83,9
	144	51,7	36,9	72,5

fipronil (dietary :µg/kg millet bran)				
No.	*Time (h)*	*ED50*	*95% confid.*	Interval
2	24	9682	3401	27561
	48	2362	1161	4807
	72	649	359	1176
	96	259	141	476
	120	175	92,4	33
	144	68,1	31	150
4	24	1444	762	2733
	48	737	412	1317
	72	677	380	1207
	96	273	153	489
	120	211	116	384
	144	102	51,4	203

lambda-cyhalothrin (topical : ng/g insect)				
No.	Time (h)	ED50	95% confid.	Interval
3	24	61,3	41,5	90,5
	48	58	39,5	85
	72	58	39,5	85
	96	55	37,8	80,2
	120	47,4	32,8	68,7
4	24	145,6	94	225,6
	48	134,3	86,9	207,6
	72	134,3	86,9	207,6
	96	134,3	86,9	207,6
	120	134,3	86,9	207,6
	147	134,3	86,9	207,6
6	24	190,2	105,7	342,4
	48	190,2	105,7	342,4
	72	190,2	105,7	342,4
	96	173,1	97,3	308,1
	120	135,4	77,8	235,4
	148	99,1	57,9	169,6
	172	99,1	57,9	169,6
	196	91,3	53,4	156,1

lamda-cyhalothrin (dietary : mg/kg millet bran)				
No.	Time (h)	EC50	95% confid.	Interval
2	24	7,52	4,04	14
	48	6,63	3,58	12,3
	72	3,17	1,65	6,09
	96	2,48	1,25	4,92
	120	2,48	1,25	4,92
	148	2,48	1,25	4,92
	172	2,48	1,25	4,92
	196	2,48	1,25	4,92
3	24	7,7	4,88	12,1
	48	4,65	2,95	7,33
	72	3,72	2,34	5,91
	96	3,48	2,18	5,53
	120	2,27	1,38	3,73
	144	1,92	1,15	3,21
4	24	7,99	4,89	13
	48	2,51	1,41	4,45
	72	2,19	0,65	7,33
	96	1,57	0,51	4,83
	120	0,46	0,19	1,11

malathion (topical : µg/g insect)				
No.	Time (h)	ED50	95% confid.	Interval
2	24	46	31,4	67,3
	48	37,4	26,7	52,3
	72	27,5	20,3	37,1
	96	21,3	15,8	28,9
	115	21,3	15,8	28,9
5	24	62,4	43,1	90,4
	48	30,8	23,5	40,5
	72	22,1	16,8	28,9
	96	18	13,6	23,9
	120	14,6	10,8	19,7
	144	14,6	10,8	19,7
	168	12,1	8,8	16,7
6	24	120,8	75,3	194,1
	48	66,2	48,1	91,1
	72	43,1	32,5	57,3
	96	33,2	25	44,2
	120	27,9	20,7	37,7
	144	26,7	19,7	36,2
	168	25,5	18,7	34,7

malathion (dietary : mg/kg millet bran)				
No.	Time (h)	EC50	95% confid. Interval	Interval
2	24	490	292	823
	48	146	90	237
	72	101	62	166
	96	60,8	36	102,8
	110	54,7	32,1	93,5
	134	49,4	28,6	85,4
4	24	543	359	821
	48	220	178	273
	72	189	154	231
	96	116	104	131
	120	113	101	127
	148	61	42	88,5
	172	61	42	88,5
	196	61	42	88,5
5	24	268	160	448
	48	139	86	226
	72	109	67	177
	96	86,5	53	141,2
	120	74,1	45,1	121,5
	144	68,7	41,7	113,1
	168	54	32,3	90,2

B: Trachyderma hispida

bendiocarb (dietary : mg/kg millet bran)				
No.	*Time (h)*	*EC50*	*95% confid.*	interval
3	24	209	129	338
	48	151	97	236
	72	133	86	205
	96	124	80	191
	120	116	76	178
	144	94	62	145
	168	94	62	145
4	24	212	140	319
	48	135	95	190
	72	94	69	129
	96	85	63	116
	120	85	63	116

chlorpyrifos (topical : µg/g insect)				
No.	*Time (h)*	*ED50*	*95% confid.*	interval
4	24	70	57,1	85,9
	48	54,3	44,3	66,5
	72	40,7	32,6	50,8
	96	40,7	32,6	50,8
	120	40,7	32,6	50,8
	144	39	31,2	48,9
	168	37,4	29,8	47,1

lambda-cyhalothrin (topical : ng/g insect)				
No.	*Time (h)*	*ED50*	*95% confid.*	interval
1	24	472	335	652
	48	472	335	652
	72	472	335	652
	96	472	335	652
	120	430	310	596
4	24	287	205	403
	48	263	188	369
	72	263	188	369
	96	263	188	369
	120	263	188	369
5	24	602	417	871
	48	294	204	422
	72	241	167	346
	96	241	167	346
	120	241	167	346
	144	241	167	346

lamda-cyhalothrin (dietary : mg/kg millet bran)				
No.	*Time (h)*	*EC50*	*95% confid.*	interval
2	24	19,7	9,17	42,5
	48	6,22	3,44	11,3
	72	3,97	2,25	6,99
	96	3,29	1,88	5,77
	120	1,88	1,06	3,34
	144	1,72	0,97	3,07
3	24	4,04	2,53	6,45
	48	3,7	2,36	5,82
	72	2,9	1,92	4,38
	96	2,26	1,6	3,21
	120	1,78	1,27	2,49
	144	1,65	1,16	2,33

malathion (dietary : mg/kg millet bran)				
No.	Time (h)	EC50	95% confid.	Interval
4	24	661	379	1153
	48	147	96	226
	72	104	68	158
	96	73	48	111
	120	73	48	111
	144	51,3	33,3	78,6
5	24	452	304	673
	48	308	216	437
	72	122	89,7	166
	96	88,1	71,7	108
	120	77	62,4	94,9
	144	54,2	38,1	76,9

CHAPTER 8:

Side-effects of chlorpyrifos and deltamethrin in a Sahelian millet agro-ecosystem

Harold VAN DER VALK and Ousmane KAMARA

SUMMARY

Fields planted with pearl millet (*Pennisetum typhoides*) in central Senegal were experimentally treated with insecticides used in locust control during the rainy season of 1993. This to study their impact on beneficial arthropods in the millet agro-ecosystem as well as the likelihood of pest resurgence after locust or grasshopper control operations.

Four fields of 1.3- 2.6 ha each were treated with chlorpyrifos at an average rate of 214 g a.i./ha; four others were sprayed with deltamethrin at on average 16.4 g a.i./ha. Treatments took place during the late flowering and early maturation stages of the crop. Four additional fields functioned as untreated controls. Presence of beneficial arthropods was assessed using malaise traps, yellow sticky traps and direct field observations. Different life stages of the millet head miner (*Heliocheilus albipunctella*), a primary candidate for resurgence, were sampled throughout the study. Bioassays were carried out to assess the toxicity of insecticide residues present on millet leaves to the parasitoïd *Bracon hebetor* (Braconidae) and the predator *Verania striata* (Coccinellidae).

Fifteen taxa were monitored by trapping. It was argued that insecticide effects could be validly evaluated for 8 of these taxa. Chlorpyrifos applications significantly reduced the activity density of Bombyliidae (77%), Ichneumonidae (66%) and coccinellid sp.2 (33%), all for at least three weeks after treatments. Deltamethrin applications caused fewer effects on the activity densities of beneficial arthropods, resulting in significant reductions of Ichneumonidae (64%, for at least three weeks) and Tachinidae (42%, during one week). A significant five-fold increase of Halictidae, which lasted one week, was also observed with this insecticide.

The bioassays showed that fresh chlorpyrifos residues on millet leaves were highly toxic to *Bracon*, but not so to *Verania*. The opposite was the case for deltamethrin, being highly toxic to *Verania* and much less so to *Bracon*. The toxicity of chlorpyrifos residues to both species diminished rapidly; deltamethrin was more persistent. Predictions based on these bioassays did not always agree with effects actually observed on trap counts in the field. Reasons why this might have been the case are discussed.

Chlorpyrifos applications resulted in an average increase in the peak density of the millet head miner (*Heliocheilus albipunctella*) of 88%. Deltamethrin did not cause significant changes in head miner densities. Rates of parasitism of head miner larvae by *Bracon hebetor* did not appear to be affected by either of the insecticides. It is hypothesized that insecticide induced mortality of flying predators such as sphecoid wasps or hemipterans may have been responsible for increased survival of the millet head miner in fields treated against locusts with chlorpyrifos.

INTRODUCTION

In spite of recent developments on mycopesticides and chitin synthesis inhibitors, control of locusts and grasshoppers still relies to a large extent on the use of broad-spectrum insecticides. Such compounds are potentially harmful to beneficial arthropods in agricultural ecosystems.

We studied the effects of fenitrothion and diflubenzuron on natural enemies in pearl millet in Senegal (Van der Valk and Kamara 1993). This crop is regularly sprayed during locust or grasshopper control campaigns. Several groups of natural enemies, all Hymenoptera, were affected by fenitrothion applications during early maturation of the crop. Diflubenzuron did not have statistically significant effects on the studied taxa. Furthermore, fields treated with the organophosphate had on average 80% higher peak densities of *Heliocheilus albipunctella*, the millet head miner. This lepidopteran millet pest is of increasing importance in the western Sahel.

We hypothesized that fenitrothion had reduced the densities of natural enemies of the millet head miner, thus allowing a larger fraction of the larvae the survive until late in the season. A second study applying the same insecticides, but slightly earlier in the growing season, did not show as much effect on beneficial arthropods, not did it result in increased head miner densities (Kamara and Van der Valk 1995). Therefore, we suggested that broad spectrum insecticides used in locust control mainly affect predators and/or parasitoïds of the millet head miner larvae rather than their eggs.

Here we present a third study on the side-effects of locust and grasshopper control in the millet agro-ecosystem. Two other insecticides were chosen for this study. Chlorpyrifos is one of the most widely used insecticides in recent Desert Locust control campaigns. It is a broad-spectrum organophosphate like fenitrothion. We were especially interested to see if chlorpyrifos applications would result in similar effects to those found earlier with fenitrothion. Deltamethrin is used on a smaller scale in locust control. It is a synthetic pyrethroid, which tends to be less toxic for vertebrates than the organophosphates mentioned above, but still showing broad-spectrum activity against arthropods. Its effects compared to the organophosphates was our main concern. Since most side- effects were to be expected with treatments carried out at the early maturation stage of the millet, the treatment schedule of the very first study (Van der Valk and Kamara 1993) was repeated.

The report was kept as concise as possible. We refer to our previous reports (Van der Valk and Kamara 1993, Kamara and Van der Valk 1995) for a more in depth discussion of the role of natural enemies in millet and the effects insecticides may have on them.

STUDY SETUP AND METHODOLOGY

General study lay-out

Twelve farmer fields planted with pearl millet (*Pennisetum typhoides*, variety Souna III) were selected for the study in late July 1993 around the villages of Nioro du Rip and Prokhane, in west-central Senegal. Treatments were assigned to four blocks of three fields each. Each block contained a control field and two fields to be treated with different insecticides. Two blocks were located close to Nioro, two others around Prokhane. Distances between neighbouring plots within a block varied from a minimum of 250 m to about 1 km. Blocks, however, were spaced up to 15 km apart.

Fields which were to be sprayed were chosen such that all of the directly neighbouring fields had crops other than millet. This was done to assure that any recolonization of the treated plots would proceed in a similar fashion, and to avoid underestimation of the impact of the insecticide which generally is applied to series of adjoining millet fields at a time. Complete randomisation of treatments within each block was not feasible because millet fields that were isolated from other millet fields, as well as sufficiently large for the experiment, were relatively rare. Control fields were therefore chosen at the start of the study, since there was no need for them to be isolated from other millet fields. Treatments were subsequently assigned in a random fashion to the other two fields in each block.

Fields that were to be treated varied in size from 1.3 to 2.6 ha. All were cultivated according to standard farmer practice, and project staff did not intervene in cultivation apart from the insecticide treatments. No other pesticide applications were made by the farmer. The preceding year all fields had been cultivated with peanuts or maize, and had not received insecticides.

Insecticide application and deposition

Four fields were treated with the organophosphate chlorpyrifos (Dursban® 240 g a.i./l ULV formulation); four others were sprayed with deltamethrin (Decis® 10 g a.i./l ULV formulation), a synthetic pyrethroid. Control fields were left untreated. Treatments took place September 21st - 22nd, 1993 on the fields near Nioro du Rip (no. 2,3,4 and 5), and September 28th - 29th on the fields near Prokhane (no. 8,9,10 and 11). Pesticide application to the latter group of fields was delayed because they had been sown later than the Nioro fields and millet development slightly lagged behind. Millet development was mostly at the late flowering and early maturation stages at the time of treatment in all fields.

Treatments were carried out with a portable Micro-ULVA® spinning disk sprayer. The sprayer was equipped with four 1.5 V dry cell batteries, resulting in a droplet volume median diameter (VMD) varying between 80 and 100 µm. Spray passes were always perpendicular to the direction of the wind (with a maximum deviation of ~40°). The sprayer head was held at the height of the millet ears (1.7 - 2 m). Track spacing was kept standard at 10 m. Spray passes were marked by flag men, one at each side of the plot.

Flow rate and operator walking speed had been calibrated before the treatments. During application walking speed was timed for every spray pass and adjusted whenever needed to assure an even coverage of the plot. Total volume of pesticide in the sprayer bottles before treatment, as well as the volume left over afterwards, were measured. The surface area of the sprayed field was estimated with a calibrated distance wheel and a compass. Area dosage was subsequently calculated based on area treated and volume of insecticide used.

Wind speed was measured regularly during the treatments using a cup-anemometer held at approximately 1.7 m above the ground. Dry and wet bulb temperatures were determined at the start and end of each treatment with an aspired psychrometer, whirled in the shade.

Insecticide residue analysis was performed on millet leaves which were sampled approximately one hour after spraying. To assess degradation rates of the insecticides under the circumstances of the trial, further vegetation samples were taken at fixed intervals after spraying. Residue analysis was carried out using a gas chromatograph with an electron capture detector. A full description of the sampling procedures, extraction and analysis is given by Gadji (1996).

Arthropod assessments

Malaise traps

Malaise traps (interception traps for flying insects) were used to capture flying beneficial insects, especially Hymenoptera and Diptera.

Two malaise traps was erected in the centre of each plot (Figure 1). It had two opposing interception areas of 1 x 1.50m. The tops of the traps were made out of fine, white mosquito netting, the lower part was, darker, Bordeaux red or red-brown mosquito netting. One trap on each plot was oriented north-south, the other one east-west. The collection bottle at the top of the trap was filled with a 4% formol solution.

Malaise traps were first installed August 2nd, 1993 on the fields around Nioro and August 9th on those around Prokhane. Thereafter, collection bottles were changed on a weekly basis until harvest of the millet, mid-October. Insect samples were taken back to the laboratory, washed with tap water, and subsequently stored in 70% ethanol while awaiting further sorting and identification.

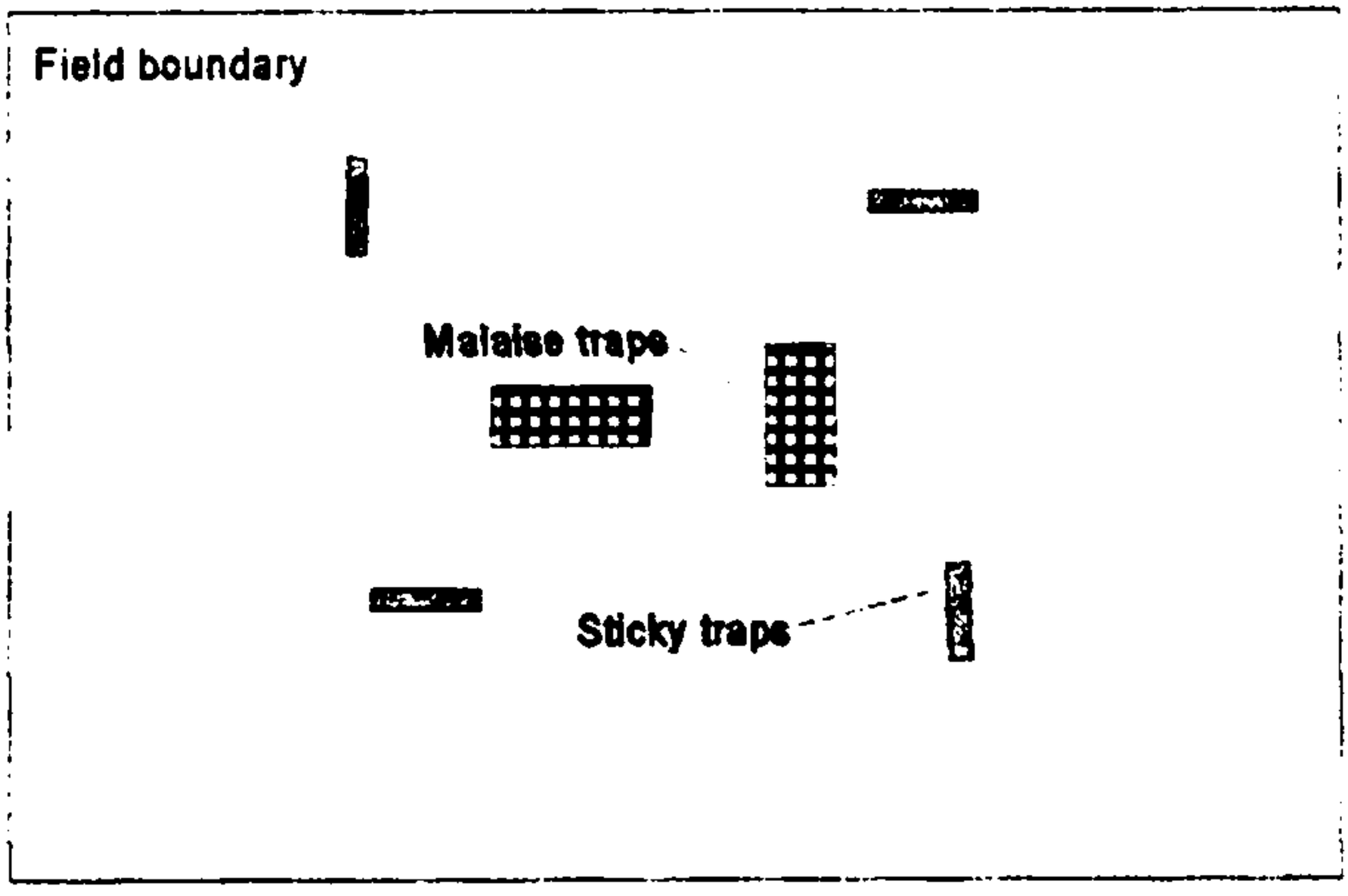


Figure 1: Placement of traps within the experimental fields.

Sticky traps

Yellow sticky traps were installed to monitor micro-Hymenoptera, Syrphidae and Coccinellidae. Four poles were erected in each plot, 25-40 m outward from the malaise traps, but assuring a minimum distance to the field border of at least 20-30 m (Figure 1). Each pole had the form of a T, 2m high with the horizontal top bar measuring 50 cm. Two flat yellow sticky traps (Agrisense Ltd, UK, 25x30 cm) were affixed vertically to each pole, at the ends of the horizontal top bar. On both sides of the plastic yellow cards a thin layer of Trappit® Glue had been applied.

Traps were first installed September 3rd, 1993 and then on weekly basis until harvest of the millet. After installation, the yellow cards were left in the field for 4 days, then taken down, carefully wrapped in kitchen cling film, and transported back to the laboratory. The cling film was used to protect the insects caught on the cards, and to allow easy observation under a stereo-microscope. The cards were stored in a refrigerator until identification and counting, to minimise the growth of fungi on the insects.

Population parameters of millet head miner

Egg incidence

On three occasions between September 6th and September 23rd, eggs of *Heliocheilus albipunctella* (hereafter simply called *Heliocheilus*), the millet head miner, were sampled in all fields, to form an idea about the incidence of oviposition by this potential pest species. In each field, 10 millet ears were cut that had emerged from the flag leaf but on which male flowers had not yet extended. *Heliocheilus* oviposits on these just emerged ears. Ears were individually put in small paper bags and taken back to the laboratory.

There, the ears and the bags were inspected thoroughly and any eggs found were counted and carefully dislodged from the ear. Undamaged eggs were individually incubated in gelatine capsules and kept at ambient temperature in the laboratory. This was done the same day that the ears were cut, or the day after; in the latter case the ears would be kept in a refrigerator overnight. After a minimum of 3 weeks, emergence of the larvae of *Heliocheilus* or the adults of *Trichogrammatoidea* sp. (Trichogrammatidae), the principal egg parasitoïd, was assessed under a stereo-microscope.

Larval incidence

Three times after the treatments, while millet ears were maturing, incidence of *Heliocheilus* larvae was estimated. Forty millet hills were inspected per field per session and the percentage of ears showing attacks, i.e the presence of galleries in the ear, noted. Hills were selected in a systematic way, eg. every 5th, 6th or 7th hill in 3 or 4 rows chosen in the centre of the plot. Consecutive assessments in the same plot did never include the same hills.

During each observation, 25-30 ears showing attacks were cut and taken back to the laboratory. After dissection of the ears, the number of larvae of *Heliocheilus* was noted.

Parasitism by Bracon hebetor

Immediately after the harvest of the millet, an assessment was carried out of the rate of parasitism by *Bracon hebetor* (Hymenoptera, Braconidae). This parasitoïd constructs tough silky cocoons in which it pupates, that persist in the galleries made by the millet head miner. Thus, inspection of millet ears showing galleries at the end of the growing season allows for a fairly precise estimate of cumulative, total, parasitism rates. It does underestimate mortality due to *B. hebetor,* though, since millet head miner larvae may be killed by the parasitoïd without the latter ovipositing on it.

In each field, between 200 and 300 millet ears were sampled at random from the harvest collection points. The percentage of ears infested with the head miner were determined. Subsequently, all

galleries on the infested ears were opened up and inspected for cocoons of *Bracon hebetor*.

Residual populations in the soil

To assess if the treatments had any effects on residual populations of *Heliocheilus* pupae in the soil as well as on certain natural enemies, soil samples were taken in each field between November 16th and 24th, 1993. Ten holes, situated on the two median axes, were dug in each field. Every hole measured 50 x 50 x 20 cm (width x length x depth). The soil was sieved over a 1 mm mesh sieve and all chrysalids of *H. albipunctella* were collected, as well as diapausing cocoons of *Cardiochiles sahelensis* (Braconidae), and *Heliocheilus* larvae mummified by the parasitoïd *Copidosoma* sp nr. *truncatellum* (Encyrtidae).

Bioassays

Bioassays were carried out to evaluate the toxicity of treated vegetation to *Bracon hebetor* (Braconidae) and *Verania striata* (Coccinellidae). Millet leaves were cut in the fields at different times after treatment. Subsequently, insects were exposed to the insecticide residues on these leaves, as well as on untreated control leaves, and effects assessed approximately 24 hours after the start of the bioassay.

Bracon hebetor

The bioassay method used for *Bracon hebetor* (Braconidae) has been described by Van der Stoep (1992) and by Danfa *et al.* (1997). Small plastic tubes (4 cm long x 2 cm Ø) were used a bioassay cages for the wasps. Both ends were closed off with mosquito netting to allow for ventilation. The interior wall of the tube was first covered with a piece of humidified filter paper. On this filter paper a piece of millet leaf was placed so that the entire interior wall of the tubes was covered (only both ends were left uncovered). Two parasitoïds (1♀ and 1♂) were introduced in each bioassay cage. After 24 hours, mortality and sub-lethal effects such as knockdown or partial immobilization were scored. For each independent replicate test, generally ten bioassay cages were used. Insects were obtained from the rearing colony at the Nioro du Rip entomological laboratory just before the start of the bioassay.

Verania striata

The bioassay with the *Verania striata* (Coccinellidae) was loosely based on the methodology described by Wiles and Jepson (1992). Plastic petri dishes (5.5 cm Ø) were used as bioassay cages. In the bottom of the petri dish a hole of approximately 3 cm Ø was cut and covered with mosquito netting to allow for ventilation of the cages. Two pieces of humidified filter paper were placed in the lid of the dish. On the filter paper a disk of millet leaf was placed so that it completely covered the lid of the petri dish. The cage was closed by placing the bottom of the dish on top of the lid. In this way the millet leaf was snugly fixed to one side of the bioassay cage. Generally 3 adult coccinellids were put in each cage. Mortality and sub-lethal effects such as knockdown or partial immobilization were assessed. Ten cages (30 insects) were used per treatment, either insecticide(s) or control. No distinction between sexes was made. All insects were field-captured and had been obtained from untreated millet, maize or peanut fields on the evening before the start of the bioassay. They were left overnight in pots filled with millet or peanut leaves, under ambient laboratory conditions, for acclimatization. No aphids were provided as food.

Data analysis

Time series

The catches from the malaise traps result in short time series of ten samples (over ten weeks) with

seven samples before and three after treatment. Sticky trap assessments resulted in a series of three samples before and three after treatment. Analysis of variance was chosen as method of statistical comparison based on the BACI (Before-After-Control-Impact) principle (Stewart-Oaten *et al.* 1986, Underwood 1991).

The effect parameter used in all analyses was the (log-transformed) difference between the treatment value (insect count from the trap) for a given sampling date, and its paired control value within the same block (Stewart-Oaten *et al.*, 1986). This pairing of treated and control values within each block was done under the assumption that variability within blocks was generally less than among blocks (see §2.1). The effect parameter was calculated as "ln(treated count + 1) - ln(control count + 1)".

One or more values of the effect parameter after treatment were then tested against the average effect parameter over the five weeks before treatment, using sampling dates as (pseudo) replicates. Otherwise said, one tests if the ratio of counts between the treated and control fields has significantly changed after treatment when compared with the average ratio before the treatment. Five pre-treatment counts were used in the statistical analysis of the malaise traps, rather than all seven, so that it was comparable to the study carried out in 1991 with fenitrothion and diflubenzuron (Van der Valk and Kamara 1993).

Since each block contained both treatments and a control plot, the study resembles a randomised complete block (RCB) design. However, a RCB analysis of variance (ANOVA) assumes that no interaction exists between blocks and treatments (Sokal and Rohlf 1981, Dutilleul 1993). This could not be excluded here *a priori*. Therefore, a two-way ANOVA, with explicit assessment of the interaction term, was applied instead.

The corresponding ANOVA table is given below (Table 1). This is a mixed model ANOVA with blocks considered a random factor and treatment as fixed (Sokal and Rohlf 1981, Bennington and Thayne 1994).

Table 1: Description of the analysis of variance (ANOVA) used to assess the effect of insecticide treatments on the activity density of beneficial arthropods.

Source of variation	degrees of freedom	F-ratio	Conditions
Blocks	3	not tested	
Before vs. After (=effect of treatment)	1	1] $MS_{bef.\ vs.\ aft.} / MS_{interaction}$	if interaction is significant
		or	
		2] $MS_{bef.\ vs.\ aft.} / MS_{error}$	if interaction is not significant
		or	
		3] $MS_{bef.\ vs.\ aft.} / MS_{pooled\ error}$	if interaction is not significant, AND 2] is not significant, AND pooling of interaction + error terms is allowed
Interaction	3	$MS_{interaction} / MS_{error}$	
Error	4[(t1-1)+(t2-1)]		
Total	4(t1+t2)-1		

t1 = number of dates (replicates) before treatment; t2 = number of dates (replicates) after treatment;
MS = mean square

The effect of the treatment ($MS_{before\ vs.\ after}$), which is our main interest in this study, is tested over the interaction term, only if the latter is significant. A significant interaction means that the effect of treatment is not generalised over all blocks. In such a case, the treatment effect will only be considered "ecotoxicologically significant", if it is significant over the interaction. When the interaction is not significant, one may decide to pool the interaction and error mean squares. There does not seem to be general agreement among statisticians if this should be done (Sokal and Rohlf 1981). Therefore, conservatively, MSs were not pooled if the F-ratio based on the error mean

square was already significant. Only when this F-ratio was not significant, and after verifying Bancroft's rules for pooling (Sokal and Rohlf 1981), were the interaction and error MS pooled and used as the denominator over which to test the treatment effect.

All calculations were programmed into a Quattro Pro v7.0 spreadsheet.

A small number of "missing values" had to be taken into account for both trap types. These were non-valid catches, mostly malaise trap collection bottles or sticky trap cards that had fallen from the trap. Also, the number of trapping days was not always the same, especially when traps had to be repaired after having been damaged. In case of the sticky traps, all counts were therefore proportionally standardised to represent the number of insects/8 traps/4 trapping days. Counts for malaise traps were proportionally standardised to represent the number of insects/2 traps/7 trapping days. Missing data were proportionally corrected, based on counts for the remaining trap(s) in the same field on the same date.

The size of the effect of treatment is expressed as the percentage reduction or increase in trap catches, compared to the 4 weeks before treatment, and corrected for any control variation. Effect size was calulated as:

$$\%\ relative\ effect = \left[1 - \frac{avg.\ TR\ after\ treat.}{avg.\ CO\ after\ treat.} \times \frac{avg.\ CO\ before\ treat.}{avg.\ TR\ before\ treat.}\right] \times 100\% \quad (1)$$

with: *avg. TR = average count in the treated plots*
avg. CO = average count in the control plots
before treat. = the 5 trapping weeks before treatment
after treat. = one or more trapping weeks after treatment

Other data sets

Whenever comparisons were made between data gathered at one specific point in time (e.g. density of larvae, percentage parasitism), RCB-design Analysis of Variance was used with blocks as replicates and treatments as the factor to be analysed. Percentages (%) were as a rule transformed to arcsin[square root (%/100)]; counts were transformed to log(count) whenever variances of the untransformed data were not homogeneous after applying an F_{max} test (Sokal and Rohlf 1981). When significant differences were found in the ANOVA, separation of means was carried out with the Least Significance Difference (LSD) test. Calculations were carried out using the MSTAT-C statistical package for personal computers (MSU 1989).

Error levels

Type I error levels (alpha) were set at 0.10 (i.e. a maximum 10% probability of concluding (wrongly) from the given statistical test that the treatments do have an effect while in reality they do not). This slightly larger error level than usual was used because it reduces the probability of making a Type II error (beta) (the chance of concluding (wrongly) from the test that the treatments do not have an effect, while in reality they do). A high probability of making a Type II error is often

neglected, but from an environmental point of view this may be just as important, or more important, than making a Type I error. A more flexible approach to setting error levels has been suggested by several authors for environmental impact studies (Fairweather 1991, Mapstone 1995).

Meteorological data

Rainfall and minimum and maximum temperatures were collected at the Nioro du Rip laboratory as well as in the village of Prokhane (Annex 1).

RESULTS

Pesticide applications

The results of the insecticide treatments are given in Table 2. The recommended application rate of chlorpyrifos against the Desert Locust is 240 g a.i./ha (FAO 1997). Actually applied rates in this experiment averaged 214 g a.i./ha, with a maximum deviation of 17% from the recommended rate. Initial residues in the top part of the millet crop ranged from 19 to 35 mg a.i./kg fresh vegetation (Gadji 1996). This corresponds well with initial deposition of treatments in previous experiments with organophosphates in millet (Gadji 1993a,b). Chlorpyrifos disappeared relatively rapidly from millet leaves, having a chemical half-life of 1-2 days.

The intended application rate of deltamethrin was 15 g a.i./ha (FAO 1997). Actual application rates in this experiment were on average 16.4 g a.i./ha. The maximum deviation was 18% from the recommended rate. Initial residues on millet vegetation ranged from 1.2 - 2.2 mg a.i./kg fresh weight. Deltamethrin disappeared more slowly from millet leaves than chlorpyrifos, with chemical half-lives ranging from 2 - 7 days.

Wind speed during treatment, one of the more important factors influencing the quality of ultra low volume drift spray applications, was rather variable. On average it surpassed 1 m/s, though, which is considered a minimum for this type of applications (FAO 1992). It appears that this variability in wind speed did not reduce insecticide deposition very much, since the measured initial residues were not particularly low given the application rates.

Effects on beneficial arthropods

Weekly catches from malaise traps were available for a period of seven weeks before and three weeks after treatment. Catches for all these weeks are shown in the graphs below, but only the five last pre-treatment counts (weeks 3 to 7) were used in the statistical analysis, as was discussed above. The three pre-treatment and three post-treatment counts from the sticky traps are shown in the graphs, and all were used in the statistical analyses.

Catches were sorted and identified for a limited number of taxa which are known to be predators or parasitoïds of millet or groundnut pests, or otherwise could be considered as beneficial. Groundnuts are often grown interspersed, and in rotation with millet in this part of Senegal, and may receive locust or grasshopper control treatments as well. Table 3 lists the identified taxa, their role in the agro-ecosystem, as well as the total number of specimens captured.

Abundance of insects in the traps depends on various factors, including their actual density, their activity, meteorological conditions, and trap placement (Muirhead-Thomson 1991). Counts in traps will therefore further be referred to as the insect's "activity density".

Diptera

The impact of the insecticide treatments was assessed for four different taxa of flies (Table 3).

The activity density of robber flies (Asilidae) peaked early in the growing season (Figure 2a), as was observed in previous studies in millet (Van der Valk and Kamara 1993). At the time of the treatments, only few Asilidae were caught in the malaise traps. A wide range species of robber flies were caught, most of which were fairly small (<1cm long). Even though a slight decrease relative to the controls is seen at the end of the assessment period in the chlorpyrifos fields, this is not statistically significant.

Table 2: Treatment parameters for the fields sprayed with chlorpyrifos and deltamethrin. All applications were carried out with hand-held Micro-ULVA® spinning disk sprayers. Flow rates were approximately 30 ml/min for chlorpyrifos and 56 ml/min for deltamethrin. Track spacing was 10 m. Initial insecticide residues were measured in the top 2-3 millet leaves; half lives were based on exponential decline of the insecticide (see Gadji 1996).

Field	Date of treatment	Hour begin-end [1]	Number of passes	Area treated (ha)	Volume applied (ml)	Area dose (g a.i./ha)	Temperature (°C) begin-end [1]	Relative humidity (%) begin-end	Wind speed (m/s) min.-max.	Initial residues (mg/kg fresh weight)	Half-life (hours)
CHLORPYRIFOS											
2	22 Sep 93	08^{55} - 10^{40}	21	1.32	1260	230	28 - 30	98 - 80	0 - 2.7	34.8	28
4	22 Sep 93	08^{55} - 10^{30}	12	2.07	1850	215	26 - 32	83 - 70	0.5 - 3	30.3	37
8	29 Sep 93	09^{00} - 10^{20}	16	1.74	1520	210	27 - 31	90 - 67	0.5 - 3	18.6	45
10	29 Sep 93	09^{05} - 10^{30}	17	1.85	1530	200	26 - 33	93 - 72	0 - 2.2	32.3	34
DELTAMETHRIN											
3	21 Sep 93	08^{15} - 09^{35}	13	2.4	3770	15.7	25 - 28	89 - 80	0.8 - 1.9	1.6	n.s. [2]
5	21 Sep 93	08^{35} - 10^{15}	24	1.99	3525	17.7	25 - 32	88 - 86	0.5 - 2	2.2	45
9	28 Sep 93	08^{10} - 10^{10}	22	2.62	4495	17.2	25 - 33	95 - 64	0.5 - 3	1.5	163
11	28 Sep 93	08^{15} - 09^{40}	21	1.91	2880	15.1	25 - 29	97 - 77	0 - 1.9	1.2	109

[1]: data for begin and end of treatment; [2]: n.s.=does not comply with exponential decline model

The bee flies (Bombyliidae) also peaked in the malaise traps before the treatments, but a second smaller increase in activity density was seen at the end of the observation period (Figure 2b). Over 90% of the catches consisted of one species, *Exoprosopa tricolor* Macquart. Its host range in the Sahel is not known. No significant effect of deltamethrin was observed, but chlorpyrifos reduced the bee fly catches with, on average, 77% over the three weeks after treatment (Table 4,8).

The tachinid flies (Tachinidae) showed their highest activity density at the end of the study (Figure 2c). Most of these catches late in the millet growing season consisted of one small-bodied species, possibly *Exorista* sp. Neither of the two insecticides appear to influence the activity density immediately after treatment. The average drop in malaise trap catches on deltamethrin plots during the very last week was significantly steeper than in the control plots (Table 4,8). However, it is not clear what may have caused such a delayed effect, nor if this has any ecological significance.

Syrphid flies or hover flies (Syrphidae) were readily caught in malaise traps, but even more effectively on yellow sticky traps. *Ischiodon aegypticus* was by far the most numerous species in both traps. On the sticky traps only this species was counted, while all Syrphidae were pooled in the malaise trap catches. However, >95% of the hover flies caught in the malaise traps were also *I. aegypticus*. The hover flies probably mainly visit the millet to feed on pollen, since aphid densities were very low. Large numbers of *I. aegypticus* larvae were found feeding on aphids in neighbouring groundnut fields. The syrphid activity density peaked during the period of spraying (Figure 2d,e). No significant reductions in this density were found in any of the traps (Table 4).

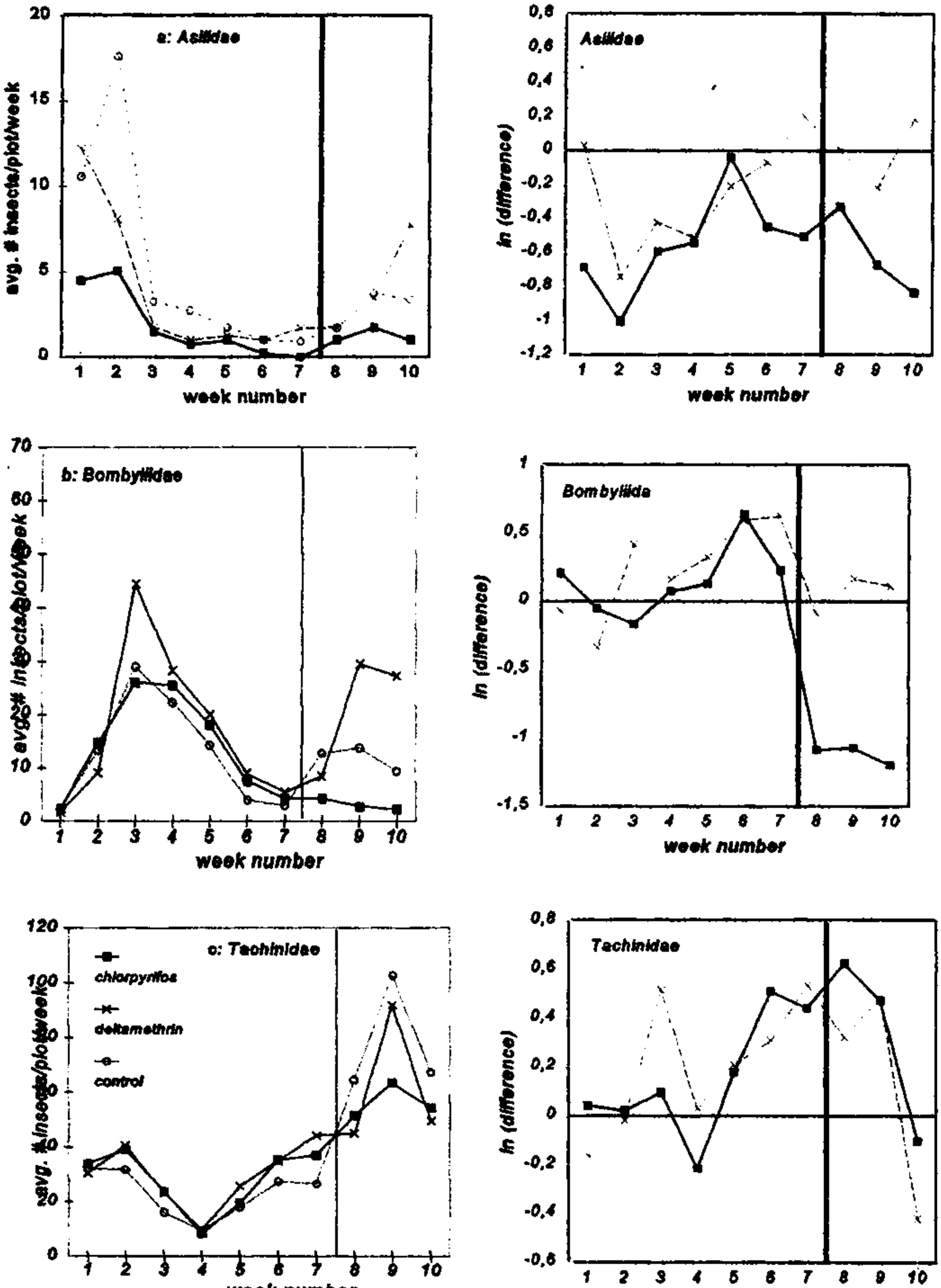
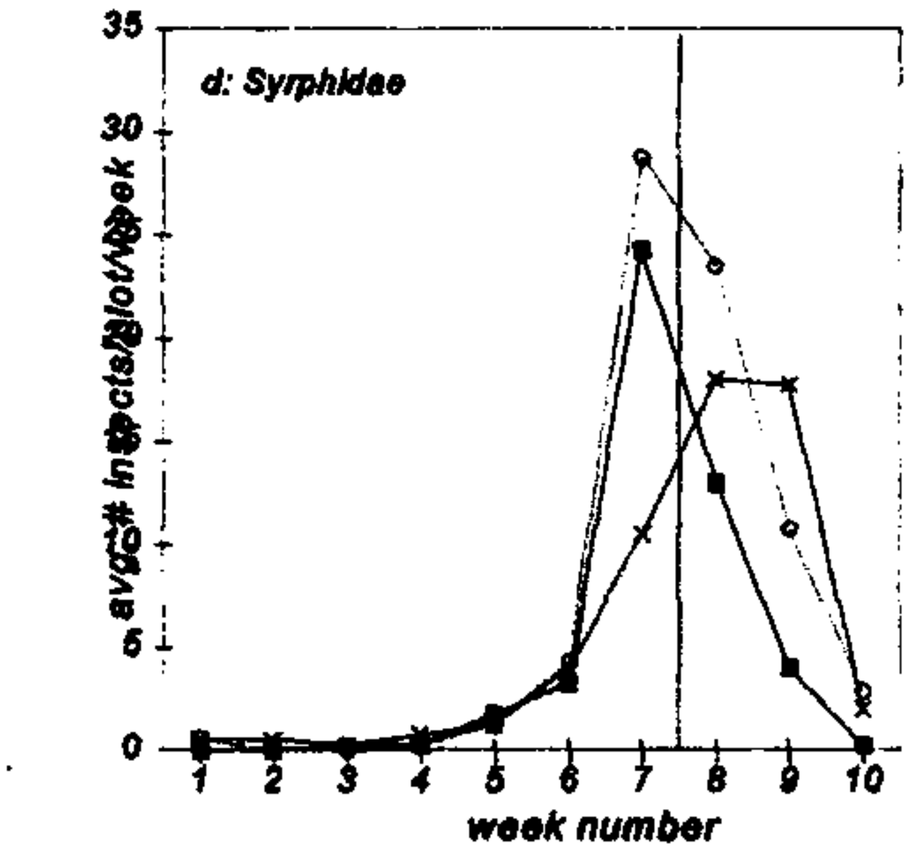
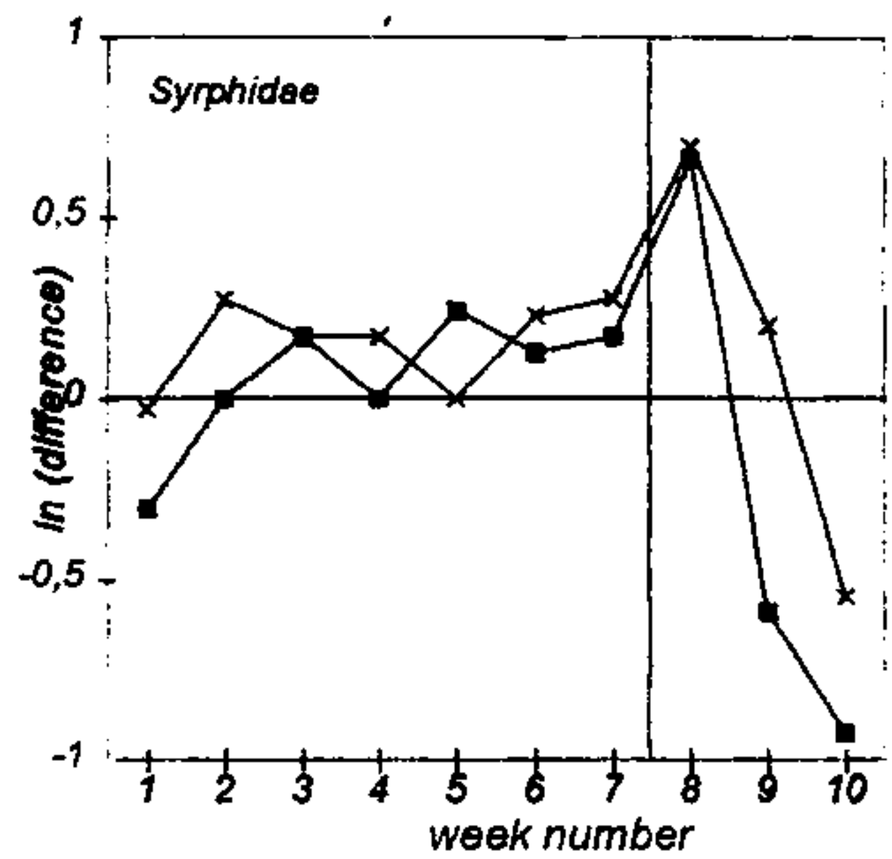
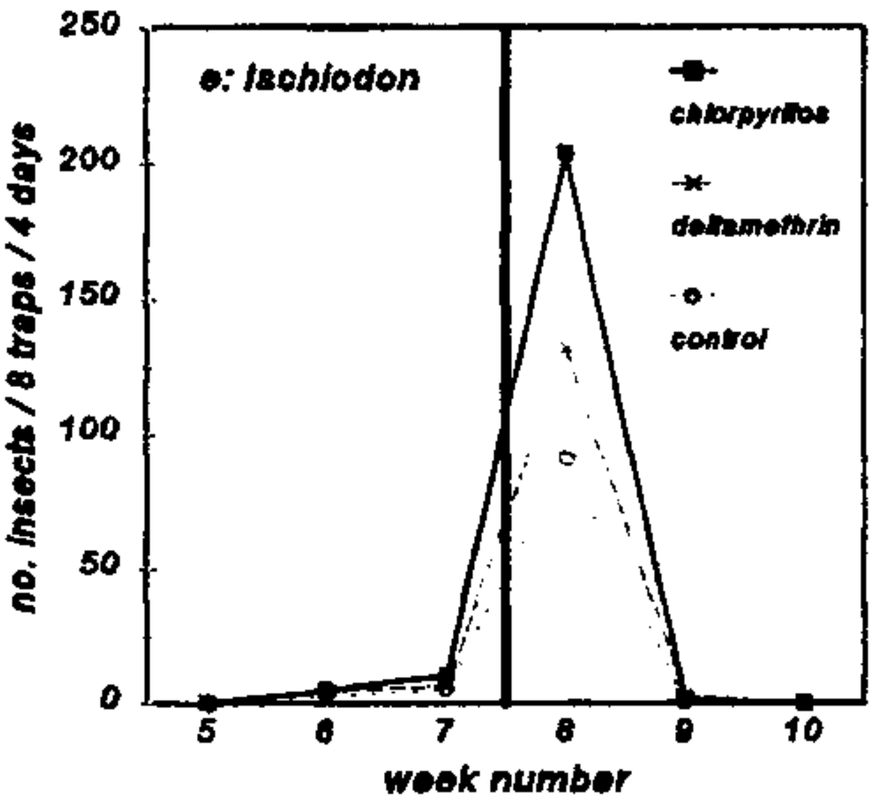


Figure 2: DIPTERA. *Left side graphs*: Average number of insects caught per field per sampling round in fields treated with chlorpyrifos, deltamethrin and in untreated controls (average of 4 fields). *Right side graphs*: Average differences between the log-transformed counts of the treatment and its paired control. Treatments were carried out between sampling week 7 and 8 (vertical line). For the results of the statistical analysis see Table 4
a: Asilidae, b: Bombyliidae, c: Tachinidae







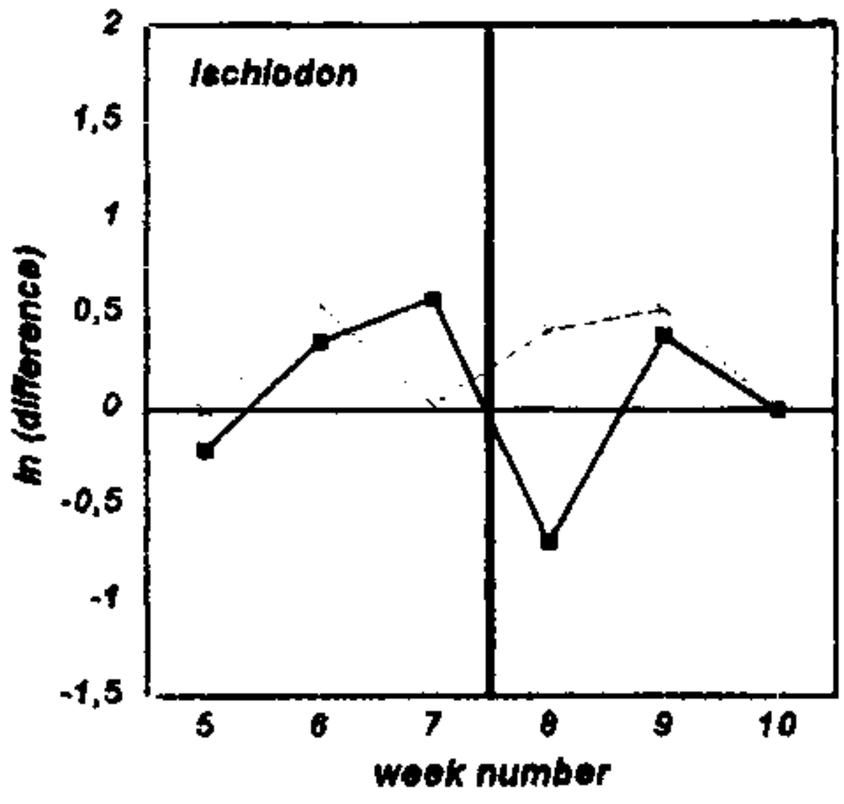


Figure 2: DIPTERA.. CONTINUED.
d: Syrphidae (malaise traps), e: Syrphidae (*Ischiodon aegypticus*) (sticky traps).

Hymenoptera

Hymenoptera were present in relatively large numbers during the whole study period (Figure 3a). Neither of the insecticides caused statistically significant reductions in activity density of the Hymenoptera at order level.

Bethylidae were caught in the malaise traps during the entire study period, but in low numbers. No statistically significant reductions in activity density could be observed for this group of parasitoïds after treatments with either of the two insecticides (Figure 3b).

Copidosoma sp. nr. *truncatellum* (Encyrtidae), the egg-larval parasitoïd *of Heliocheilus albipunctella*, was caught in large numbers on the yellow sticky traps, especially in the weeks preceding the treatments. Their activity in millet tends to coincide with the female flowering of the millet, when *Heliocheilus* oviposits on the ears. Even though especially appeared to cause a drop in activity density relative to the controls (Figure 3c), applications of neither of the two insecticides resulted in statistically significant effects. This was mainly due to large statistical interactions between treatments and blocks, suggesting that effects did occur, but not generalised over the whole study area.

Table 3: Taxa of beneficial arthropods assessed in this study in either malaise traps or sticky traps, and their hosts in millet or groundnuts in Senegal. The total number caught per taxon during the study in 1993 are listed, as well as the trap type used.

Taxon	Class	Role in agro-ecosystem	Total standardized catch [1]	Trap type [2]
Diptera				
Asilidae: total	predator	polyphagous predators of e.g. grasshoppers, Lepidoptera [1,2]	414	M
Bombyliidae	predator	Certain species known to attack grasshopper egg pods [2], or Lepidoptera larvae in millet [1]	1659	M
Syrphidae: total (mainly *Ischiodon aegypticus*)	predator	predators of aphids: observed on *Aphis craccivora*, *Rhopalosiphum maidis* in Senegal [1,4]	699	M
Syrphidae: *Ischiodon (=Xantogramma) aegypticus* (Wiedemann)	predator	as above	1865	S
Tachinidae: total	parasitoïd	attacks several lepidopteran pests in Senegal [1]	4633	M
Hymenoptera				
Hymenoptera: total	parasitoïd predator pollinator	attacks many pests in Senegal [1,2,4]; locally important pollinators [8,9]	9579	M
Bethylidae: total	parasitoïd	parasitoïds of Lepidoptera and Coleoptera; *Goniozus* attacks the millet stem borer *Coniesta ignefusalis* in the Sahel [1,10]	264	M
Braconidae: total	parasitoïd	attacks several lepidopteran pests in Senegal [1]	1598	M
Braconidae: *Cardiochiles* spp.	parasitoïd	Several species of this genus attack *Heliocheilus albipunctella* and *Helicoverpa armigera* in Senegal [1,5]	588	M
Encyrtidae: *Copidosoma* sp. nr. *truncatellum*	parasitoïd	egg-larval parasitoïd of *Heliocheilus albipunctella* in the Sahel [1]	6608	S
Halictidae: total	pollinator	para-social bees; pollinators [9]	202	M

Ichneumonidae: total	parasitoïd	attacks several lepidopteran pests in Senegal [1]	700	M
Sphecidae: *Tachytes* spp.	predator	predators of grasshopper nymphs [2,6]; status in Sahel unknown.	1029	M
Tiphiidae: *Mesa* spp.	parasitoïd	attacks larvae of Scarabaeidae, Tenebrionidae [7]; status in Sahel unclear.	113	M
Coleoptera				
Coccinellidae: *Verania (=Alesia) striata*	predator	attacks aphids on groundnut, maize and millet; seen to attack young *Hellocheilus* larvae on millet ears [3,4]	393	S
Coccinellidae: "species2"	predator	observed in large numbers on millet and groundnuts. Probably aphid predator [3]	1294	S

1: Total catch during the study, standardized as no. insects/8 traps/4days (sticky traps) or no. insects/2 traps/7 days (malaise traps) during each sampling round.
2: M= malaise traps; S= sticky traps

References: [1]: Bhatnagar 1987 [2]: Greathead *et al.* 1994 [3]: this study [4]: Risbec 1950 [5]: Huddleston and Walker 1988 [6]: Bohart and Menke 1976 [7]: Kimsey 1991 [8]: Crane and Walker 1983 [9]: Roubik 1989 [10]: Smith *et al.* 1993

Almost 17% of the Hymenoptera caught in the malaise traps consisted of Braconidae. Their activity density peaked just before the treatments, but they remained present afterwards (Figure 3d). There was no statistically significant effect of either of the insecticides.

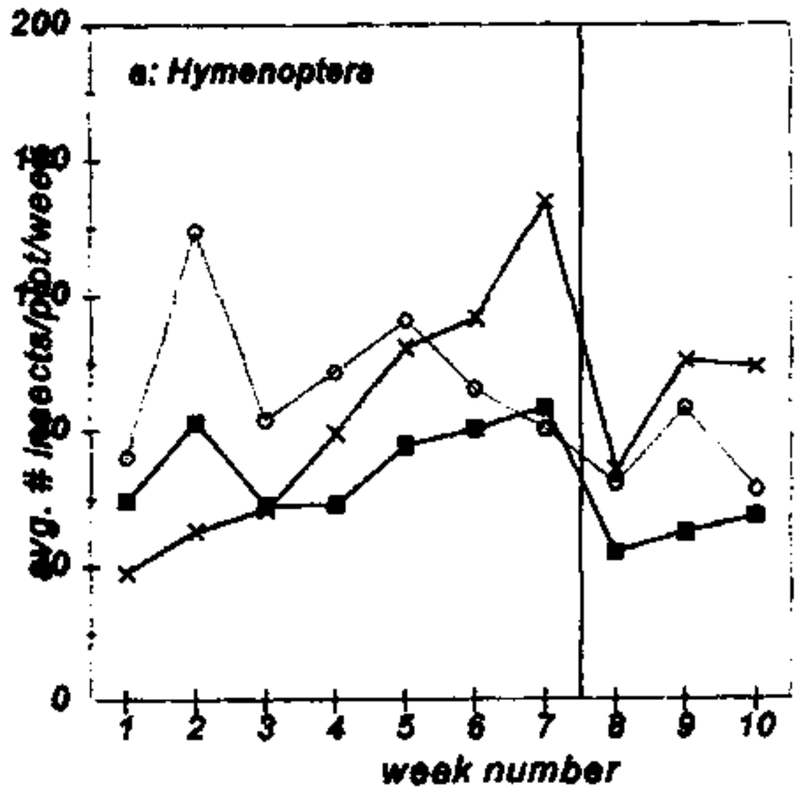
Cardiochiles was the most numerous genus among the Braconidae, with 37% of the catches. *Cardiochiles sahelensis*, the parasitoïd found to attack the millet head miner, was only encountered in low numbers. Catches were largely dominated by, what appeared to be, *Cardiochiles testaceus*; no host is known for this species (Huddleston and Walker 1988). *Cardiochiles* spp. also peaked just before the treatments were carried out (Figure 3e). Neither of the insecticides caused statistically significant effects.

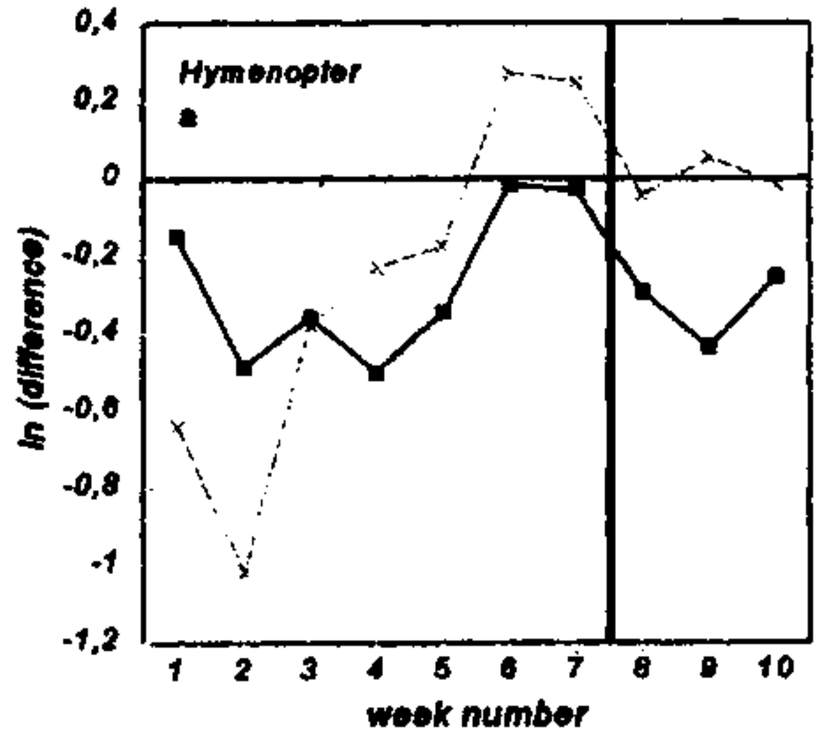
Ichneumonid parasitoïds were encountered less frequently in the malaise traps, totalling 7% of the Hymenoptera caught. Like the Braconidae, the Ichneumonidae peaked in the weeks before treatment (Figure 3f). Chlorpyrifos applications resulted in a statistically significant reduction of the activity density of, on average, 66% during the three weeks after treatment (table 4,8). No recovery had been observed at the end of the study. The same was the case for deltamethrin treatments, that caused an average reduction in the activity density of 64% over the three weeks after spraying.

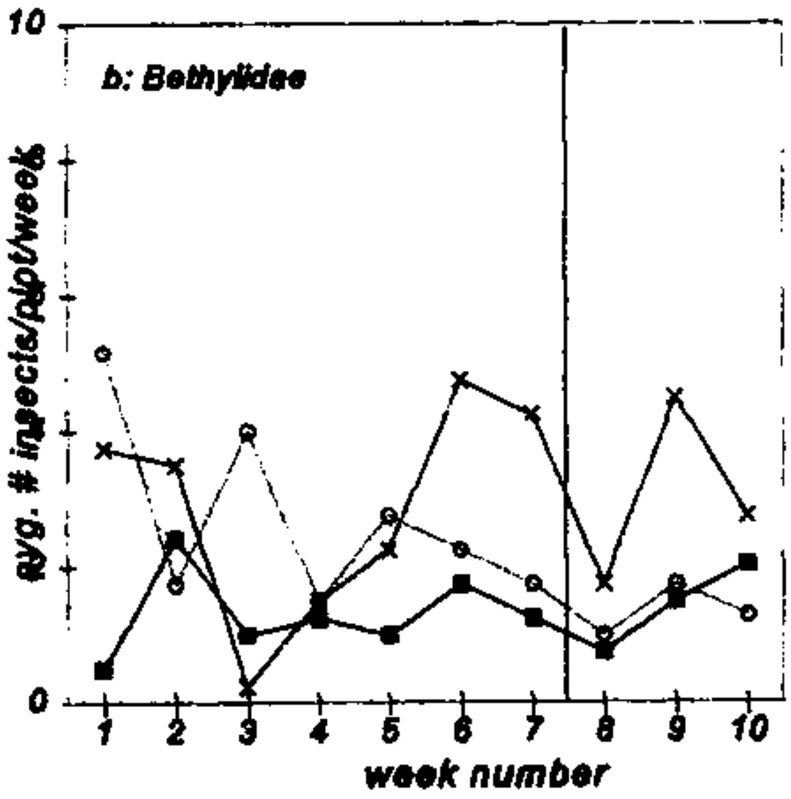
Halictid bees were present in fairly low numbers during the whole study period (Figure 3g). Chlorpyrifos treatments did not cause statistically significant effects on the malaise trap catches. A fivefold increase in the activity density of Halictidae was observed after treatments with deltamethrin, on the other hand (table 4,8). This effect lasted one week, and was not statistically significant anymore afterwards.

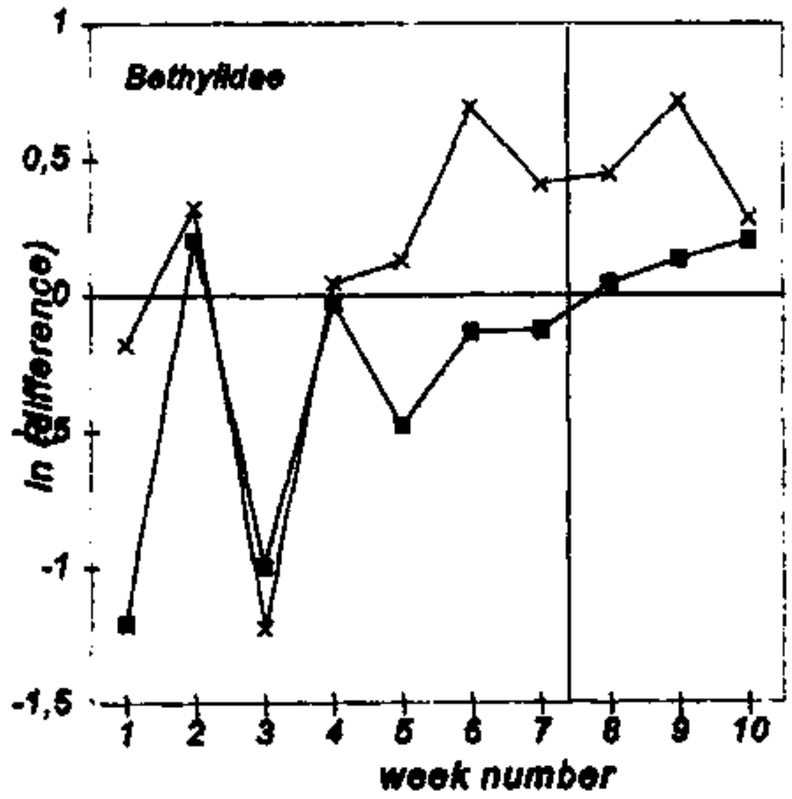
The sphecoid predatory wasps of the genus *Tachytes* were very numerous in the first weeks of the study, but catches decreased continuously afterwards (Figure 3h). They almost disappeared after treatment in all fields. No statistically significant effects of either of the insecticides could be observed.

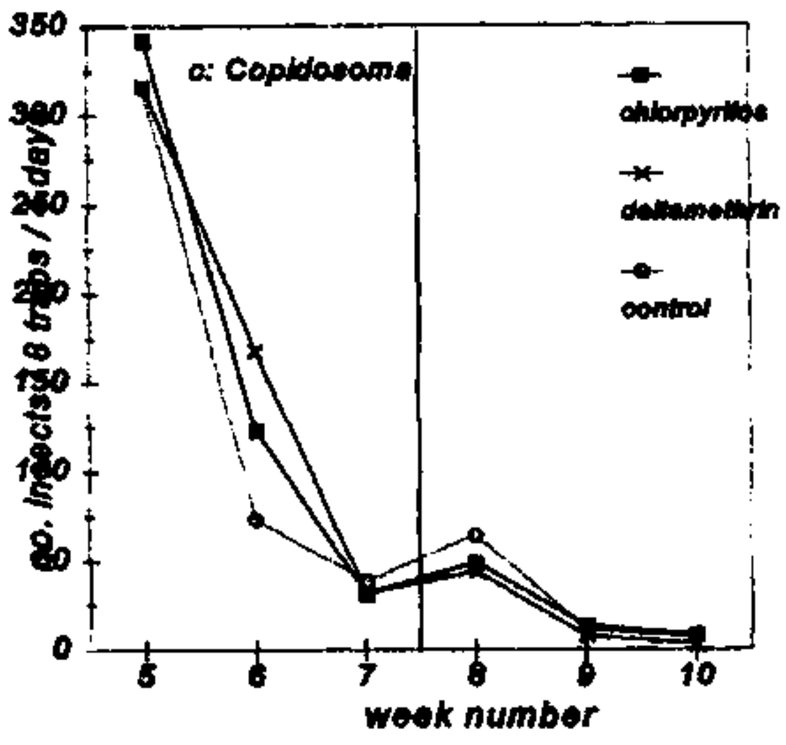
Mesa spp. was the most numerous tiphiid wasp genus found in the malaise traps. Absolute numbers caught of this genus were low, and they peaked before the treatments (Figure 3i). No significant impact of the insecticides was observed.











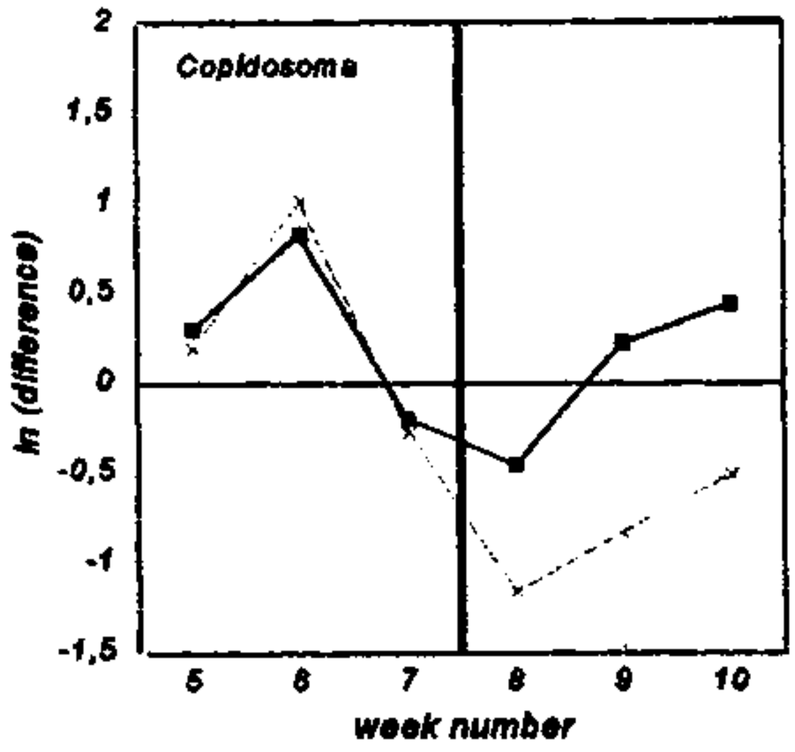
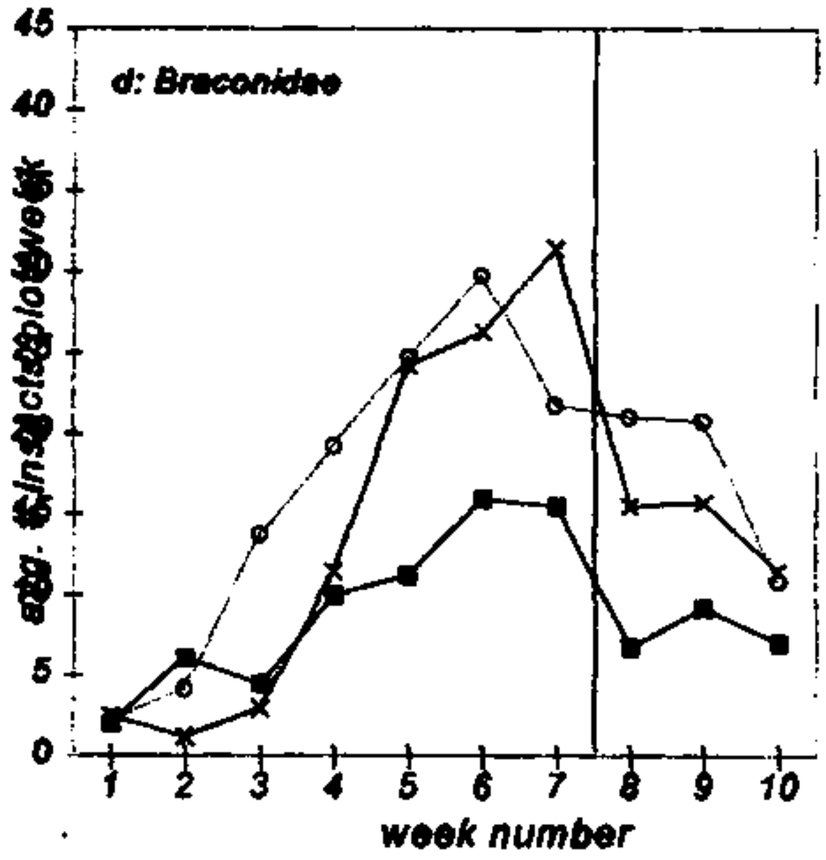
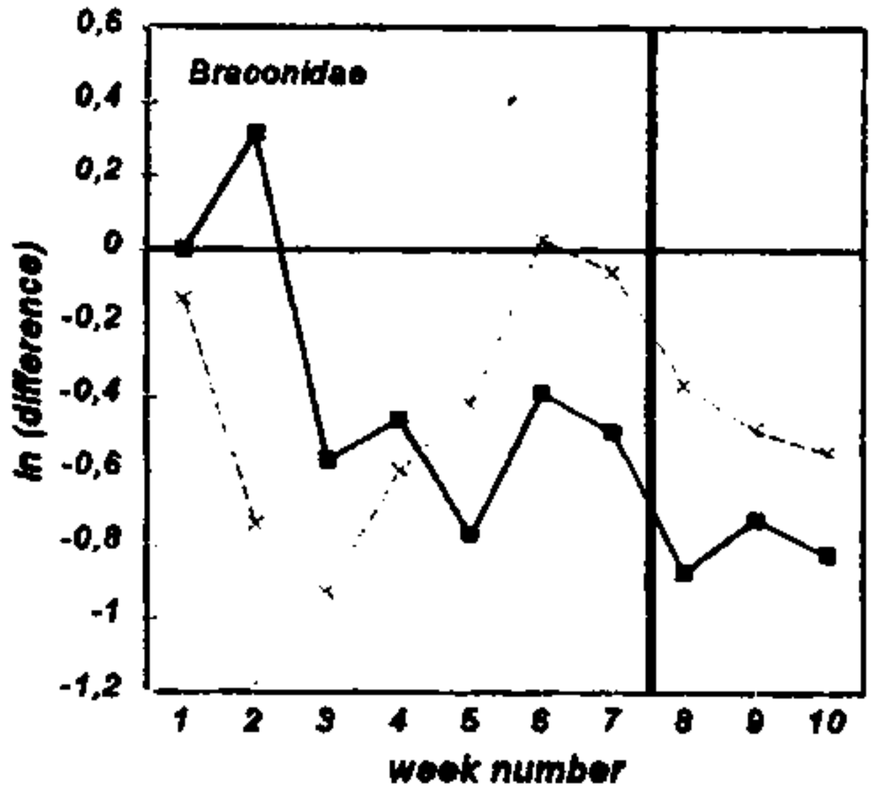
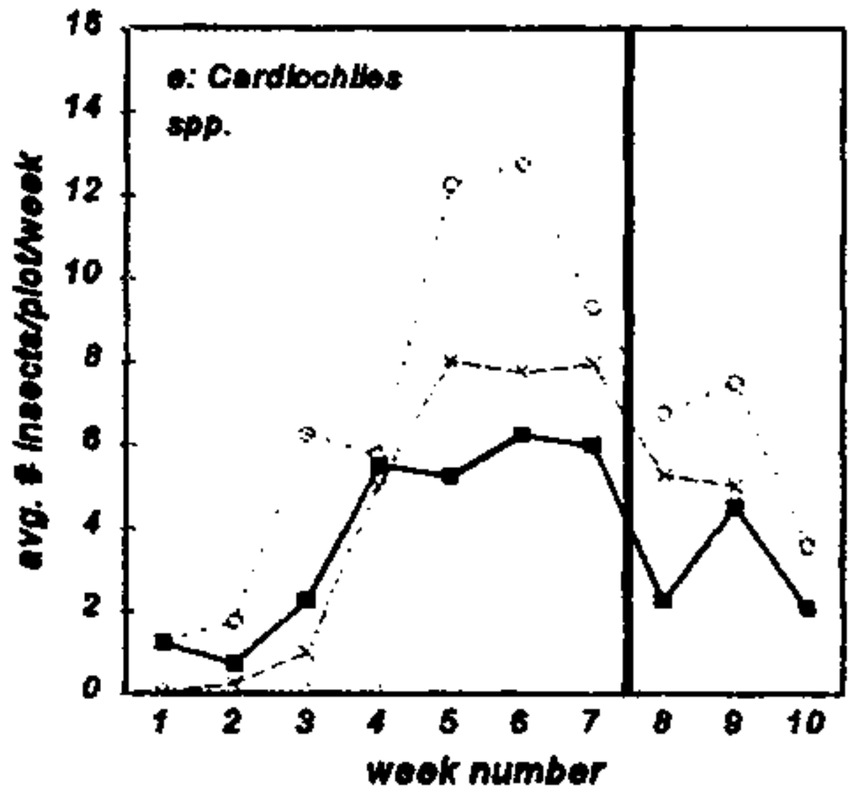
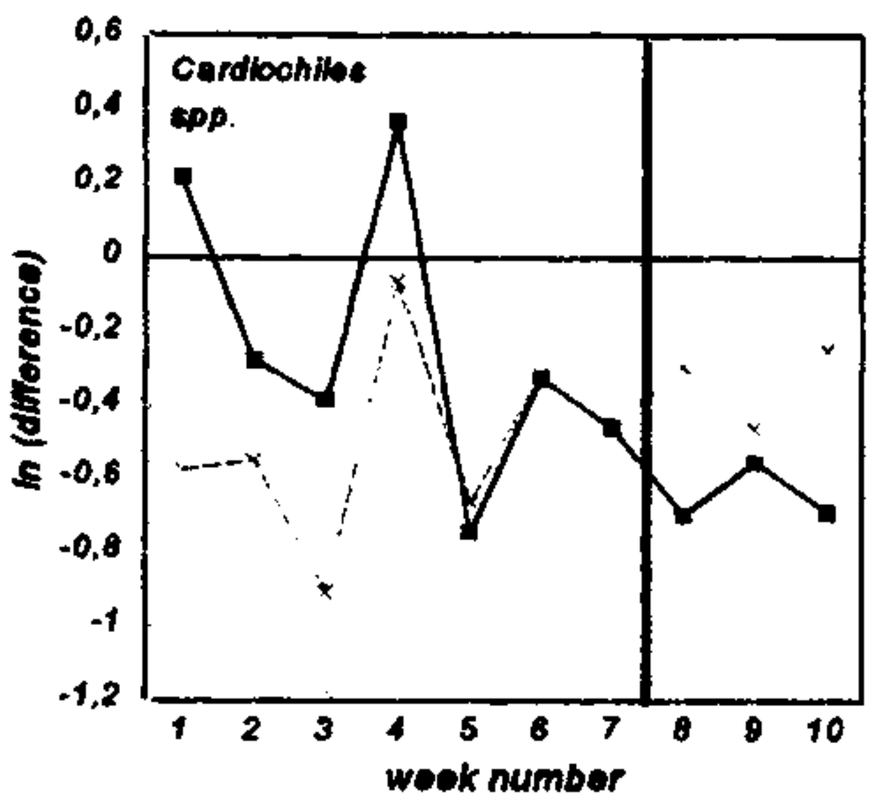


Figure 3: HYMENOPTERA. *Left side graphs*: Average number of insects caught per field per sampling round in fields treated with chlorpyrifos, deltamethrin and in untreated controls (average of 4 fields). *Right side graphs*: Average differences between the log-transformed counts of the treatment and its paired control. Treatments were carried out between sampling week 7 and 8 (vertical line). For the results of the statistical analysis see Table 4



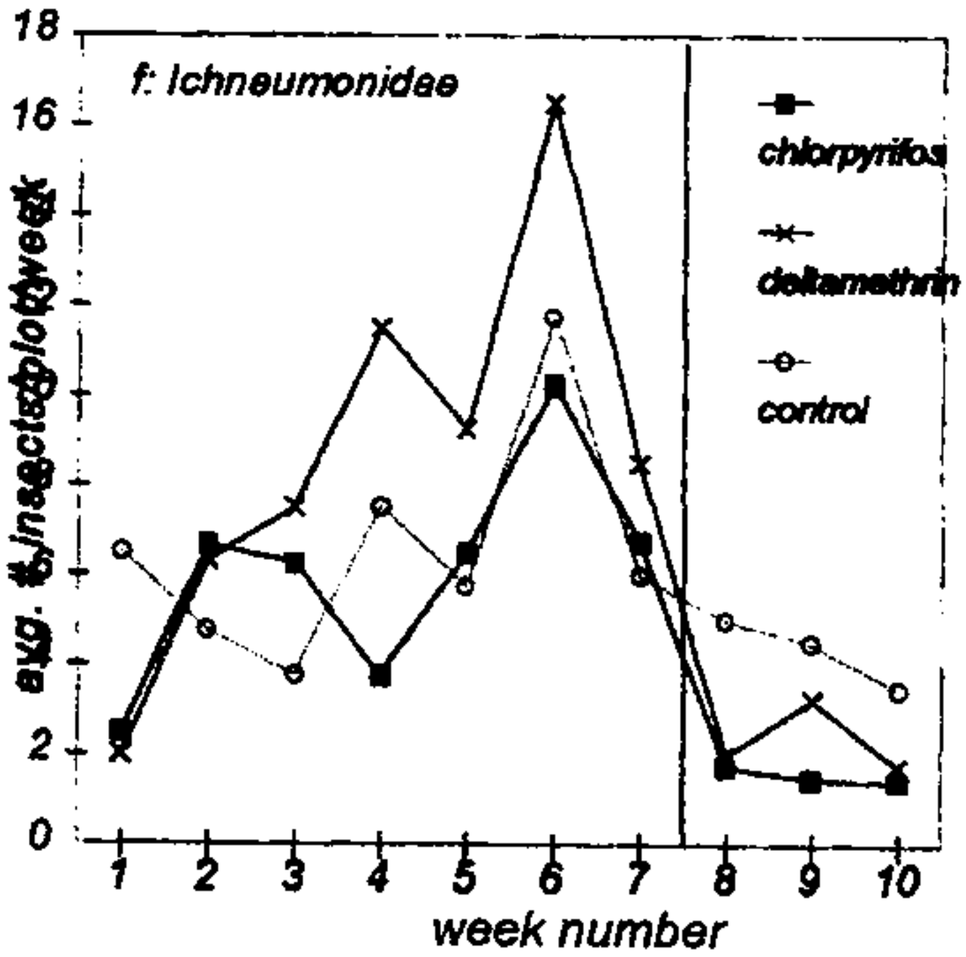


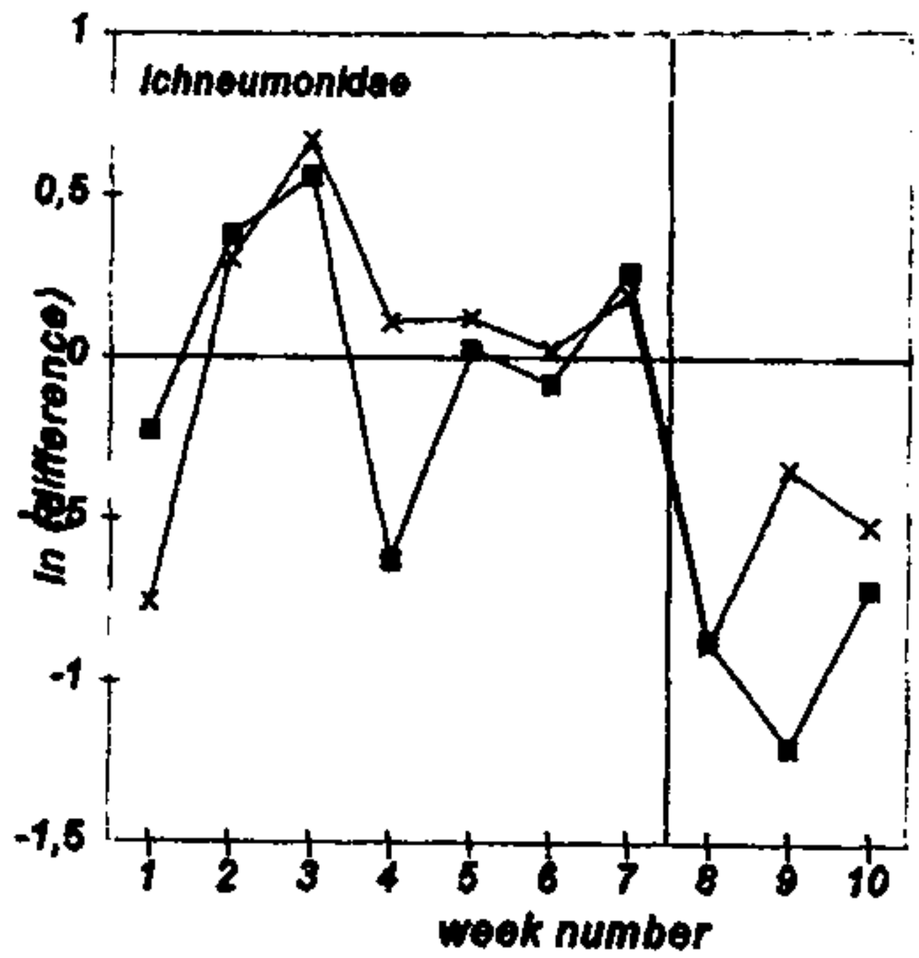


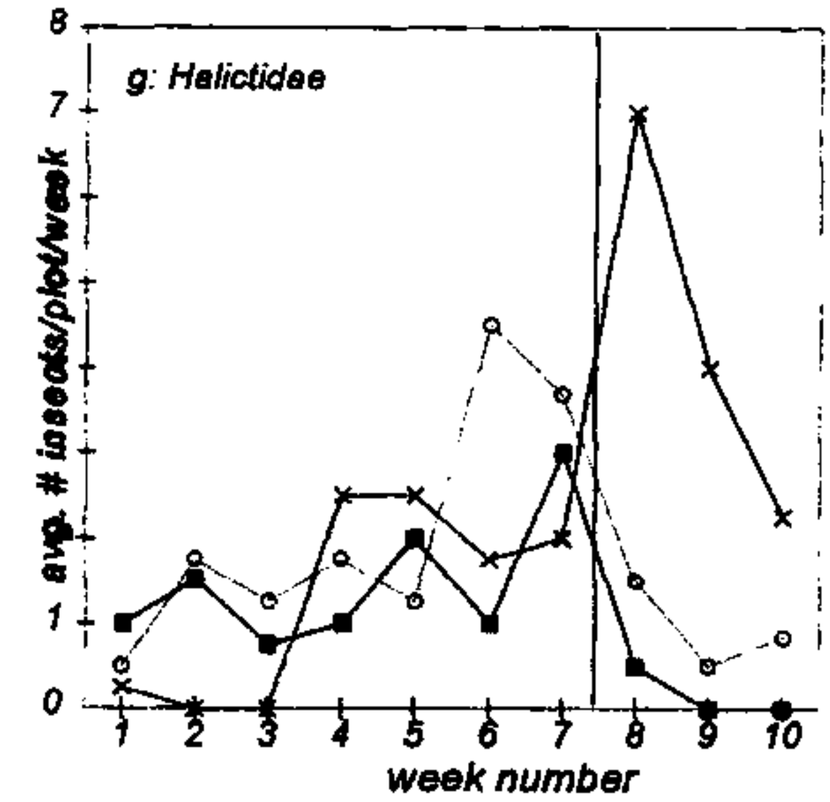


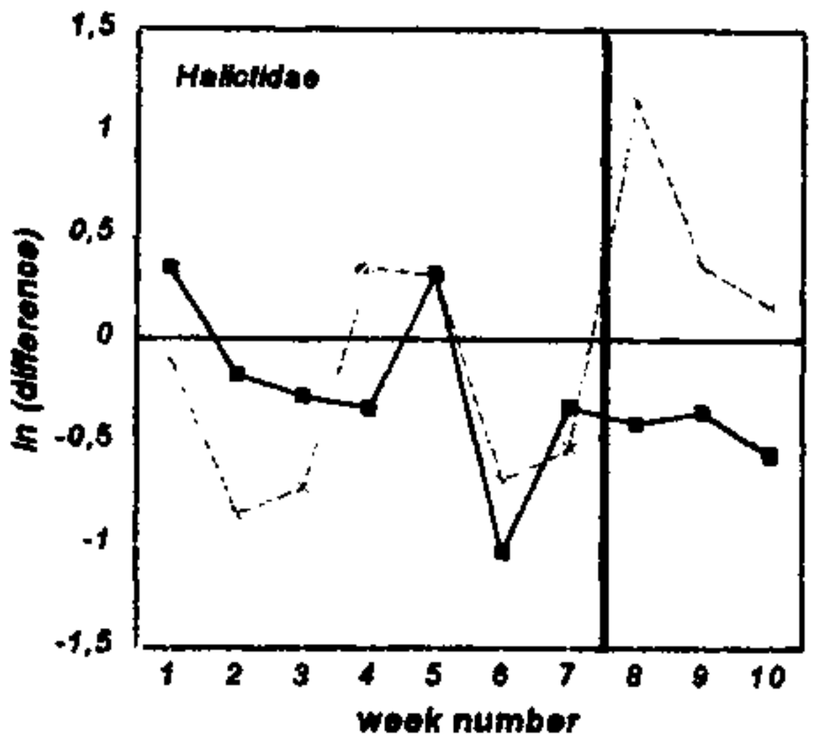
a: total Hymenoptera; b: Bethylidae; c: *Copidosoma* sp. nr. *truncatellum* (Encyrtidae)

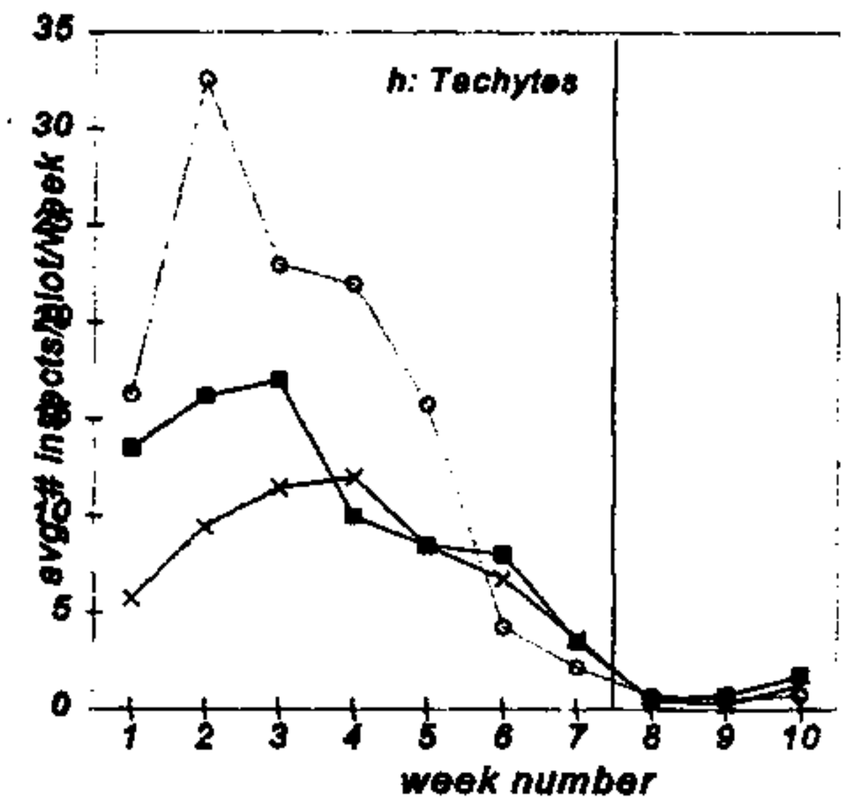
Figure 3: HYMENOPTERA. Continued
d: Braconidae; e: Cardiochiles spp. (Braconidae); f: Ichneumonidae

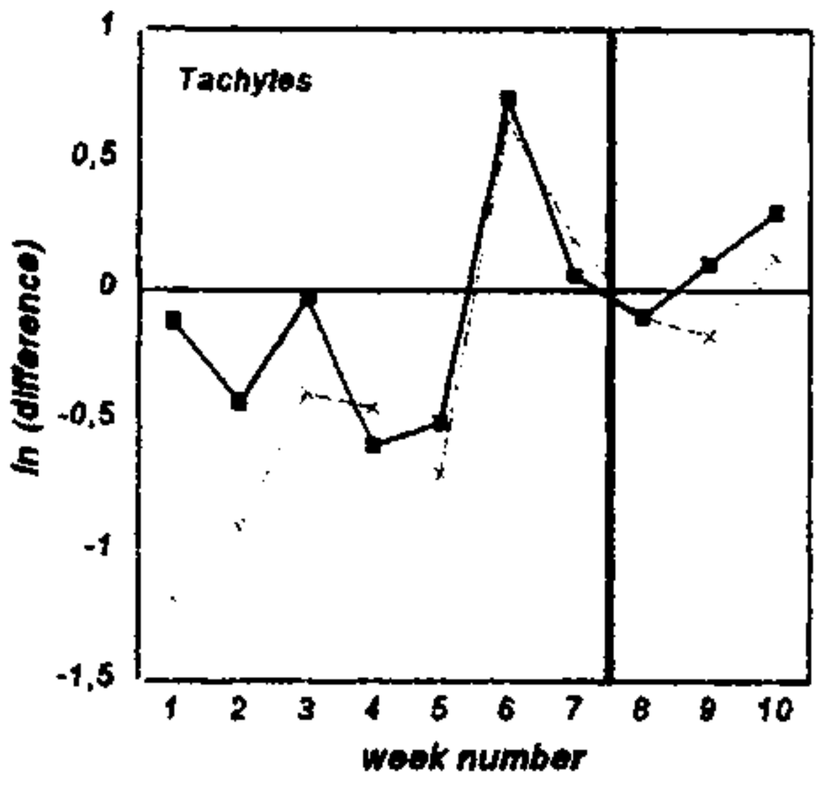

f: Ichneumonidae
chlorpyrifos
deltamethrin
control
avg. Ichneumonidae/week
week number

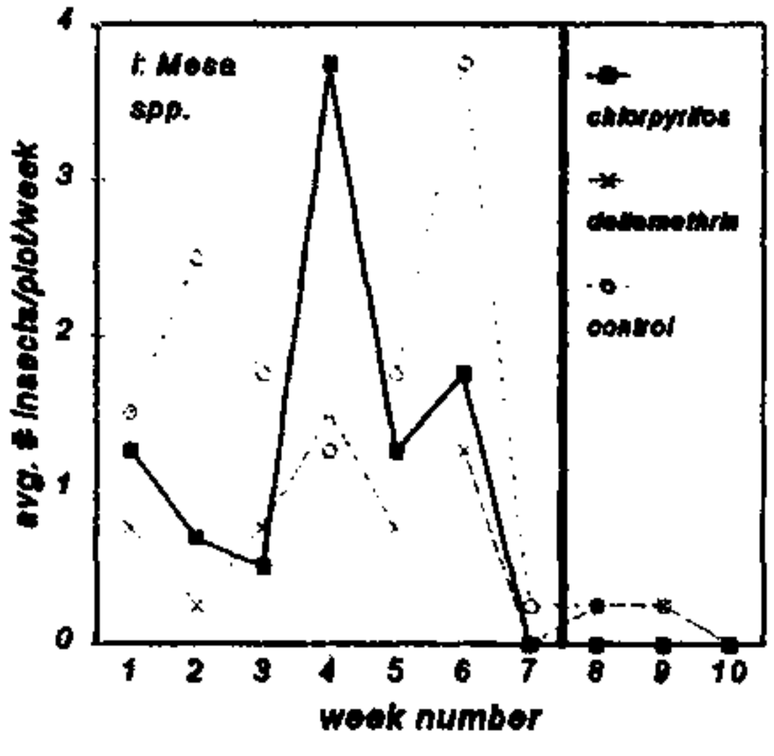

Ichneumonidae
ln (difference)
week number











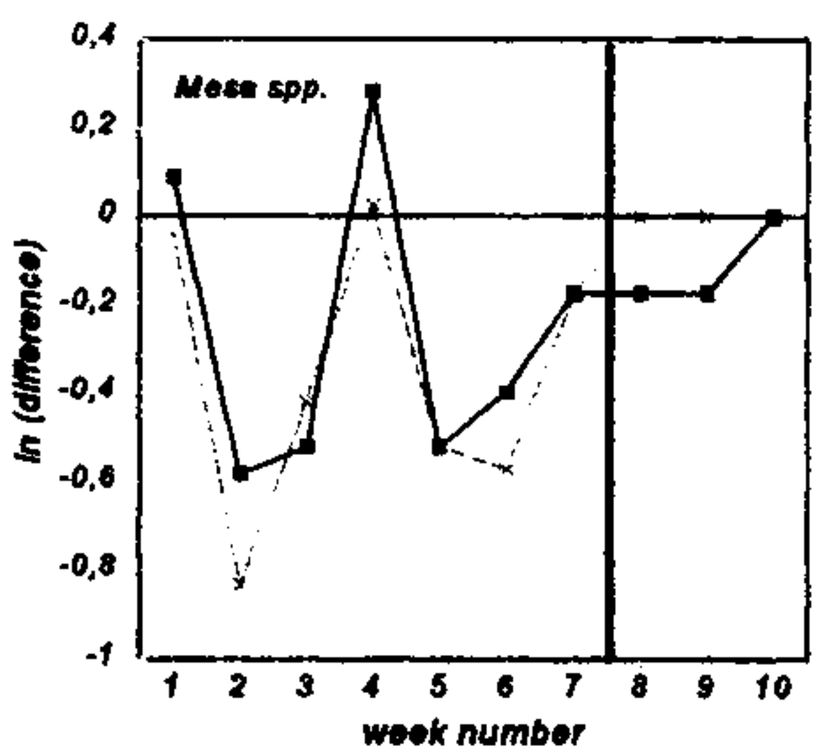


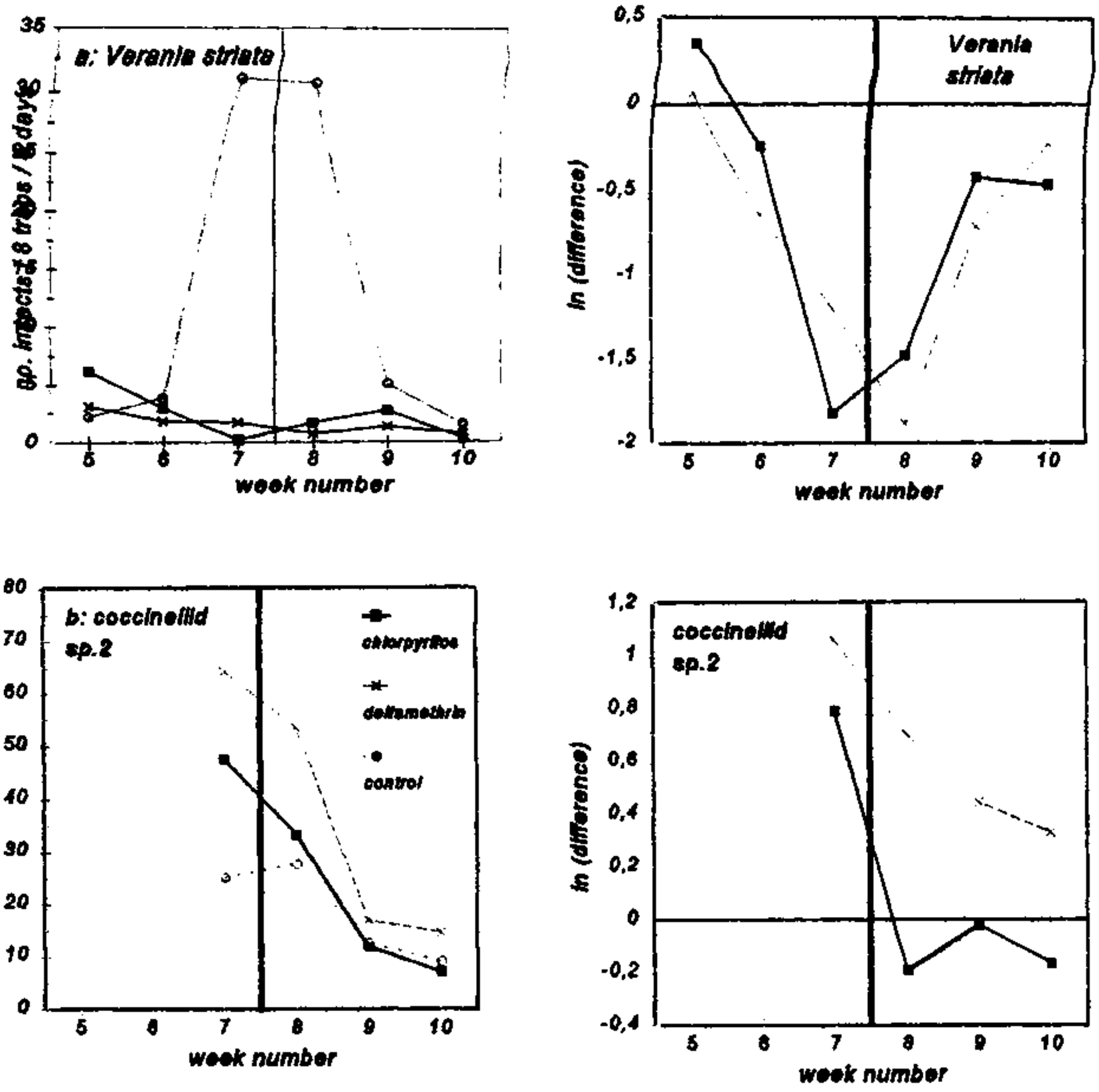
Figure 3: HYMENOPTERA. Continued
g: Halictidae; h: *Tachytes* spp. (Sphecidae); i: *Mesa* spp. (Tiphiidae)

Coleoptera

Coccinellid beetles were caught most effectively on the yellow sticky traps. They were dominated by an as yet unidentified species (coccinellid sp.2). It strongly resembled *Verania striata*, another species encountered in large numbers in the traps. Coccinellid sp2. is slightly smaller than *V. striata*, but has a similar colour and stripe pattern on its elytra, and is quite probably of the same genus.

A drop of 87% in the activity density of *Verania striata* was observed for one week after treatments with deltamethrin (Figure 4a), but this was not statistically significant. Nor effects of chlorpyrifos were observed either.

No statistically significant effects of deltamethrin on coccinellid sp.2 were detected, even though their activity density was reduced when compared to control fields (Figure 4b). Chlorpyrifos, on the other hand, reduced the catches of this species with on average 33% over the 3 weeks after



treatment (Table 4,8). It should be noted that for coccinellid sp.2 only one pre-treatment sample was available, which reduces the reliability of the observed effects.

Figure 4: COLEOPTERA. *Left side graphs*: Average number of insects caught per field per sampling round in fields treated with chlorpyrifos, deltamethrin and in untreated controls (average of 4 fields). *Right side graphs*: Average differences between the log-transformed counts of the treatment and its paired control. Treatments were carried out between sampling week 7 and 8 (vertical line). For the results of the statistical analysis see Table 4
a: *Verania striata* (Coccinellidae); b: Coccinellid sp.2 (Coccinellidae)

Table 4 : Summary of the Analyses of Variance carried out on the different taxa. The probablities (p) that the treatment effects were due to chance are given for all $p \leq 0.10$. The denominator Mean Square used for the calculation of F was always the MS_{error} (see Table 1).

Taxon	Week number (after treatment)					
	Chlorpyrifos			Deltamethrin		
	8	9	10	8	9	10
Diptera						
Asilidae	ns [1]	ns	ns	ns	ns	ns
Bombyliidae	<0.0001			ns	ns	ns
Tachinidae	ns	ns	ns	ns	ns	0.067
Syrphidae (M)	ns	ns	ns	ns	ns	ns
Syrphidae (S)	ns	ns	ns	ns	ns	ns
Hymenoptera						
Hymenoptera (total)	ns	ns	ns	ns	ns	ns
Bethylidae (total)	ns	ns	ns	ns	ns	ns
Braconidae (total)	ns	ns	ns	ns	ns	ns
Braconidae: Cardiochiles spp.	ns	ns	ns	ns	ns	ns
Encyrtidae: Copidosoma sp. nr. *truncatellum*	ns	ns	ns	ns	ns	ns
Halictidae (total)	ns	ns	ns	0.008	ns	ns
Ichneumonidae (total)	0.003			0.006		
Sphecidae: *Tachytes* spp.	ns	ns	ns	ns	ns	ns
Tiphiidae: *Mesa* spp.	ns	ns	ns	ns	ns	ns
Coleoptera						
Coccinellidae: *Verania striata*	ns	ns	ns	ns	ns	ns
Coccinellidae: coccinellid sp.2	0.026			ns	ns	ns

[1] ns : not significant at α=0.10

Population parameters of the millet head miner

Oviposition and egg emergence

Since the treatments took place after most of the millet flowering had occurred, their effect on emergence or parasitism of *Heliocheilus* eggs could not be assessed. Data on egg incidence and parasitism levels are therefore only used to facilitate interpretation of differences observed in larval densities. The data in Table 5 show that oviposition was not homogeneous over all the blocks. More eggs were found in the fields near Prokhane (blocks III and IV). This difference was consistent during all three sampling periods (data not shown). It may have been due to the delay in millet development in the Prokhane fields, which might have increased the synchronisation between emergence of *Heliocheilus* moths and the availability of appropriate millet ears for oviposition. The difference in oviposition between blocks justifies an analysis based on a randomised complete block design for any subsequent larval statistics.

Table 5: Incidence of eggs of *Heliocheilus albipunctella* in the millet fields used in the study (total of 3 sampling rounds), and the degree of success of emergence. That is, the average percentage of eggs which were not parasitized with *Trichogrammatoidea sp.* nor infertile.

Insecticide	Block I		Block II		Block III		Block IV		Average eggs/ 100 ears
	eggs/ 100 ears	% success	eggs/ 100 ears	% success	eggs/ 100 ears	% success	eggs/ 100 ears	% success	
Chlorpyrifos	130	46	40	50	1020	55	430	70	405
Deltamethrin	60	17	100	33	480	46	1070	49	428
Control	130	73	140	17	440	38	580	31	323
Average	107 a [1]	45	93 a	33	647 b	46	693 b	50	

Statistics for differences between treatments: eggs/100 ears : ANOVA (RCB) p=0.176
Statistics for differences between treatments: % success : ANOVA (RCB) p=0.394
Statistics for differences between blocks: eggs/100 ears : ANOVA (RCB) p=0.052

[1]: means followed by the same letter are not significantly different (Least Significant Difference Test. $\alpha = 0.10$)

Larval populations

The percentage of ears showing galleries made by the millet head miner are given in Table 6. On average, approximately half the millet ears showed signs of infestation with *Heliocheilus*, but there is no significant difference between treatments for this parameter.

Differences between treatments did show up for the peak larval density of *Heliocheilus* (Table 7). These were not significantly different from the controls in the fields treated with deltamethrin. But fields treated with chlorpyrifos had considerably higher densities of millet head miner larvae in 3 out of 4 fields. On average, chlorpyrifos treated fields showed an 88% increase in *Heliocheilus* peak larval density when compared to the controls (Figure 5).

Table 6: Percentage of millet ears showing galleries made by the millet head miner, *Heliocheilus albipunctella*. Shown are the peak incidence levels of 3 observations.

Treatments	Blocks								Average
	I		II		III		IV		
	n [1]	%	*n*	%	*n*	%	*n*	%	%
Chlorpyrifos	*271*	54	*208*	21	*164*	63	*161*	67	51
Deltamethrin	*377*	60	*250*	32	*151*	58	*211*	67	54
Control	347	43	283	33	195	72	*155*	65	53
Average		52		29		64		66	

[1]: n = number of millet ears sampled; % = percentage of ears with *Heliochellus* galleries.

Statistics for differences between treatments: percentage of ears with galleries: ANOVA (RCB) p=0.175

Table 7. Number of *Heliocheilus albipunctella* larvae per 100 millet ears for treated and untreated fields. Given are the peak densities of 3 observations.

Treatment	Number of *Heliocheilus* larvae per 100 millet ears					
	Block				Mean [1]	
	I	II	III	IV		
Chlorpyrifos	172	16	168	184	135	a
Deltamethrin	60	31	102	57	62	b
Control	61	40	58	131	72	b

Statistics for differences between treatments: larval peak density: ANOVA (RCB) p=0.096

[1]: means followed by the same letter are not significantly different (Least Significant Difference Test, α = 0.10)

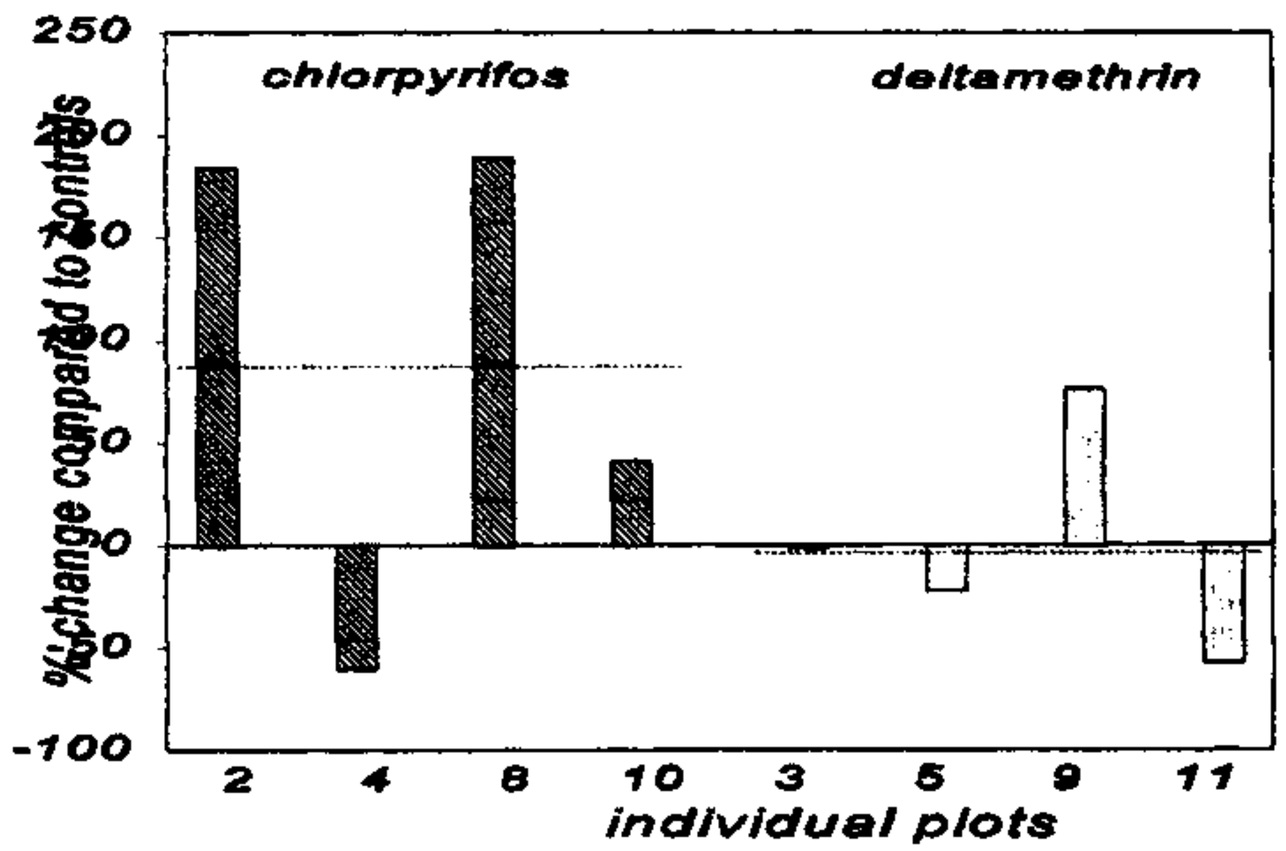
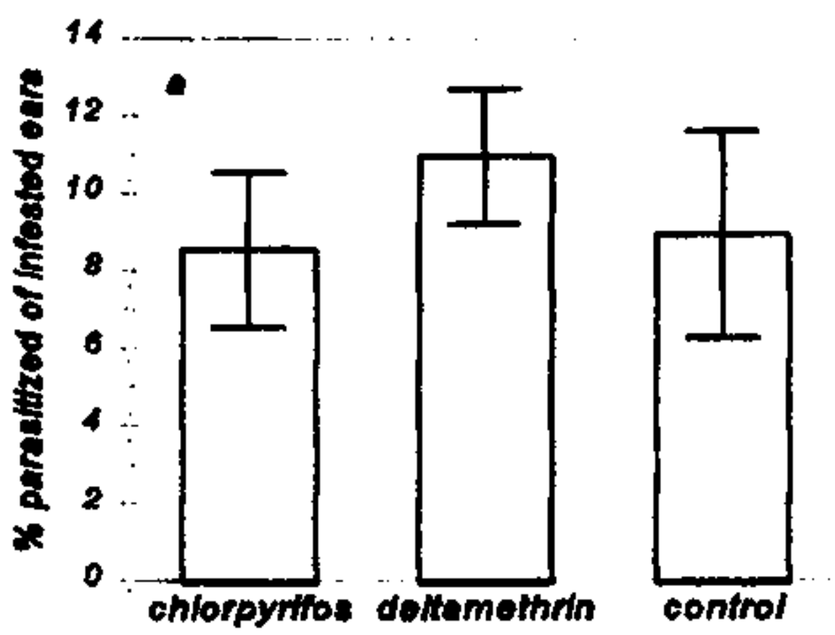


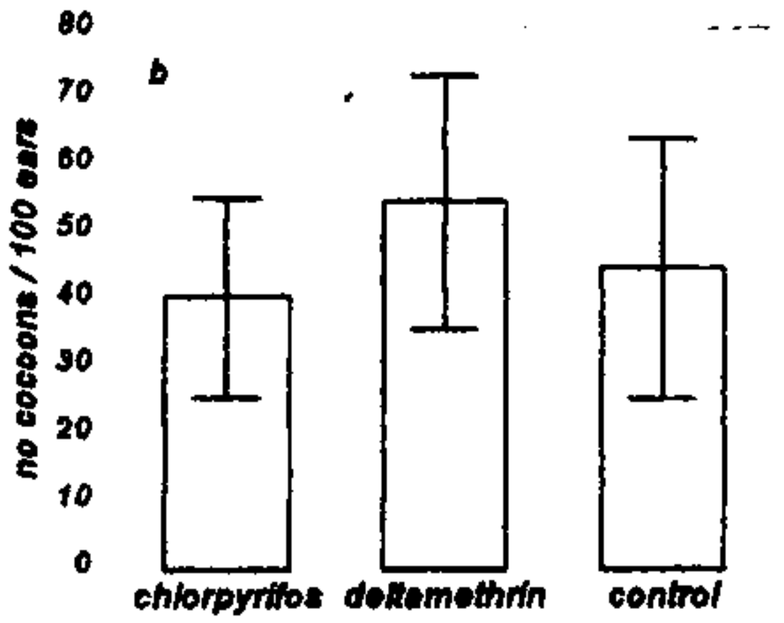
Figure 5: Percentage change in the peak density of *Heliocheilus albipunctella* in the study fields when compared to their paired controls, after locust control treatments with chlorpyrifos or deltamethrin. The horizontal hatched line represents the average change.

Larval parasitism by Bracon hebetor

The degree of parasitism of larvae of *Heliocheilus* by the braconid parasitoïd *Bracon hebetor* is shown in Figure 6. Parasitism rates are calculated in different ways. The percentage of ears infested with head miner on which one or more cocoons of the parasitoïd were found is given in Figure 6a. This parameter is not very precise, though, and does not take into account the success rate of the parasitoïd. Therefore, the number of cocoons produced per 100 millet ears is pictured in Figure 6b as a measure of overall reproduction success of *Bracon hebetor*. Since host density may also influence parasitoïd succes, independent of any insecticide effects, the number of cocoons produced by *B. hebetor* as a fraction of the peak density of *Heliocheilus* larvae is shown in Figure 6c.

All three graphs provide a similar picture, with *Bracon hebetor* appearing slightly more successful in fields treated in deltamethrin than in the others. None of these differences was statistically significant, though.





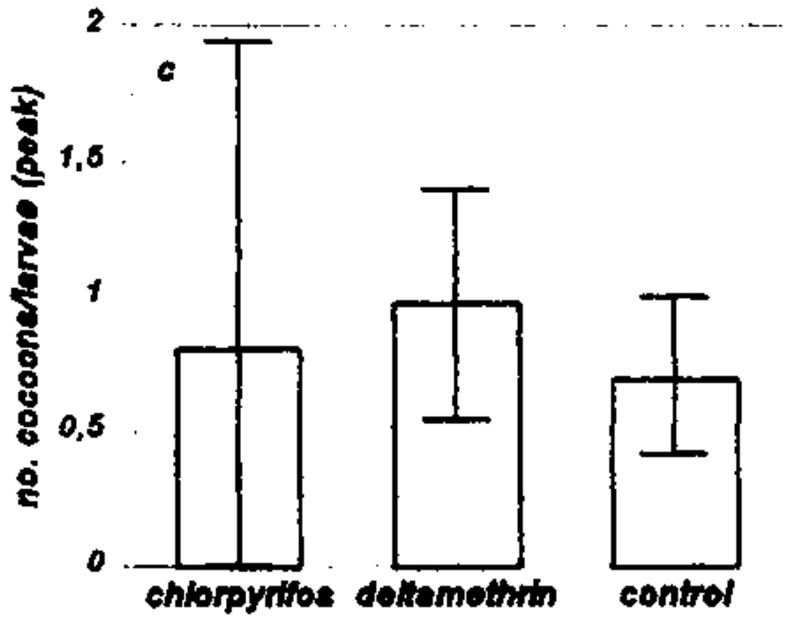


Figure 6: a: The percentage of millet ears infested with *Heliocheilus albipunctella* on which one or more cocoons of *Bracon hebetor* were found [RCB-ANOVA for differences between treatments: p=0.25]. b: the number of cocoons of *B. hebetor* produced per 100 millet ears [RCB-ANOVA for differences between treatments: p=0.37]. c: the number of cocoons produced by *B. hebetor* as a fraction of the peak density of *Heliocheilus* larvae [RCB-ANOVA for differences between treatments: p=0.26].

Residual populations in the soil

Only 35 chrysalids of *Heliocheilus* were found in the 120 soil samples taken after the millet harvest, as well as 5 larvae mummified by *Copidosoma sp. nr. truncatellum* and 5 cocoons of *Cardiochiles sahelensis*. These number were too low for any valid comparisons between treatments and no further analysis was carried out.

Bioassays

The results of the bio-assays are shown in Figure 7. The effect parameter is the joint mortality and immobilization percentage after 24 hours of exposition to the treated millet leaves. Generally very little recovery was observed from immobilization, even after having left the insects on clean humidified filter paper for another 24 hours. Therefore, we consider that insects being immobilized after 24 hours of exposure in the laboratory would most probably die in the field. This justifies combining the dead and immobilized fraction in one effect parameter. The effect percentages shown in Figure 7 are corrected for control mortality using Abbott's formula (Busvine 1971).

Bracon hebetor exposed on chlorpyrifos treated leaves that were only 5 hours old resulted in 100% effect (Figure 7a). This had dropped to 15% after 30 hours of weathering of the residues. Deltamethrin residues proved much less toxic to *Bracon*. Slightly more than 20% were affected after exposure to freshly treated leaves. Toxicity declined only slowly afterwards, however.

When the coccinellid beetle *Verania striata* was exposed to chlorpyrifos residues on millet leaves that had weathered only a few hours, on average 20% were affected (Figure 7b). No increased mortality was observed after exposure on leaves that had been in the field for 30 hours. Deltamethrin residues, on the contrary, were highly toxic to the coccinellid beetle. This toxicity declined only very slowly with increasing weathering periods. Even after 11 days, millet leaves previously treated with deltamethrin were still toxic for 35% of the coccinellids.

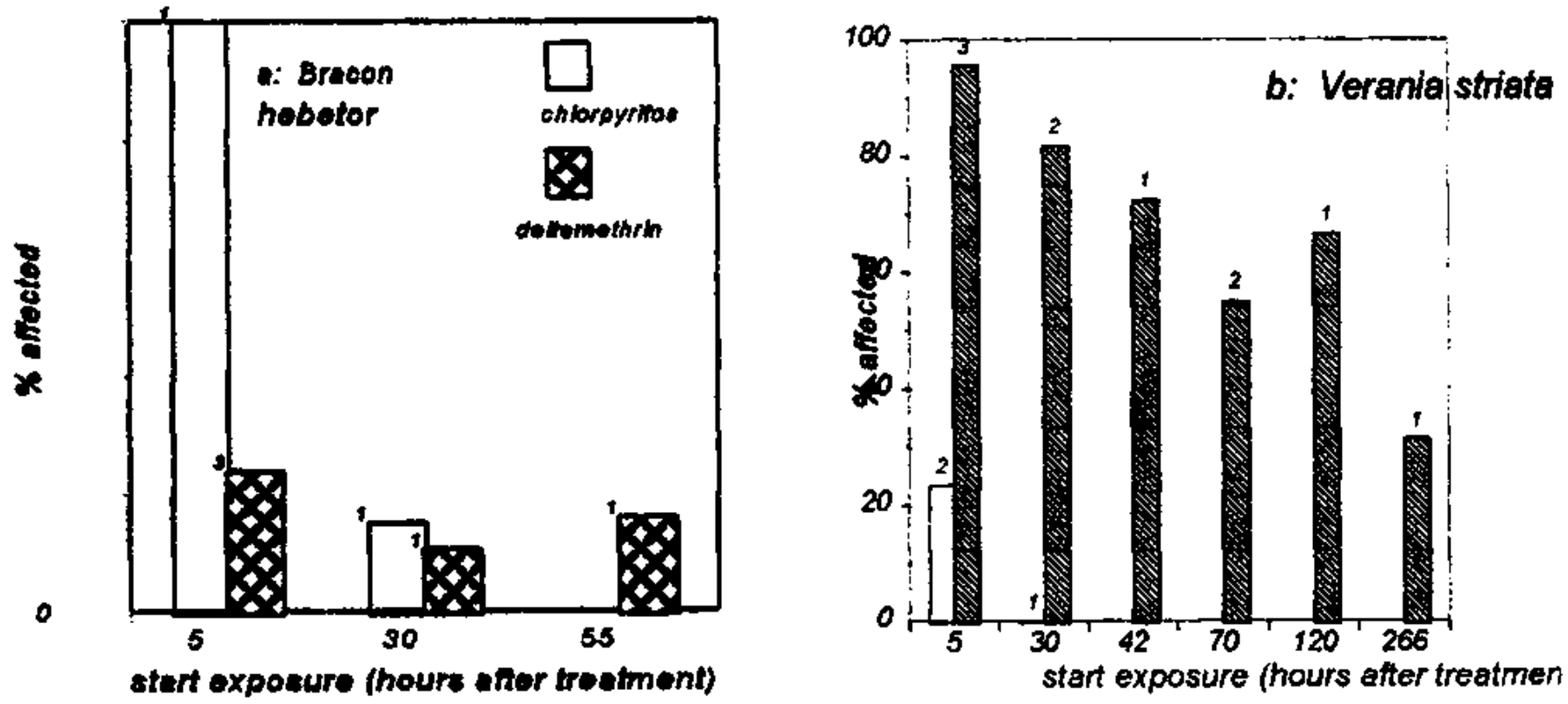


Figure 7: Results of the bioassays carried out with a: *Bracon hebetor* (Braconidae) and b: *Verania striata* (Coccinellidae). Shown are the average 24-hour effect percentages (effect was defined as death or immobilization) on sprayed millet leaves that had weathered for different periods (shown on the horizontal axis). The label on top of each bar refers to the number of independent bioassays carried out for that specific weathering period.

DISCUSSION

Effects on beneficial arthropods

A total of 15 taxa were monitored by trapping in this study. Three taxa were significantly reduced (from a statistical point of view) after treatments with chlorpyrifos. These reductions were generalized over the entire study site. The bombyliid flies showed an average reduction, relative to levels in control fields, of 77% that lasted for at least three weeks, the duration of the post-treatment monitoring period. A reduction in catches of 66% was observed for the ichneumonid parasitoïds, also lasting for the whole post-spray period. A smaller reduction in catches of 33% was found for coccinellid sp.2.

Deltamethrin applications affected fewer taxa than chlorpyrifos. The Ichneumonid catches were reduced in a very similar fashion to the chlorpyrifos fields; a 64% reduction over at least three weeks. No effects on bombyliid flies were found, but this might have been due to a large increase in activity density in one of the four deltamethrin fields. Tachinid flies were affected in the third week after treatment, and showed a reduction of catches of 42%. It is doubtful that this has large ecological relevance, given the time in the growing season and the fact that control counts were going down as well by that time. A large relative increase, but of very short duration, of halictid bee activity was observed in the first week after treatment. This was largely due to higher catches in one field.

One may conclude from this that chlorpyrifos treatments at Desert Locust control rates have more severe effects on the monitored beneficial arthropods than deltamethrin applications.

As a comparison, treatments with fenitrothion, an organophosphate very similar to chlorpyrifos, caused statistically significant reductions lasting two weeks or longer in 3 out of 12 monitored taxa (Van der Valk and Kamara 1993). All these taxa were Hymenoptera. Ichneumonidae were also affected by fenitrothion, but only for one week. At the time we suggested that this might have been an underestimate of real effects (Table 13 in Van der Valk and Kamara 1993). There is no overlap in any of the other affected taxa.

One would be inclined to conclude that both deltamethrin and chlorpyrifos had only limited effects on beneficial arthropods in millet since at most 20% of the monitored taxa were affected. However, the absence of a statistically significant reduction (or increase) in trap counts does not necessarily mean that no effect occurred. There are several reasons why an ecologically significant effect might not show up. Either the taxon may not be present in the traps in sufficient numbers to allow for a meaningful analysis; or natural variability in the effect parameter is so large that it obscures statistical significance (the statistical power of the study method is too low); or an effect may have occurred, but was missed due to the ecology of the organism. We will shortly discuss these possibilities hereafter.

If the active stage of a given taxon is practically absent at the moment of spraying, it is often unlikely that side-effects which are directly attributable to the insecticide will occur (although exceptions exist, e.g. when larval stages of a parasitoïd are affected inside their host). Indirect effects may occur, on the other hand, when the food base is depleted for subsequently arriving polyphagous predators or parasitoïds (Settle *et al.* (1996) provide an enlighting example of the latter in tropical rice agro-ecosystems). In our study, the tiphiid wasp *Mesa* spp. as well as the Bethylidae and the Halictidae were present in very low numbers throughout the study (on average <1.5 individuals per field per trapping date). The sphecoid wasp *Tachytes* spp. peaked early in the growing season and was practically absent at the time of the treatments. It is unlikely that any effect of an insecticide would show up for these taxa in a statistical test, short of complete annihilation.

Insect counts may be so variable over time that the statistical test cannot distinguish natural variability from insecticide induced effects unless the latter are very large. Consequently, the statistical power of the study is too low for ecologically significant effects to show up. It can be seen from many right hand graphs in Figures 2, 3 and 4 that pre-treatment variability in the effect parameter was indeed often high. We did not carry out a formal power analysis, however, to quantify the probability that certain effects could be found at all with the present study setup. It is suggested that in the near future a power analysis is carried out for the different types of

ecotoxicological field studies used to date to assess the effects of locust control. This will allow for a quantification of type II errors occurring (the probability of (wrongly) concluding that no ecologically significant effect occurred while in reality it did). This may improve the design of future field studies.

The third process which can mask insecticide side-effects may occur when the monitored taxon has its source outside the treated area, but is highly mobile and disperses outside its source area. In our study this may have happened with both the syrphid flies and the coccinellid beetles. Both of these groups are numerous in groundnut fields, where they oviposit and their larvae feed on aphids. The adult stages, on the other hand, appear to move into millet fields mainly to feed on pollen. It is well possible that large numbers of these insects were killed during and after the treatments in the millet fields, but that continuous immigration from neighbouring peanut fields masked such effects. Results from the bioassays seem to suggest that at least deltamethrin would have killed a large fraction of the coccinellids present in millet for several days after spraying. Clearly, for mobile beneficial arthropods that have such source populations outside the sprayed zone, monitoring populations within that zone may underestimate real effects considerably.

If we take the above considerations into account, we can now assess the taxa that were affected by the insecticides as a fraction of the monitored taxa that were likely to show effects. Only eight taxa, rather than 15, were likely to show effects. Three out of eight were affected by chlorpyrifos; one out of eight taxa was significantly affected, from an ecological point of view, after spraying with deltamethrin.

Bioassays

The bioassays showed a rather striking difference in the susceptibility of the two species tested. *Bracon* was highly susceptible to the chlorpyrifos residues but hardly to deltamethrin, while the assays with *Verania* resulted in exactly the opposite. This underlines that even though both insecticides are considered to have broad-spectrum activity, large differences may exist as to their risk for individual species.

In spite of these differences in susceptibility between species, the speed of reduction of effect over time was very similar. For both species the toxicity of chlorpyrifos residues diminished very fast, and was insignificant within one day. The effects of deltamethrin residues, however, were much more persistent for both species. This agrees with the difference in chemical half-lives (Table 2).

Unfortunately, when Table 8 and Figure 7 are compared, it becomes clear that the predictive value of the bioassays are not at all straightforward. Based on the bioassays with *Bracon*, no effects on trap counts of braconids were to be expected with deltamethrin. This is confirmed by the field data. One would expect, on the other hand, that chlorpyrifos treatments would affect braconid catches at least for a short while. This did not occur.

A possible explanation may come from the lack of persistence of the residues of chlorpyrifos. In a previous study (Van der Valk and Kamara 1993), we found long-lasting reductions in millet of *Cardiochiles* counts (the most common braconid in the traps) after fenitrothion treatments. Like chlorpyrifos, fenitrothion showed a large initial effect in the bioassay with *Bracon hebetor* (Danfa *et al.* 1997). However, fenitrothion residues were toxic for slightly longer, still showing 60% effect after one day, and becoming similar to control levels after two days of field weathering. It may be that a sort of "low risk space" develops fast enough in vegetation treated chlorpyrifos for a large part of the population to survive, while more than 24 hours of high risk millet vegetation after fenitrothion treatments results in large overall mortality. If this is indeed the case, then quite small differences in the biological half-life of an insecticide may be a more important parameter for the determination of ecological risk for beneficials than presently suggested.

Based on the bioassay with *Verania striata* one would not expect large effects on coccinellid beetles in the field after chlorpyrifos treatments. This did indeed not occur for *V. striata*, but coccinellid sp.2 appeared affected to a limited extent. The fact that statistics for this species were based on only one pre-spray count warrants a certain caution, however. The bioassays with deltamethrin suggest that large reductions in trap counts should be expected. These were not observed. A possible reason for this has been already discussed above, and refers to the source function of neighbouring groundnut and maize fields.

Effects on millet head miner dynamics

The most striking effect observed is the increase with on average 88% of peak larval densities of *Hellocheilus albipunctella*, the millet head miner, in chlorpyrifos treated fields (Figure 5). A very similar effect had been observed previously after treatments with fenitrothion at about the same time in the growing season (Van der Valk and Kamara 1993). No such increase was found after deltamethrin applications.

The fact that no effect of chlorpyrifos on gallery incidence was observed (Table 6), but an effect on larval density appears to exist, may seem inconsistent. However, it should be kept in mind that gallery incidence was defined as the percentage of millet ears that contained one or more galleries. It did not allow for any differences in the number or the length of these galleries, nor for the number of head miner larvae found in them.

The apparent inconsistency may be explained by the oviposition behaviour of the head miner and the subsequent effects of any natural enemies. *Heliocheilus* moths tend to oviposit in a patchy manner, either depositing several eggs on a millet ear, or none at all. As a result, more than one gallery is frequently found on an infested ear and/or several larvae inside the same gallery. Natural enemies will, for several reasons, often not attack and kill all hosts in a given patch (Van Driessche and Bellows 1996). Thus, higher natural enemy densities will likely reduce the density of larvae more than it will affect the percentage of infested ears.

Given the above, we hypothesize that the higher *Heliocheilus* density after treatments with chlorpyrifos were caused by a reduction in natural enemy activity. The absence of an increase in gallery incidence is not incompatible with such an effect. Additional support for the natural enemy hypothesis was recently provided from life table studies on the millet head miner in Senegal (Thiam and Van der Valk 1996, Sarr 1997). They found that most mortality due to natural enemies occurred in middle instar larvae, and not in eggs or very young larvae, nor in late instar larvae. Treatments in the study reported here were carried out while head miner larvae were mostly in their middle instars. Indeed, treatments with fenitrothion at the time of oviposition and early larval development did not result in significantly increased *Heliocheilus* populations (Kamara and Van der Valk 1995).

While such studies provide complementing information that all points in the same direction, a problem arises when the natural enemies actually responsible for *Heliocheilus* mortality are to be pinpointed. None of the three taxa affected by chlorpyrifos (bombyliid flies, ichneumonid parasitoïds and coccinellid beetles) are known to be important natural enemies of middle instar head miner larvae (Bhatnagar 1987). On the other hand, taxa that potentially are (tachinid flies or *Bracon hebetor*), did not seem to be affected by the treatments. So we cannot, in the underlying study, functionally link effects occurring on selected taxa of natural enemies with the dynamics of the millet head miner. The life table studies already mentioned may provide further clarification, though. They show that parasitoïds of the larval stages play a very limited role in the generational mortality of *Heliocheilus*. Most mortality appears to be the result of predation, quite probably due to flying predators such as Hemiptera or Sphecidae (other than the orthopteran specialist *Tachytes*). Such predators were not monitored in our ecotoxicological field study, mainly because their importance had previously been underestimated (e.g. Bhatnagar 1987).

The above discussions show very clearly the great importance of having proper understanding of the ecology of the agro-ecosystem under evaluation. The assessment of insecticide risk in real ecosystems is indeed considerably more complicated than the sum of results of a number of toxicity tests carried on "standard" non-target species. The latter approach is generally turned to for the pesticide registration, and is presently strongly *en vogue* for the Sahel as well. We argue from the above that detailed field studies, both ecological and ecotoxicological, continue to be of primary importance for judicious pest management in the Sahel.

Table 8. Summary of the effects observed on beneficial arthropods in millet after treatments with chlorpyrifos and deltamethrin as monitored with malaise traps and yellow sticky traps.

Taxon	Susceptible stage present[1]	Chlorpyrifos			Deltamethrin			Remarks
		impact[2]	mean % reduction[3]	duration of effect[4]	impact	mean % reduction	duration of effect	
DIPTERA								
Agromyzidae	yes	no			no			peaks before treatment
Bombyliidae	yes	yes	- 77%	> 3 weeks	no			absence of effects by deltamethrin possibly masked by catches in one field
Syrphidae: *Ischiodon aegypticus*	yes	no			no			
Tachinidae	yes	no			yes	- 42%	week 3	peaks after treatment
HYMENOPTERA								
Hymenoptera (total)	yes	no			no			
Bethylidae	yes	no			no			low numbers
Braconidae (total)	yes	no			no			
Braconidae: *Cardiochiles* spp.	yes	no			no			
Encyrtidae: *Copidosoma* sp. nr. *truncatellum*	yes	no			no			peaked before the treatments. Indications of effects of deltamethrin in part of the fields.
Halictidae	yes	no			yes	+ 570%	1 week	low numbers; increase in deltamethrin plots largely due to one field
Ichneumonidae	yes	yes	- 66%	> 3 weeks	yes	- 64%	> 3 weeks	
Sphecidae: *Tachytes* spp.	yes	no			no			peaks well before treatment
Tiphiidae: *Mesa* spp.	yes	no			no			peaks before treatment; very low numbers
COLEOPTERA								
Coccinellidae: *Cerania striata*	yes	no			no			
Coccinellidae Coccinellid sp.2	yes	yes	- 33%	> 3 weeks	no			only one pre-treatment sample available

[1]: if a susceptible life stage of the taxon is expected to have been present during treatment. [2]: statistically significant effect when compared to average of 5 weeks before treatment levels.
[3]: corrected for control fluctuations (- = reduction, + = increase). [4]: three weeks after treatments harvesting started in the study fields and trapping was ended.

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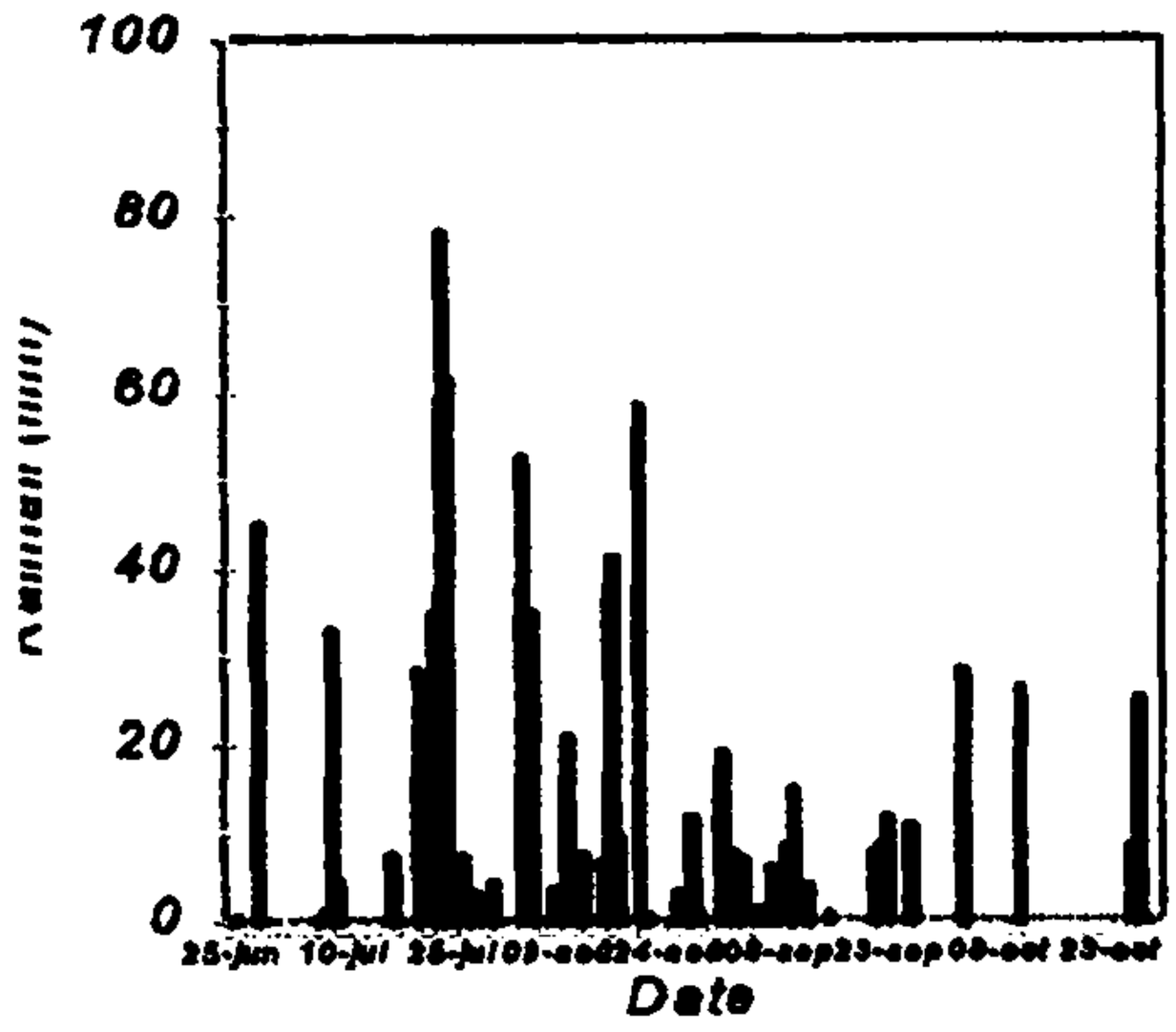
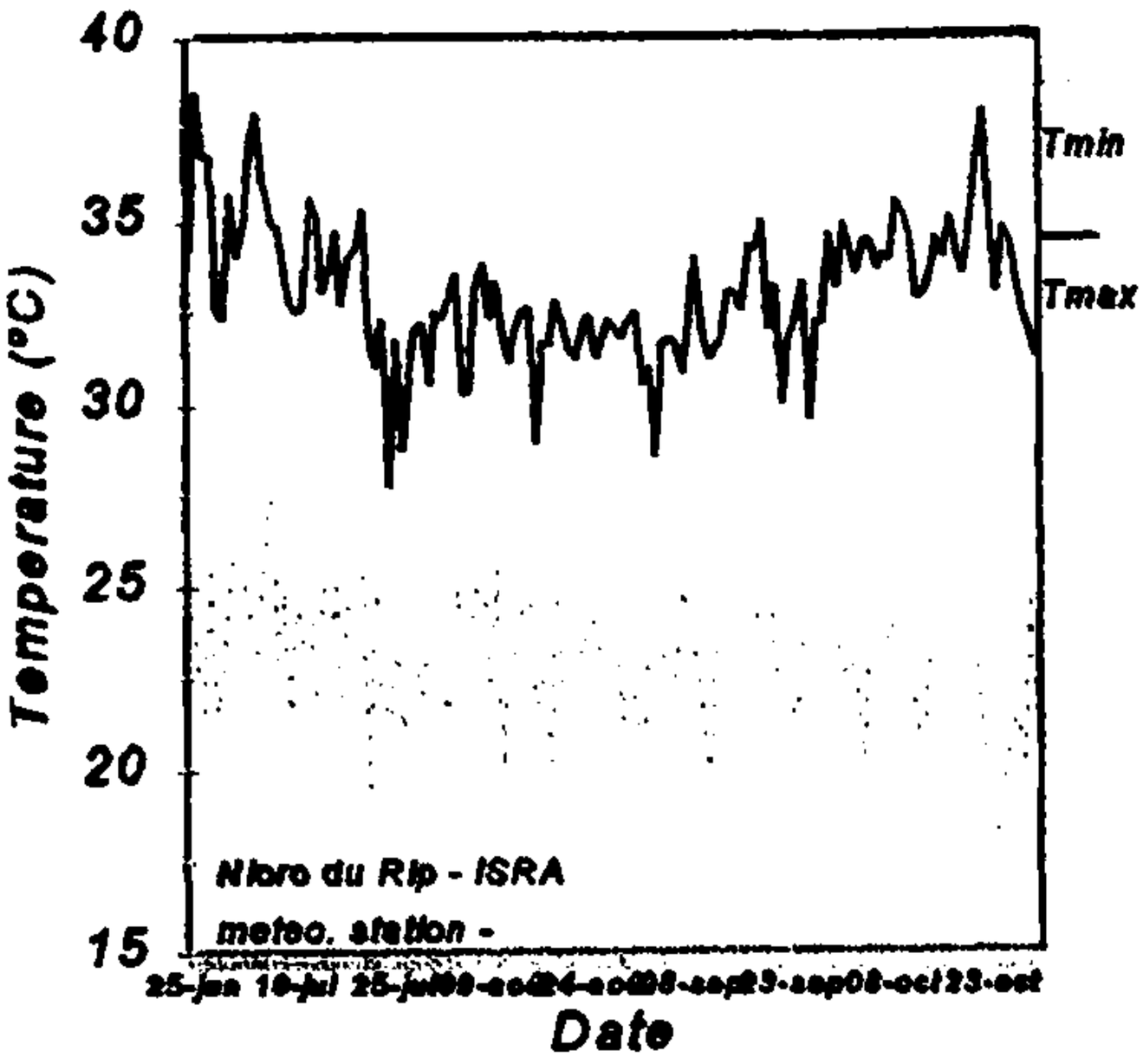
ANNEX 1: Meteorological data at Nioro du Rip and Prokhane during the millet study of 1993.





CHAPITRE 9:

Long-term effects of chlorpyrifos and fipronil on epigeal beetles and soil arthropods in the semi-arid savanna of northern Senegal

Abdoulaye DANFA, Amadou Lamine BA, Harold van der VALK,
Corinne ROULAND-LEFEVRE, Wim C. MULLIE &
James William EVERTS

SUMMARY

Four blocks of three plots measuring 125 x 125 m and containing one untreated control per block, were established in the Sahelian savanna of northern Senegal, at Fété-Olé (16°13'N 15°05W) to study the effect of two locust insecticides on some beneficial arthropods. The plots were treated with chlorpyrifos and fipronil in September, 1996. One year before treatment, ant and termite populations were studied in four of the 12 plots in order to assess normal fluctuations in population pattern and to establish monitoring methods to be used in the study. The activity of the insects was monitored using pitfall traps for beetles and ants, 'Pearce' traps for termites (predominantly *Psammotermes hybostoma*) and the 'Berlese' trap for Collembola and mites.

Chlorpyrifos was applied on pasture at an average rate of 207 g a.i./ha (between 134 and 258 g a.i./ha). Effects were usually delayed by one week, and generally were short-term. The most notable acute effects were a significant reduction ($p<0.05$) in carabid beetle catches from the second to the fourth week after treatment. On the contrary, the Tenebrionidae showed a steady but significant ($p<0.01$) increase in numbers in the treated plots, relative to the controls. This was mainly caused by an increase of *Zophosis trilineata*, the dominant tenebrionid beetle.

Fipronil was applied at a mean rate of 11 g a.i./ha (between 10 and 12.5 a.i./ha). In all the tests, reduction in catches of all epigeal beetle families was observed with the exception of the Scarabaeidae. This reduction was significant after two weeks in the Carabidae, and after three weeks in the Histeridae, Curculionidae and Elateridae ($p<0.05$). A highly significant reduction ($p<0.001$) was observed in the Tenebrionidae, one to four weeks after treatment. In ants, there was a significant reduction in catches one ($p<0.05$) and three weeks ($p= 0.001$) after treatment. One year after treatment, Elateridae were still significantly reduced ($p<0.05$), as compared to the controls, in one out of four catches. The other beetle families were no longer different from the controls.

Fipronil had a long term effect on termites. Termite catches gradually decreased after treatment in the treated plots. Five weeks after treatment, catches were significantly lower than in the controls ($p<0.001$). Monitoring one year after treatment (1997) showed that termites, particularly *P. hybostoma* and to a lesser extent ants and mites, were significantly less abundant in treated plots. After two years (1998), termites were still captured in significantly lower numbers in the treated plots. Visual observations in March 1999 and with traps in October and November 1999 revealed the re-occurrence of *P. hybostoma* in fipronil-treated plots. Observations on termite galleries, however, indicated that the activity in fipronil treated plots was still reduced by 70%, as compared to either controls or chlorpyrifos-treated plots. Full recovery was only achieved in 2000, four years after treatment. The study clearly indicated the possibility of a long-term effect of locust control with fipronil at the rate of 10-12 g a.i./ha, on epigeal termites and ants. Because the observations are limited to one termite species and to a very high dose, it is necessary to carry out more detailed studies taking into account all the useful soil termites an ants, as well as applying doses currently recommended by the manufacturer, before drawing conclusions on the long term risks of the product.

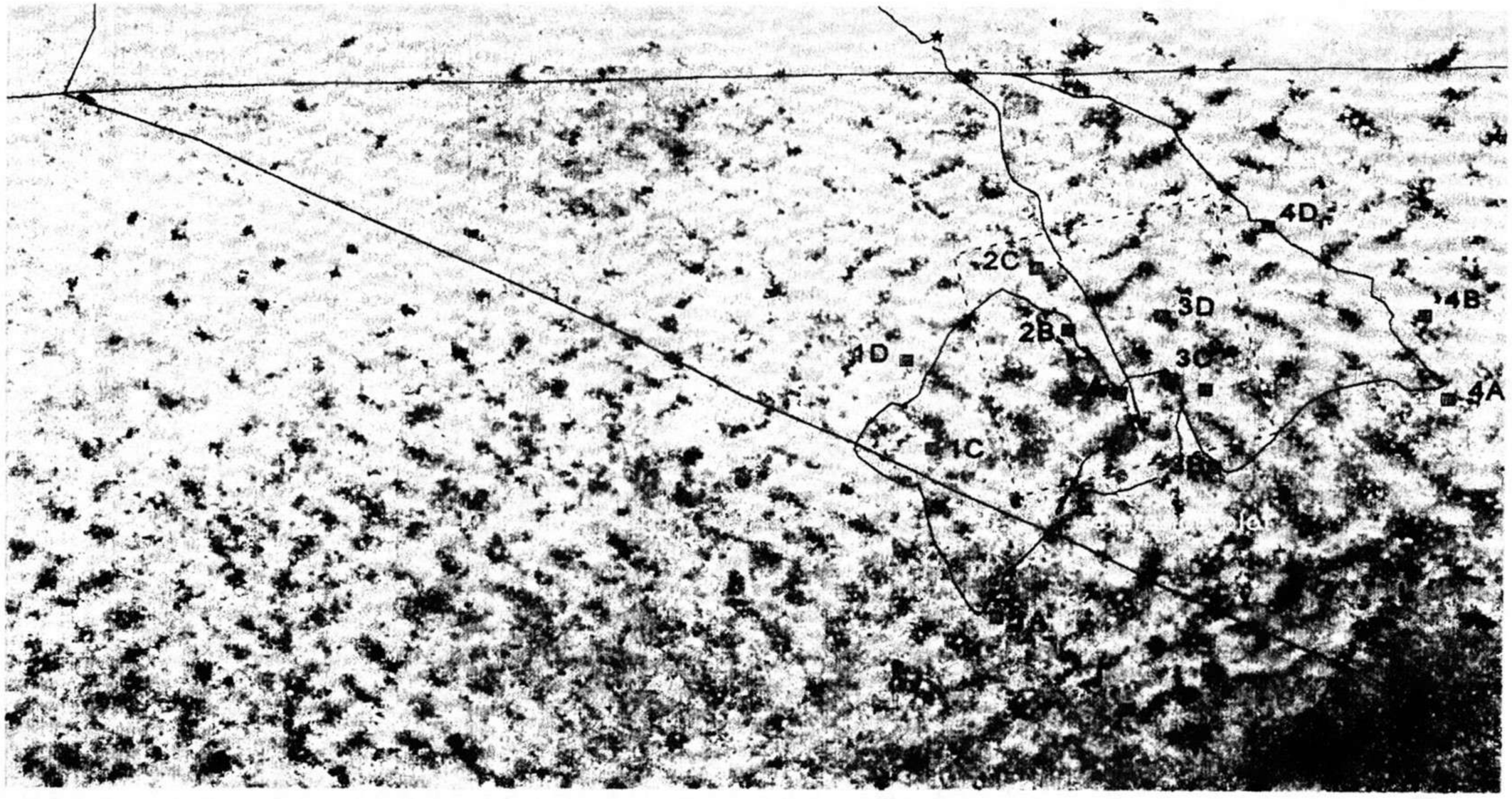


Figure 1 : Map showing position of the study plots relative to the former ORSTOM-study site at Fété-Olé. The aerial photograph (courtesy IGN, Dakar) was taken in 1980. The dark patches on the photograph are high tree densities in depressions (see Fig. 2 also).

INTRODUCTION

Crops are often infested by locusts in the Sahel. Some species like the Desert Locust (*Schistocerca gregaria)* are very voracious and mobile, capable of covering large areas and destroying thousands of hectares of crops. These insects have therefore become a real menace in the Sahel where most countries use chemical control. Other control methods which are more costly, require some technicity which does not facilitate their immediate use in the Sahel.

However, the countries using them and the donors of these pesticides are worried about the potential danger of the abusive use of these locust control products. More attention is now being paid to their secondary effects on the environment (Everts 1990, Peveling *et al.* 1994, Balança & de Visscher 1995). Studies on the risk posed by these insecticides in the Sahelian zones where there is increase in locust control are still very few (Matteson 1992). Research is therefore necessary to fill this gap. The Locustox Project has been working along these lines for a number of years. An evaluation was made on the side-effects of chlorpyrifos and fipronil on epigeal beetles and other arthropods in the arid savanna of Fété-Olé (16° 13 N - 15° 5W) in northern Senegal. The activity of the different arthropods was estimated by trapping.

Chlorpyrifos is an organophosphate, with a long standing history as a locust insecticide. Its side-effects on non-target arthropods are relatively well studied under Sahelian conditions (Van der Valk 1990, FAO 1996). It served in the present study as a positive control. Fipronil is a relatively new compound, belonging to the phenyl pyrazoles, whose impact on non-target arthropods under Sahelian field conditions had only partially been assessed, when the current study was initiated (1995).

The soil arthropods studied were epigeal beetles, termites, ants, Collembola and mites. Epigeal beetles included the Carabidae, the Tenebrionidae, the Histeridae, the Scarabaeidae, the Curculionidae and the Elateridae. These families were chosen in relation to their importance in the Sahelian ecosystem, and because a previous study had shown that several locust insecticides belonging to different chemical families were capable of inducing effects which lasted until one year after treatment (Van der Valk *et al.* 1999).

The Carabidae are polyphagous predators capable of attacking a large number of arthropods. The larvae of genera like *Abecetus*, *Harpaglossus*, *Homalolachnus* and *Chlaenius* were reported as predators of locust egg pods in Africa by Greathead *et al.* (1994). In Senegal several carabid species have been identified as predators of the millet head miner, *Heliocheilus albipunctella* (De Joannis), (Bhatnagar 1987, Ndoye 1991). The Tenebrionidae are important detritivores of the arid ecosystems (Crawford 1981). Larvae of some species (e.g. *Pimelia senegalensis*) are predators of grasshopper egg pods (Greathead *et al.*. 1994). The Histeridae are predators and microphagous. Larvae of the genus *Saprinus* are known to attack locust egg pods (Greathead *et al.* 1994). The Scarabaeidae captured are generally coprophagous detritivores which feed mainly on cow-dung. Fété-Olé is a sylvo-pastoral zone where the Scarabaeidae are useful as they efficiently incorporate the dung into the soil thereby reducing nutrient loss through climatic factors. Some species like *Onthophagus maculatus F.* are in addition predators of myriapods and diplopods in the Sahel. The Curculionidae are phytophagous. *Smicronyx umbrinus* infests *Striga hermonthica*, which is a parasite of millet and cowpea in the Sahel (Faragalla *et al.* 1985 , Smith *et al.* 1993). The Elateridae are generally phytophagous but the larvae of some species are predators (Fry 1989).

Termites are social insects considered as the key group in the arid and semi-arid ecosystems (Whitford 1991, Black & Okwakol 1997). The effect of termites depends on the trophic group considered. There are three distinct trophic groups: the xylophagous species which consume wood at different stages of decomposition, the fungus-growers which are in direct symbiotic relationship with fungi which they tend under plant debris in their nests and the humivores which feed on soil organic matter. The material used in constructing above-ground or subterranean nests as well as the diversified nature of their food endows each species with a particular effect on the environment. Through their nest building activities, termites affect the physico-chemical properties of the surrounding soil (Wood & Sands 1978, Loby de Bruyne & Conacher 1990, Jones 1990, Nash & Whitford 1995). The humivores enrich the soil with organic matter while the fungus-growers mineralize the soil (Granier-Sillam *et al.* 1989). The ability of fungus-growers to decompose plant material makes them destructive (Martius, 1994) while the humivores play a very important role in the nutrient cycle (Holt 1996).

Ants are organisms of practical importance in almost all ecosystems of the world (Hölldobler & Wilsson, 1990). The biomass and the abundance of these insects are as high in the desert as in the semi-arid ecosystems (Mackay 1991, Heatwole & Muit 1991). Although some pests exist (example: Dolichoderinae), ants play an important but less spectacular role than termites in the physicochemical structure of the soil. They increase the porosity and aeration of the soil (Lobry de Bruyn & Conacher 1994, Dean & Yeaton 1993). Soils surrounding ant nests are enriched with organic matter and mineral elements (Lobry de Bruyn & Conacher 1990, Mackay 1991, Dean & Yeaton 1993, Whitford & Di Marco 1995). In Africa there are at least seven ant genera used in biological control (Way & Khoo 1992).

Soil microarthropods (Collembola and mites) play an important role in the ecosystem. They serve in soil restoration with some species being fungivorous and others predators (Whitford & Parker 1989, Siepel 1994 & 1995). According to Koehler (1992), their high sensitivity to external agression combined with their importance to the ecosystem make them very vital in ecotoxicology. They are therefore considered by many authors as good indicators of the effect of pesticides in the environment (Joy & Chakravorty 1991, Koehler 1992, Krogh 1994) .

MATERIALS AND METHODS

Study area

The study was done at Fété-Olé (16° 13' N - 15° 5' W), about 50 km south of the ISRA Research Station at Fanaye where the team was based. The site is situated in and around a formerly fenced plot of 1 km² established in 1969 by ORSTOM-Sahel (now IRD) which carried out ecological research in the framework of the International Biological Programme (Bille *et al.* 1972). At the beginning of the 80s, the fencing wire was removed by the population. Figure 1 shows the position of the study plots in relation to the IRD site.

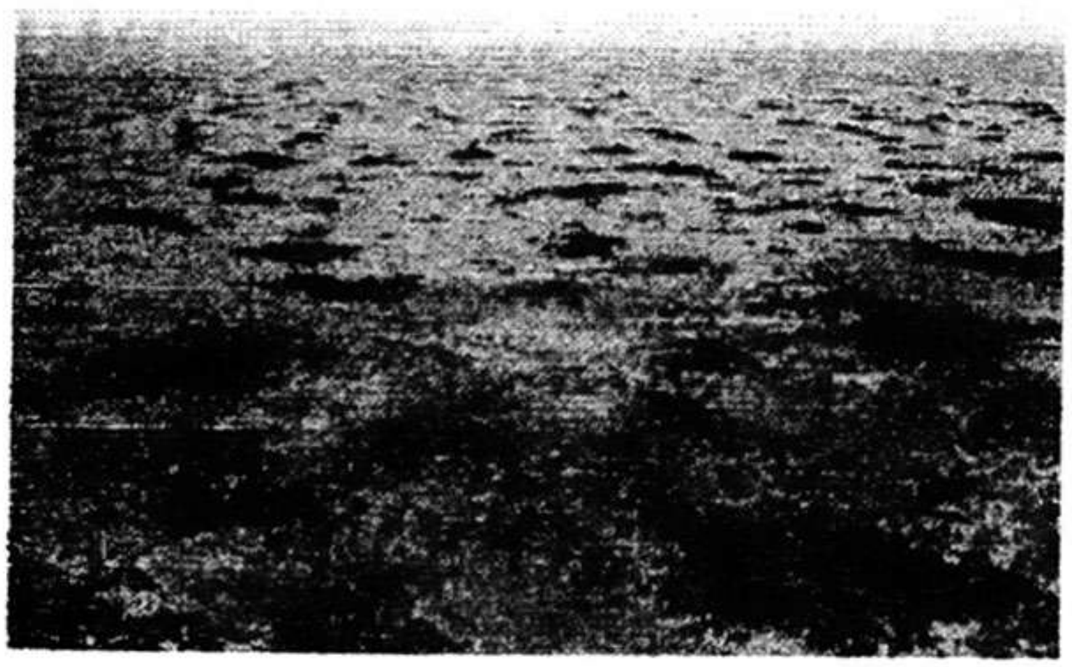


Figure 2. Typical view of the northern Ferlo savanna with regularly dispersed depressions and a temporary pond, supporting high tree densities (Photo- USAID/USGS).

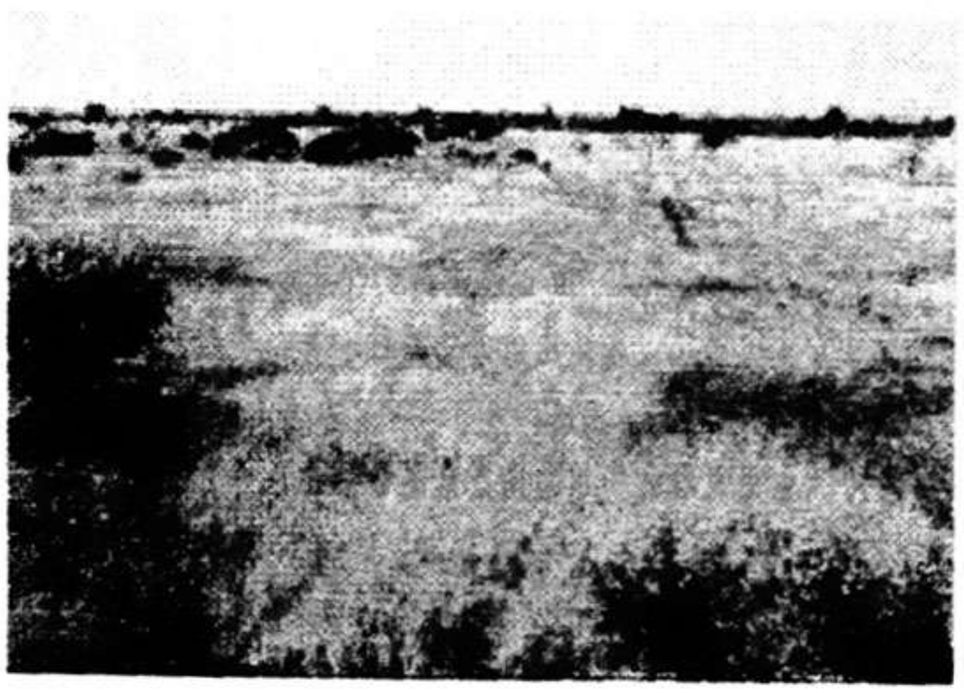


Figure 3. One of the study plots (IA) at the end of the rainy season. A few *Boscia senegalensis* bushes are visible in the foreground.

The Fété-Olé zone is situated in the northern Ferlo. It is characterized by a plateau gently sloping towards the river. A 1-3 m layer of sand representing an 'erg' (ancient dunes subdued by time) covers ancient deposits. Following the classification of Michel (n.d.) soils in the Fété Olé area belong predominantly to the brown-red type (iso-humic soils), according to the morphology of their profile and

dominant colour. A few large dunal bands oriented towards the north-east and south-west, cover the region. In the interdunes (valley lying between the dune masses), are depressions which sometimes form temporary ponds (Bille *et al.* 1972; Fig. 2). The soils in these depressions are slightly argilous and this is responsible for their low permeability.

On the basis of some Sudanian biome elements, Bille & Poupon (1972a) classified the vegetation of the Fété-Olé zone in 1970 as Sahelo-sudanian. Currently, the vegetation of the northern part of Ferlo is considered as part of the woody herbaceous type of the Sahel which extends from the Atlantic coast of Senegal, Mauritania in the West and to the Red Sea of the Sudan in the East (White 1986). The experimental plots which were used can be considered as characteristic of this vegetation type (Fig. 3). In the 70s, the Fété-Olé vegetation was made up of annual herbaceous plants capable of completing their cycle in two to three months. Bille & Poupon (1972a) identified eight vegetation types in the periphery of the depressions and at the dune summit and slope. Among others, the species characteristic of the dunes are: *Aristida mutabilis*, *Blepharis linariiflolia*, *Schoenefeldia gracilis*, *Cenchrus bifloris*, *Chloris prieurii* and *Panicum laetum*. The depressions are characterized by the presence of: *Cassia tora*, *Triumfetta pentendra*, *Panicum humile*, *Eragrostis* spp., *Andropogon* sp., *Zornia glochidiata* and *Echinochloa colona*.

In 1969, a 500m x 500 m subplot, within the IRD site was fenced as a reference plot for long-term monitoring (Fig.1). In 1976, the average number of trees and shrubs per hectare in this reference plot varied between 147 to over 2400 (average: 680) depending on the presence or absence of depressions. Ninety-nine percent of these trees belonged to six species: *Boscia senegalensis* (59.9 %), *Gulera senegalensis* (28.6 %), *Balanites aegyptiaca* (6.9 %), *Grewia bicolor* (2.1%), *Acacia senegal* (0.9 %), *Commiphora africana* (0.5 %) and other species like the baobab (*Adansonia digitata*) made up only 1 % (Poupon 1980).

Since studies by Bille & Poupon (1972a & b) and Poupon (1980), rainfall in the Ferlo has decreased drastically from 350-550 mm annually (Bille *et al.* 1972) to 200-400 mm in 1990 and 1994 (Consère 1996). Rainfall decline is evident all over the region. For example, the average annual rainfall in Saint Louis for the normals 1931-1961 and 1961-1989 was 347 and 258 mm respectively and equivalent to 26 % reduction (Tourand 1993). The Fété-Olé vegetation shows the first step in the degradation process which is characterized by an upsurge of toxic plants such as *Callotropus procera* since the 90s, a rapid dispersal of *Tribulus terrestris* characteristic in the intensely grazed and trampled zones (White 1986), and the presence of important variations in the age structure of the populations of the different tree species (Vincke 1995). The net primary production of the herbaceous stratum was estimated at 800-1200 kg dry matter per hectare from 1988-1995, with values rarely attaining 1000-1500 kg/ha and less than 500 kg/ha from 1992-1995 (Consère 1996).

The tree and shrub density has equally decreased, a phenomenon characteristic of the northern Sahel (Chamard & Courel 1999). Climatic effects are combined with anthropic action to explain this regression in the river valley. On the contrary, that observed in the Sahelian zone of Fété-Olé can be justified only by the climate. Between 1976 and 1983 in the reference plot, *Boscia senegalensis* has decreased by 4.6 %, *G. senegalensis* by 20 %, *Balanites aegyptiaca* by 2 %, *Acacia senegal* by 20 %, *Grewia bicolor* by 14.7 % and *Commiphora africana* by 10.4 % (Consère 1996).

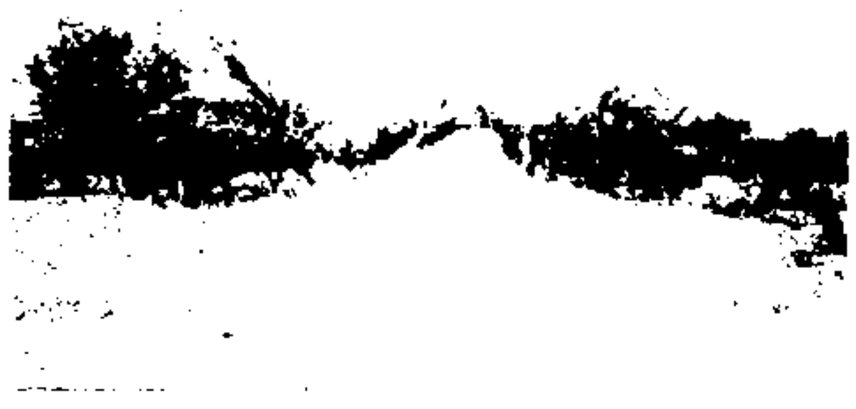


Figure 4. Dead Grewia bicolor on abandoned Macrotermes subhyalinus nest.

Figure 5. Treatment of plots by drift spraying using a spinning disk sprayer.

In 1995, the reference plot was restudied (Vincke 1995). When compared to 1983, a clear increase was observed in *B. senegalensis*, a species adapted to drought. This is typical of the 'Sahelinization' of the zone as confirmed by Miehe (1990) who indicates that the sandy Ferlo ecosystems are evolving into drier savanna independently of grazing. Some regeneration has been observed in *C. africana* and *G. bicolor*, but these species are no longer present in the dunes and hillsides, but are only maintained in the depressions. Compared to 1976, reduction in numbers of these species is highly significant. This is also valid for *A. senegal*, a species which is not adapted to low rainfall and continues to disappear. In 1999, this species was no longer present in the study plots (Table 1). Compared to 1976, four tree species have totally disappeared from the zone.

According to Amath Sow, Chief of the Fété-Olé village and assistant during the IRD studies, *B. senegalensis* and *C. procera* have increased appreciably in the 90s to the detriment of *A. senegal* and *G. bicolor*. The latter species has totally disappeared from the dunes, leaving only skeletons (Fig. 4). A change has also been observed in the ruminant herds which visit the zone. Cattle (grazers) have decreased in favour of sheep and goats (browsers). There has also been a change in the termite communities with respect to the 70s. Three *Trinervitermes* species have almost completely disappeared, leaving only *T. trinervius*, in some depressions away from the study zone. The current ecological conditions appear also to have affected *M. subhyalinus* which is still abundant, but many of its nests are actually abandoned (Fig. 4). On the contrary, a new species, *Odontotermes latericius*, which is characteristic in the arid regions, has established.

Within the 12 study plots, five are situated in the IRD site, of which plot 2A is within the limits of the reference plot (Fig. 1). The other seven plots are to the east, south and west of the IRD quadrant. The plots are situated at the summit of the dunes and a census of the woody vegetation was taken in 1999 (Table 1). Plot 2A shows a high similarity to the 1995 census of the reference plot. The dominance of *B. senegalensis* is evident, although some regression appears to have taken place since 1995.

Table 1. Composition and density (trees/ha) of the woody vegetation of the study plot compared to the 1995 situation in the reference plot.

Species	1995 reference plot Vincke 1995	Plot 2A, 1999 (Locustox)	12 plots, 1999 (Locustox)
Boscia senegalensis	125	94.1	70.1
Guiera senegalensis	1.1	0.6	1.1
Balanites aegyptiaca	4.3	4.5	12.5
Commiphora africana	0.3	-	0.5
Calmotropis procera	*12.8*	*10.2*	*4.7*
Euphorbia balsamifera	*2138,5*	-	*0.8*
Average density	***146.7***	***109.4***	***89.7***

Rainfall during the study

Rainfall for 1996 is indicated in Fig. 6. During the year, the ISRA Station at Fanaye recorded 260mm in 22 rainy days in 1996, 161mm in 15 rainy days in 1997 and 282mm in 19 rainy days in 1998.

The monthly totals show that in 1996, August was the wettest month with a total of 133.3 mm (Fig. 6) and a homogenous decade distribution . On the contrary in 1997 and 1998, September was the wettest month with 70.2 mm and 149.3 mm respectively and a heterogenous 10-day distribution:

1997(first decade = 15.9mm, second decade = 54.3mm and third decade = 0mm).
1998(first decade = 27.8mm, second decade = 107.0mm and third decade = 14.5mm).

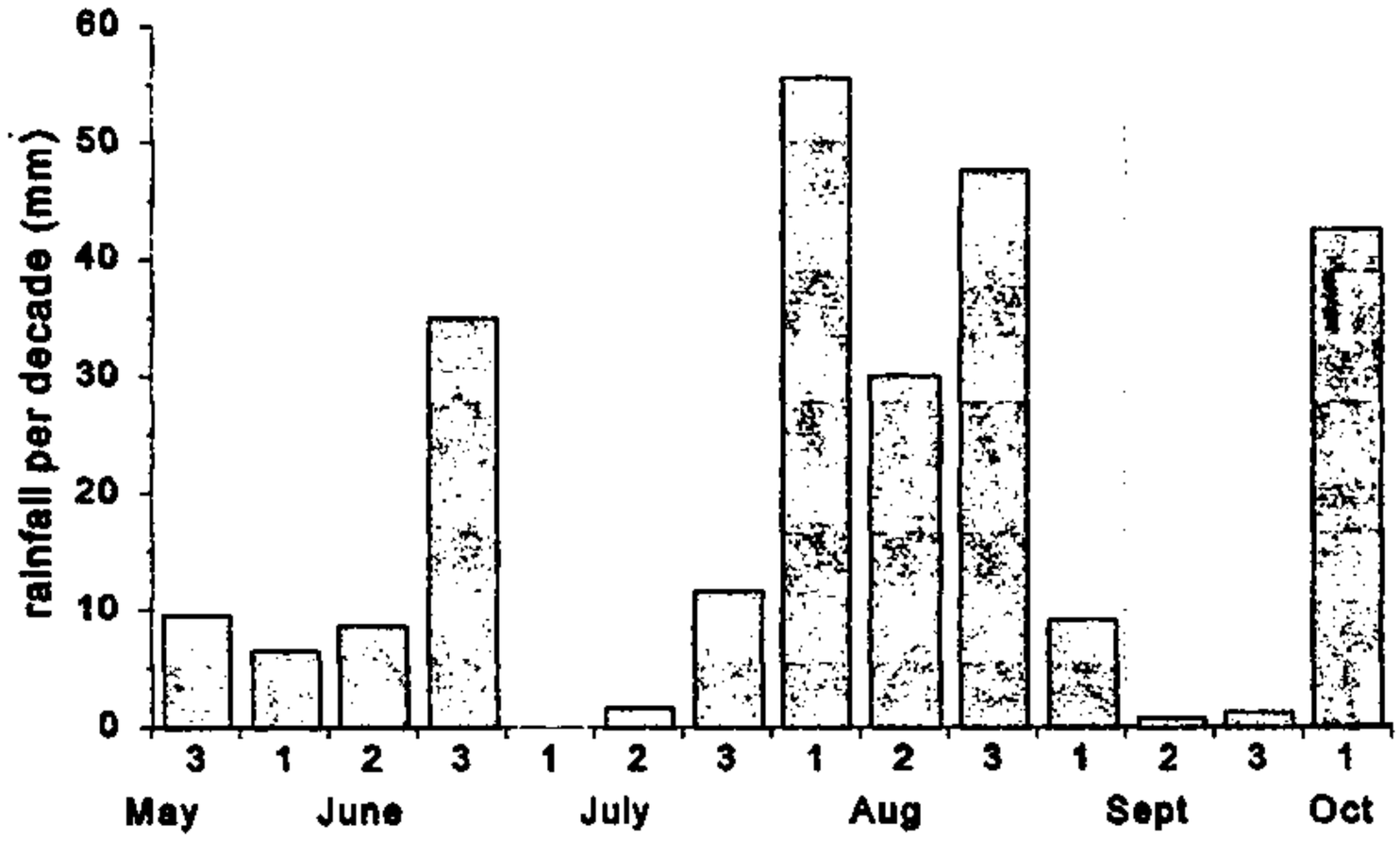


Figure 6. Rainfall recorded at the ISRA Fanaye Station in 1996 (the broken vertical line marks the moment of treatment).

Study design

Four blocks of three plots measuring 125 m x 125 m, of which two were treated and one left as the untreated control, were used .

The plots were chosen in such a way that the topography, soil and vegetation types characteristic of the site appeared homogenous. A guard row of 500-800 m séparated the different blocks. In each block, the plots were separated by a 200 m guard row (Fig.1).

The study was designed according to the Before-After-Control-Impact (BACI) principle described by Stewart-Oaten *et al.* (1986). In the design, samples are collected simultaneously in all the plots , several times before and after the insecticide treatments.

Insecticide applications

Treatments were made on 10 and 11 September 1996. Two insecticides were used : chlorpyrifos (Dursban® 240 g/l UL) (an organophosphate) (0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate) and fipronil (Regent ® 7,5 g/l UL) (a phenyl pyrazole) (RS)- 5-amino-1-(2,6-dichloro-aaa-trifluoro-p-tolyl)-4-trifluoromethyl-sulfinylpyrazole-3-carbonitrile).

The insecticies were applied with the spinning disc sprayers (Micro-ULVA ®) powered with five 1.5 V dry cell batteries (Fig. 5). According to the manufacturer's information data, this was expected to generate a median droplet diameter (VMD) averaging 60-80 µm. The sprayers and the walking speed of the spray operator were calibrated prior to treatment. Temperature and wind speed were measured

at the beginning and the end of treatments. At the end of each treatment, the amount of insecticide left over was measured to precisely determine the amount of the product actually used.

Table 3 gives details of the insecticide applications. For fipronil, the rates applied on plots IC and IVD were very close to those recommended at the time of treatment (1996) for blanket spraying against Desert Locust. There was under-dose in plots IIB and IIIB. For chlorpyrifos, over-dose was observed in plot IIA and under-dose in three others (ID, IIIC and IVA).

Evaluation of insect activity

In 1995, one year before the study started and the treatments took place, the future plots were studied for three months. During this study, the trapping methods were validated, and adapted if necessary. In addition, trapping data were collected for most taxa, usually in a limited number of plots. Only those for termites (Pearce traps and gallery counts), are used in this study. Since most of these data are from only four out of 12 plots, average catches fluctuate more widely than those obtained during the actual study period, which lasted from 1996 to 2000.

During the first year (1996), sampling began on 29 July and ended on 16 October of the same year. Up to four years after treatment, the plots were resampled from 5 September to 10 October 1997, 10 November to 14 December 1998, 14-31 October 1999 and 4-11 October 2000 to estimate long-term effects.

Trap catches were collected once a week. Notwithstanding the trap type, collections were made precisely a week after installation. The insects were collected in formaldehyde, cleaned and stored in 70 % alcohol.

Epigeal beetles and ants

Sampling of epigeal beetle and ant populations was done with an improved pitfall trap (Beye *et al.* 1998; Fig. 9). Two 500 ml cups were buried in the soil in such a way that only the upper rim was at the same level with the soil surface. They were separated by a roofing sheet screen of 80 cm x 8 cm with the lower end pushed into the soil to a depth of 3 cm and held in place with a peg stuck in centrally. Each pot was covered with a 15 x 15 cm roof held over the pots by two thick steel bars. The screen was supposed to direct the insects towards the pots which were two-thirds full of 5 % formaldehyde and a drop of detergent. Eight traps were installed along two diagonals (4 per diagonal). At the entrance of each plot, the first trap was placed 10 m from the edge while the second was installed 60 m along the diagonal. The four traps placed at 60 m were collected together in a small bucket and considered as one sample. Those placed at 10 m made up the second sample.

Termites

Termites were captured with a trap described by Pearce (1990) ('Pearce trap'; Fig. 9). It is made of two glass plates (20 cm x 12 cm x 0.2 cm) and two square pieces of cardboard of 10 cm x 10 cm. The cardboard pieces were placed 1 cm apart with an overlap of 0.5 cm on each side of the plates and held in place with a rubber band. The cardboards were previously soaked in a 1:3 solution of molasses and water. Molasses was retained after prior efficacy tests with several attractants.

Eight traps set 5 m apart were placed at the center of the plot. They were buried to the depth of 2-3 cm. The traps were therefore capable of capturing surface termites or those found just below the soil surface. After installation, the traps were moistened with a watering can. Collection was made between 8 and 11.30 am before the soil became hot. All the glass plates were collected together and labelled as a single sample. The collected plates were wrapped in 3 l plastic bags to prevent the termites from escaping. Four traps were put into each bag with two bags making a sample. After cleaning, the termites were conserved in 70 % alcohol.

In 1995, one year before treatments and during eight weeks, the number of termite galleries was assessed in two 2 meter wide bands across each plot. The number of galleries encountered was noted and averages were calculated for each plot. In 1999, the gallery count was repeated for three weeks.

Collembola and mites

Sampling of microarthropods was done with a core sampler placed along the diagonals of the plots 10 m from the edge and every 30 m thereafter. Five core samples were taken per diagonal at various depths: 2 cm, 4 cm, 8 cm and 12 cm. At each depth, the samples were mixed into one. Therefore in each plot, four samples were kept, representing different depths. The samples were placed in a Berlese trap for the extraction of the soil microarthropods. This trap is made of a wooden framework (plywood) into which four funnels are fixed. On the lower end of each funnel is a joint which holds a vial containing 5 % formaldehyde. The samples are poured into the sieves which are placed in the funnels. Four 25 W electric bulbs are installed on the lid of the trap. Each bulb overhags a sieve. The heat from the bulbs drives the microarthropods into the funnels and the vials. The samples are exposed for 48 hours for maximum Collembola and mite catches.

Residue analysis

Herbaceous vegetation and soil were sampled at different periods after insecticide treatment for residue analysis. Sampling was done along the diagonal of each plot. Soil samples were taken from the surface to a depth of 4 cm (Gadji *et al.* 1997 & 1998).

Data analysis

The effect parameter used in all analyses was the log-transformed difference between the treatment value (count or measurement) for a given sampling date, and its paired control value within the same block (Stewart-Oaten *et al.* 1986). This pairing of treated and control values within each block was done under the assumption that variability within blocks was generally less than among blocks. The effect parameter was calculated as "ln (treated count + 1) - ln (control count + 1)". Percentages were transformed using an arcsine transformation (Sokal & Rohlf 1981), resulting in an effect parameter calculated as "treated arcsine sq.root (%/100) - control arcsine sq. root (%/100)".

One or more values of the effect parameter after treatment were then tested against the average effect parameter before treatment, using sampling dates as (pseudo) replicates. Otherwise said, one tests if the difference of counts between the treated and control plots has significantly changed after treatment when compared with the average difference before the treatment. Since each block contained the two treatments and a control plot, the study resembles a randomised complete block (RCB) design. However, an RCB analysis of variance (ANOVA) assumes that no interaction exists between blocks and treatments (Sokal & Rohlf 1981, Dutilleul 1993). This could not be excluded here *a priori*. Therefore, a two-way ANOVA, with explicit assessment of the interaction term, was applied instead.

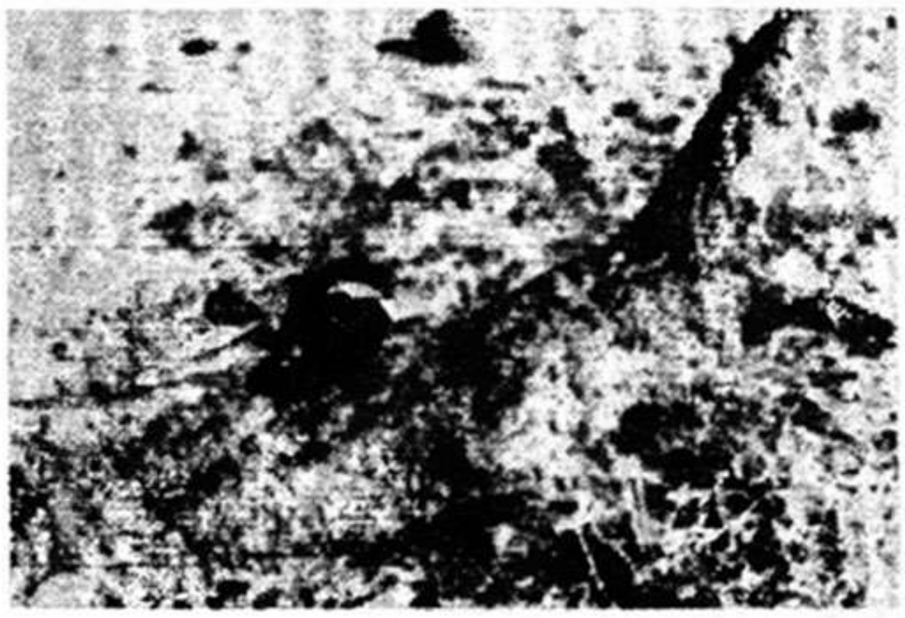
The corresponding ANOVA table is given below (Table 2). This is a mixed model ANOVA with blocks considered as a random and treatment as a fixed factor (Sokal & Rohlf 1981, Bennington & Thayne 1994). The effect of blocks is not formally tested. This is because one is not free to randomly assign a plot to any one block, as the three plots within any block are limited to that block. As a result, there is a high probability that a "restriction error" has to be taken into account , which means that there is no suitable denominator mean square (MS) over which to test the block MS (Sokal & Rohlf 1981). Anyway, one is not interested in block effects, since they are expected to exist and this was the reason why the treatments were blocked in the first place.

The treatment effect (MS before vs. after), which is our main interest in this study, is tested over the interaction term if the latter is significant. A significant interaction means that the treatment effect is not generalized over the blocks. In such a case, the treatment effect will be considered "ecotoxicologically significant", if it is significant over the interaction. When the interaction is not significant, one may decide to pool the interaction and error mean squares. There does not seem to be general agreement among statisticians if this should be done (Sokal & Rohlf 1981). Therefore, conservatively, MSs were not pooled if the F-ratio based on error mean square was already significant. Only if this was not the case, and after verifying Bancroft's rules for pooling (Sokal & Rohlf 1981), were the interaction and error MS pooled and used as the denominator over which to test treatment effect.

Figure 7. *Pimelia senegalensis*

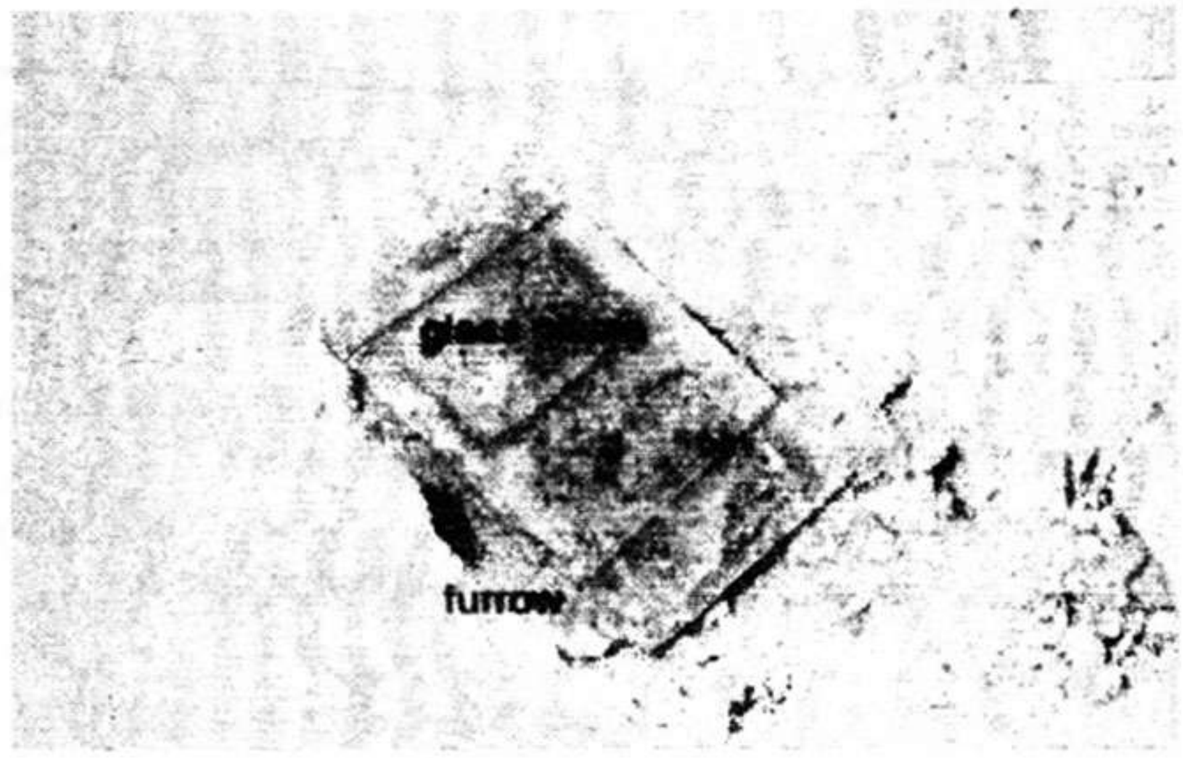
Macrotermes subhyalinus

Psammotermes hybostoma

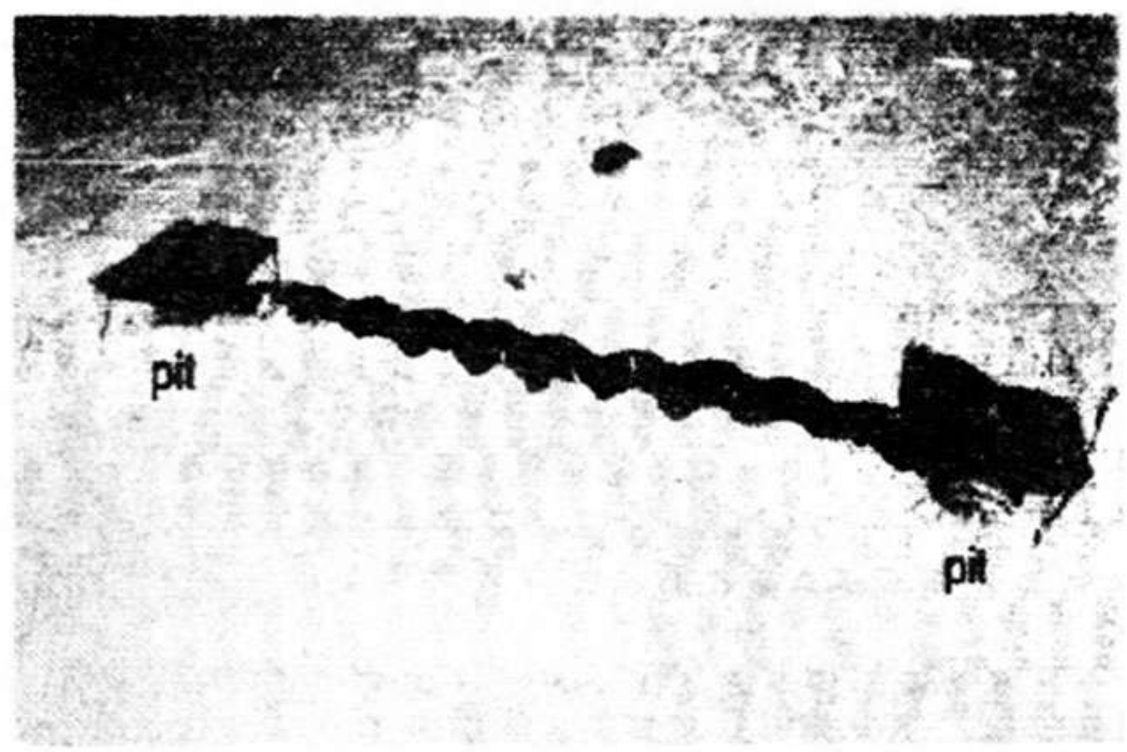


Odontotermes nilensis

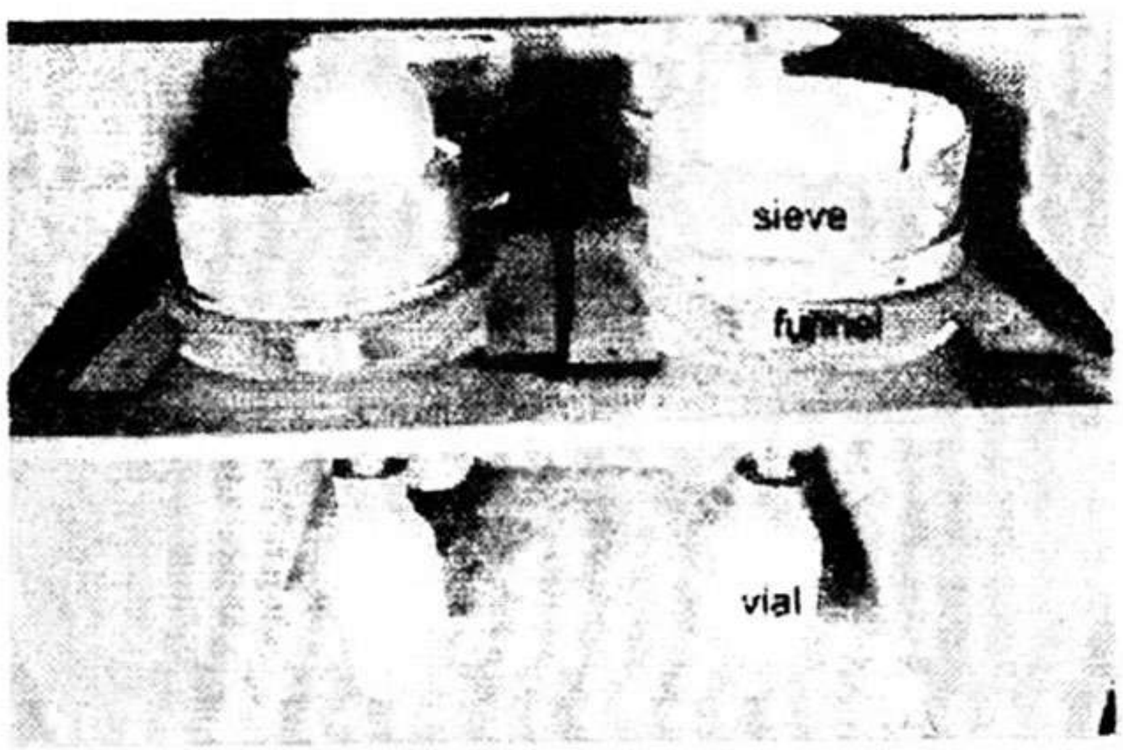
Figure 8. Galleries of the three of the most common termite species.



Pearce trap



Pitfall trap



Berlese trap

Figure 9. Traps used for arthropod sampling.

Table 2. Characteristics of analysis of variance (ANOVA) used in this study to evaluate the impact of chlorpyrifos and fipronil on epigeal beetles, ants and termites.

Source of variation	Degrees of freedom	F value	Conditions
Block		not tested	
Before and after treatment (= treatment effect)	1	1) MS before and after/MS interaction	if interaction is significant
		or 2) MS before and after/MS error	if interaction is not significant
		or 3) MS before and after/MS grouped error	if interaction is not significant
Interaction	3	MS interaction/MS error	
Error	4((t1-1) + (t2-1)		
Total	4 (t1 + t2) - 1		

t1 = dates (replicated) before treatment ; t2 = dates (replicate) after treament : MS = Mean Square (estimated variance)

RESULTS

Pesticide residues

The results of analysis of vegetation and soil residues (Gadji *et al.* 1997, 1998) are presented in Table 3. Chlorpyrifos was applied at dosages which varied between 138 and 250 g a.i./ha. With the exception of the initial residues in vegetation for the under-dosed plot (IIIC), there was generally good agreement between applied dose and vegetation or soil residues.

For fipronil, no relationship between the applied dose and the initial residue levels in both vegetation and soil could be established, but differences in applied dosages among the plots were rather small (9.9-12.5 g a.i./ha; CV= 8 %).

The half-life periods of the two insecticides (Gadji *et al.* 1997 & 1998) varied 'a factor 2' for chorpyrifos, with 56 hours for plot IIA and 115 hours for plot IVA. The same variability was observed for half-life periods of fipronil (414 and 212 hours). A year after treatment, no trace of the two products or their metabolites was identified in the soil.

Taxonomic composition

Epigeal beetles

About 112 400 beetles from 11 families were captured in the pitfall traps. The Tenebrionidae was the most abundant family (84 %) followed by the Carabidae (7 %), the Scarabaeidae (4.5 %), the Histeridae (2 %) and the the Elateridae (1 %). The other families (Curculionidae, Trogidae, Staphelinidae, Cicindellidae, Meloidae and the Chrysomelidae) were sparsely represented.

The taxonomic level which was generally used in analysis was the family. Species was used only for the Carabidae and Tenebrionidae. However, the use of a codified system, permitted the identification of 118 taxa (Appendix 1). The Tenebrionidae was dominated by *Zophosis trilineata* (78 %) followed by *Pimelia senegalensis* (11.5 %), *Zophosis quadrilineata* (5.5 %), *Vieta senegalensis* (3 %) and *Polpogonia* sp. (2 %).

Table 3. Treatment parameters of 125 m x 125 m plots. Applications were made with a hand-help spinning disk sprayer (Micro-Ulva). Meteorological date were recorded at the beginning and end of treatments. Vegetation and soil were sampled for residue analysis (Gadji et al. 1997 & 1998).

Treatments (plots)	Dates	Volume applied (l)	Dose applied (g al/ha)	Temp. (° C)	Relative humidity (%)	Wind speed (m/s)	Initial residue (mg a.i./kg)	
							Vegetation	Soil
Chlorpyrifos								
I D	11.09.66	1.510	223.7	27	51	1.5-4	26.5	0.10
II A	10.09.96	1.740	257.7	32	39	2.5-3	28.5	0.12
III C	11.09.96	1.520	208.0	27	51	2-3.5	42.4	0.10
IV A	10.09.96	0.930	137.7	32	39	2-3	15.5	0.08
Fipronil								
I C	10.09.96	2.705	12.52	32	39	2-3	2.7	4.7*
II B	11.09.96	2.280	10.99	27	51	2-3.5	5.1	4.0*
III B	10.09.96	2.070	09.90	32	39	2-3.5	2.8	4.7*
IV D	11.09.96	2.60	12.04	31	51	2-3	5.6	.36*

* = ln µg a.i./kg n.a. = not analyzed

Ants

About 51 300 ants belonging to the Formicidae and three sub-families (Formicinae, Myrmicinae and Ponerinae) were captured. An identification of the ants revealed 19 taxa (Appendix 2). The Myrmicinae was by far the most abundant and was dominated by *Monomorium* (91%; Fig. 11) and *Crematogaster* (2%; Fig.11). The first is a cosmopolitan genus distributed worldwide (Bolton 1994). The second most abundant sub-family was the Ponerinae with the genus *Pachycondyla* (4.6%) being the most abundant followed by the Formicinae: *Lepisiota* (1.2%) and *Cataglyphis* (1%) while the unidentified genera made up 0.2%.

Termites

About 19 500 termites were captured with the Pearce trap of which three taxa were distinguished:
- *Microcerotermes* sp. (Termitidae) ;
- *Odontotermes nilensis* (Emerson) (Termitidae) ;
- *Psammotermes hybostoma* (Desneux) (Rhinotermitidae).

A sub-sample of the 1996 pre-treatment catches was used to identify the different species trapped. For the analysis, these data are not taken into account and only total termite catches have been analyzed.

The most abundant species was the sand termite, *Psammotermes hybostoma* (80 %). It is a primitive xylophagous termite which consumes both plant material and exposed wood. It consists of a complex social group (with two worker and three soldier castes). It is found in dry African regions (the Sahara Desert and other sub-desert zones) including Madagascar (Pearce & Waite 1994). *Microcerotermes* sp. made up 17 % of the total catch. It is a superior xylophagous species which attacks dead wood, plant debris as well as leaves (Harris 1971, Lepage 1972). It is frequently encountered in the tropical zone. The fungus grower, *Odontotermes nilensis*, made up only 3 % of the termite catch. This species feeds on dead wood and plant debris. In the anthropized zones, it is a pest of trees and crops (Harris 1971).

It is important to note that *Macrotermes subhyalinus*, whose nests were extremely abundant at the site (Fig. 4), was not recorded in the trap catches. Therefore, a potential effect of the treatments on this species could not be assessed. Though termites constitute the most abundant arthropod group in the arid and semi-arid zones (Lee & Wood 1971, Lal 1988, Jones 1990, Mando 1997), they were the least abundant in our catches.

Effects of chlorpyrifos and fipronil

The effects were evaluated generally at the family or sub-order level, with the exception of the Tenebrionidae (4 species), the Carabidae (1 species), and ants (5 species). The evaluation was made on families that were either sufficiently represented in the catches and/or of ecological importance.

For the different arthropods and depending on the time of capture, the 1996 analysis involved the periods indicated below:
- epigeal beetles : catches made 6 weeks before and 5 weeks after treatment ;
- ants : catches made 5 weeks before and 3 weeks after treatment ;
- termites : catches made 5 weeks before and after treatment.

The long term monitoring data (1997: ants, epigeal beetles; 1997-2000: termites) involved samples taken during a 1-5 week period between September and November.
For the Collembola and mites, analysis was restricted to 1997 and 1998 data (one and two years after treatment).

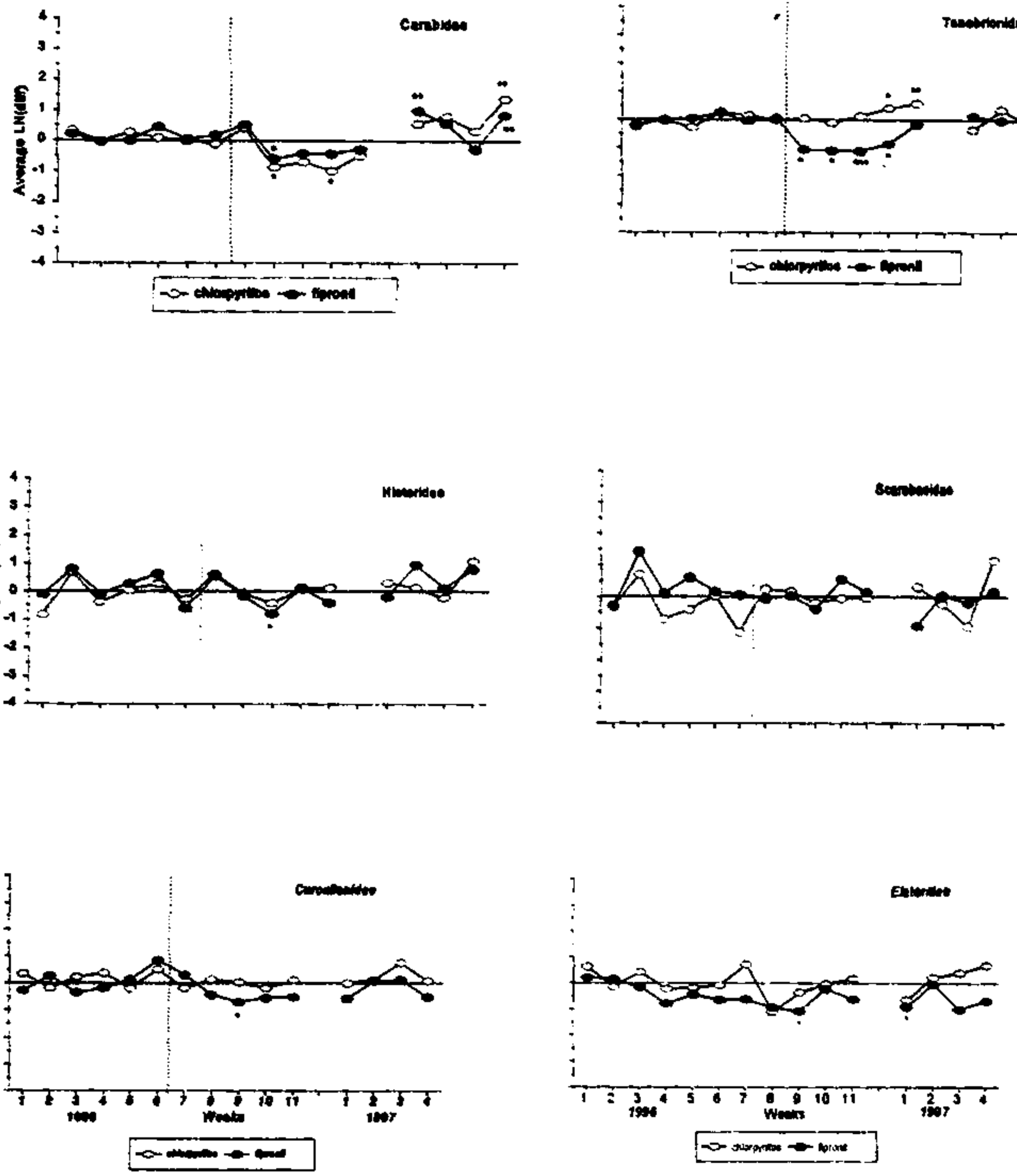


Figure 10. Average weekly pitfall trap catches (of 4 plots) of various epigeal beetle families. The log transformed data used are the differences between the treated and corresponding control plots. The vertical broken line marks the moment of treatment (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

Family and higher taxonomic level

Figures 10 and 12-14 indicate the effect of the two insecticides on the different arthropod taxa studied.

Chlorpyrifos

In the Carabidae, a significant ($p<0.05$) decrease in catches was apparent two and four weeks after

treatment. One year after treatment, average carabid catches were higher than those in controls, which was significant ($p<0.01$) in one out of four catches (Fig. 10).

Contrary to the Carabidae, catches of the Tenebrionidae gradually increased in the chlorpyrifos treated plots. Four and five weeks after treatment they became significantly more abundant than those trapped in control plots ($p<0.01$) (Fig. 10). One year after treatment, no differences between treated and control plots were found. Four other beetle families studied (Histeridae, Scarabaeidae, Curculionidae and Elateridae), were at par with the control plots after treatment.

Neither total ants trapped in pitfall traps in chlorpyrifos-treated plots the same year and one year after treatment (Fig. 12), nor collembolans and mites, trapped respectively one and two years after treatment (Fig. 14), differed from control plots.

Termites decreased in numbers after treatment, but this was significant ($p<0.05$) only three weeks after treatment. On the contrary, one year after treatment, termites were strongly reduced ($p<0.001$) in treated plots, as compared to the controls. In the chlorpyrifos plots, differences with the controls were no longer noted from the 2nd to the 4th year after treatment (Fig. 13).

Fipronil

Generally, fipronil caused a decline in catches of the different coleopteran taxa studied, with the exception of the Scarabaeidae (Fig. 10). The decrease in the number of individuals of the Carabidae was significant two weeks after treatment ($p<0.05$). In addition, three weeks after treatment, there was a significant reduction ($p<0.05$) in the Histeridae, Elateridae and Curculionidae catches. A sharp and significant decline ($p<0.001$) was observed in the Tenebrionidae in the first four weeks after treatment. One year after treatment, beetle populations had generally recovered. A signicantly lower number of Elateridae was still observed in one of four catches ($p<0.05$). On the other hand, and as in chlorpyrifos treated plots, Carabidae showed an increase in numbers ($p<0.01$) one year after treatment, as compared to controls (Fig. 10).

Ant catches decreased immediately after treatment, but this was significant ($p<0.001$) only in week one. However, one year after treatment a significant reduction in ant activity ($p<0.001$) was still apparent (Fig. 12).

Although termite catches gradually decreased and were seemingly affected from the second week onwards following treatment (Fig. 13) (which was of borderline statistical significance ($p=0.06$) at four weeks after treatment), a significant decrease ($p<0.001$) was recorded only five weeks after treatment. Also one year (1997) and two years (1998) after treatment, termite catches were strongly reduced ($p<0.001$) in fipronil-treated plots, as compared to controls. Visual observations made in March 1999 showed that *P. hybostoma* had become active again. In the fipronil treated plots this was confirmed by the Pearce traps in October and November of the same year which showed that, although still less numerous than in the treated plots, the difference between the treated and control plots was small ($p=0.06$). However, counts of termite galleries on the soil surface in October 1999 still showed a large difference (t-test; $p>0.001$) between control and chlorpyrifos treated plots on the one hand and fipronil treated plots on the other. There was a 70 % reduction in actvity in the latter as compared to the other two types. In 1995, a year before treatments were made, counts taken during eight weeks on the same plots did not reveal any differences between them . In October 2000 there was a complete recovery in termite activity (Fig. 13).

For the Collembola, no decrease was observed in fipronil treated plots compared to the control. On the contrary, in mites, a distinctive difference was observed in relation to the control for samples taken at 2, 8 and 12 cm, a year after treatment (Fig. 14). Two years after treatment, no difference was observed any longer between the treated and control plots.

Genus and species level

Figures 15 and 16 indicate the effect of the two insecticides on the different species of beetles and

ants.

Chlorpyrifos

Abecetus sp. was the only genus separately studied in the Carabidae. It was not affected by chlorpyrifos. Four species and one genus were studied in the Tenebrionidae *(Pimelia senegalensis* (Fig. 7), *Vieta senegalensis, Zophosis quadrilineata, Zophosis trilineata* and *Polpogonia* sp.*)*. Among these five taxa, *Z. quadrilineata* showed a non-significant decrease with subsequent recovery, from week one after treatment . On the contrary, *Z. trilineata* showed an increase ($p<0.05$) in week 4 after treatment (Fig. 15).

In ants, where five genera were studied, none was affected (Fig. 16) and this situation remained unchanged after a year.

Fipronil

The Carabidae were generally affected by fipronil with the exception of *Abecetus* sp.. In the Tenebrionidae, no effect could be demonstrated by fipronil on *Pimelia senegalensis* and *Vieta senegalensis*. On the contrary, there was a decrease in catches in three other taxa (Fig. 15) :

- two weeks after treatment in *Zophosis quadrilineata*;
- weekly for four weeks after treatment in *Zophosis trilineata* ;
- first three weeks after treatment in *Polpogonia* sp.

The effect of fipronil was observed only in two ant species. *Monomorium* sp. and *Lepisiota* sp. were significantly affected ($p<0.001$), one week after treatment (Fig. 16).

Long term studies (a year after treatment) revealed statistically significant ($p<0.05$) effect of fipronil on four ant taxa : *Monomorium* sp. (Fig. 11), *Lepisiota* sp., *Crematogaster* sp. (Fig. 11) and *Cataglyphis* sp.

Crematogaster sp.

Monomorium sp.

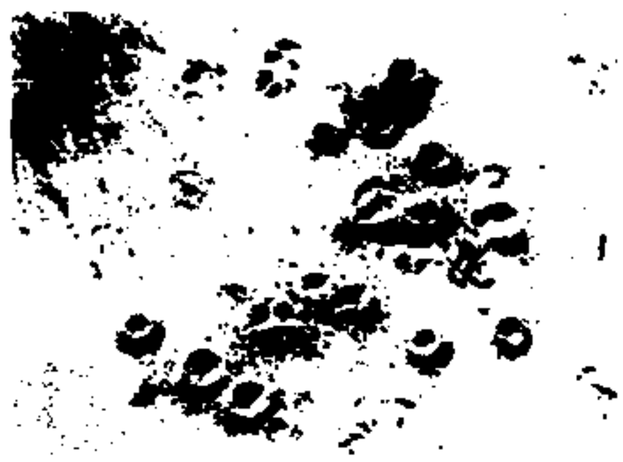


Figure 11. Examples of nests of two of the most common ant genera trapped.

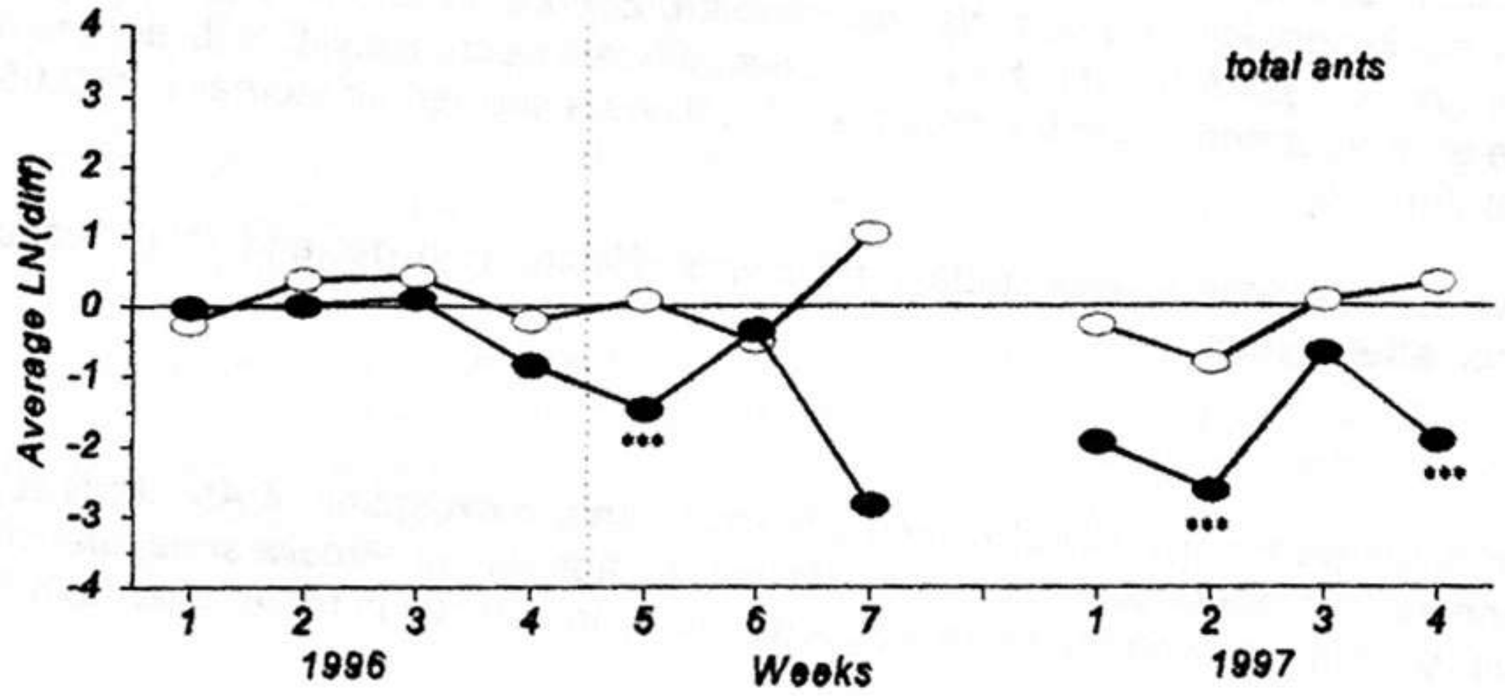


Figure 12. Average weekly pitfall trap and catches (of 4 plots). The log transformed data used are the differences between the treated and corresponding control plots. The vertical broken line marks the moment of treatment. (* $p<0.05$, *** $p<0.001$).

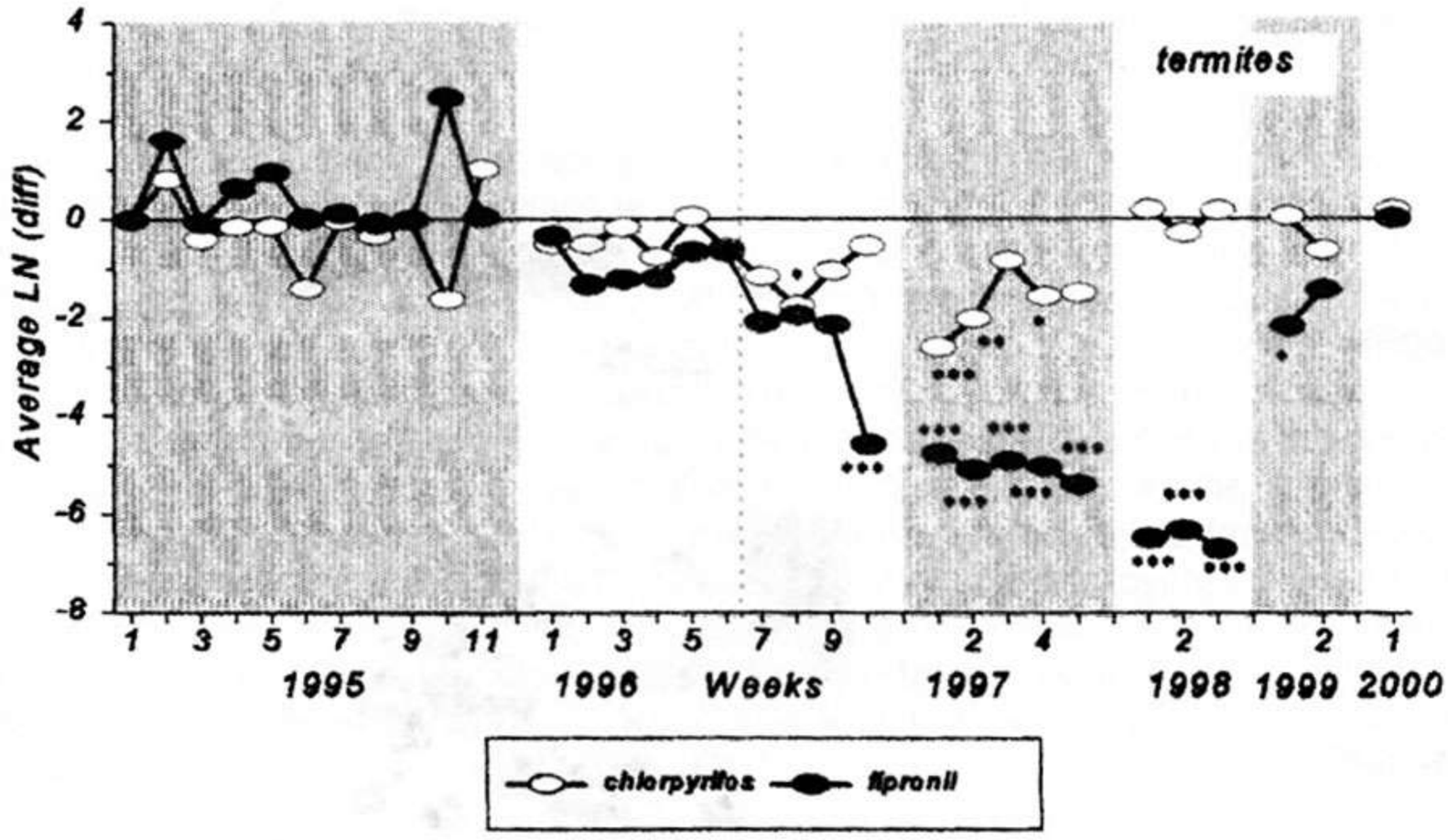


Figure 13. Average weekly Pearce trap termite catches (of 4 plots). The log transformed data used are the differences between the treated and corresponding control plots. The vertical broken ligne marks the moment of treatment (* $p<0.05$, ** $p<0.01$, *** $p<0.001$).

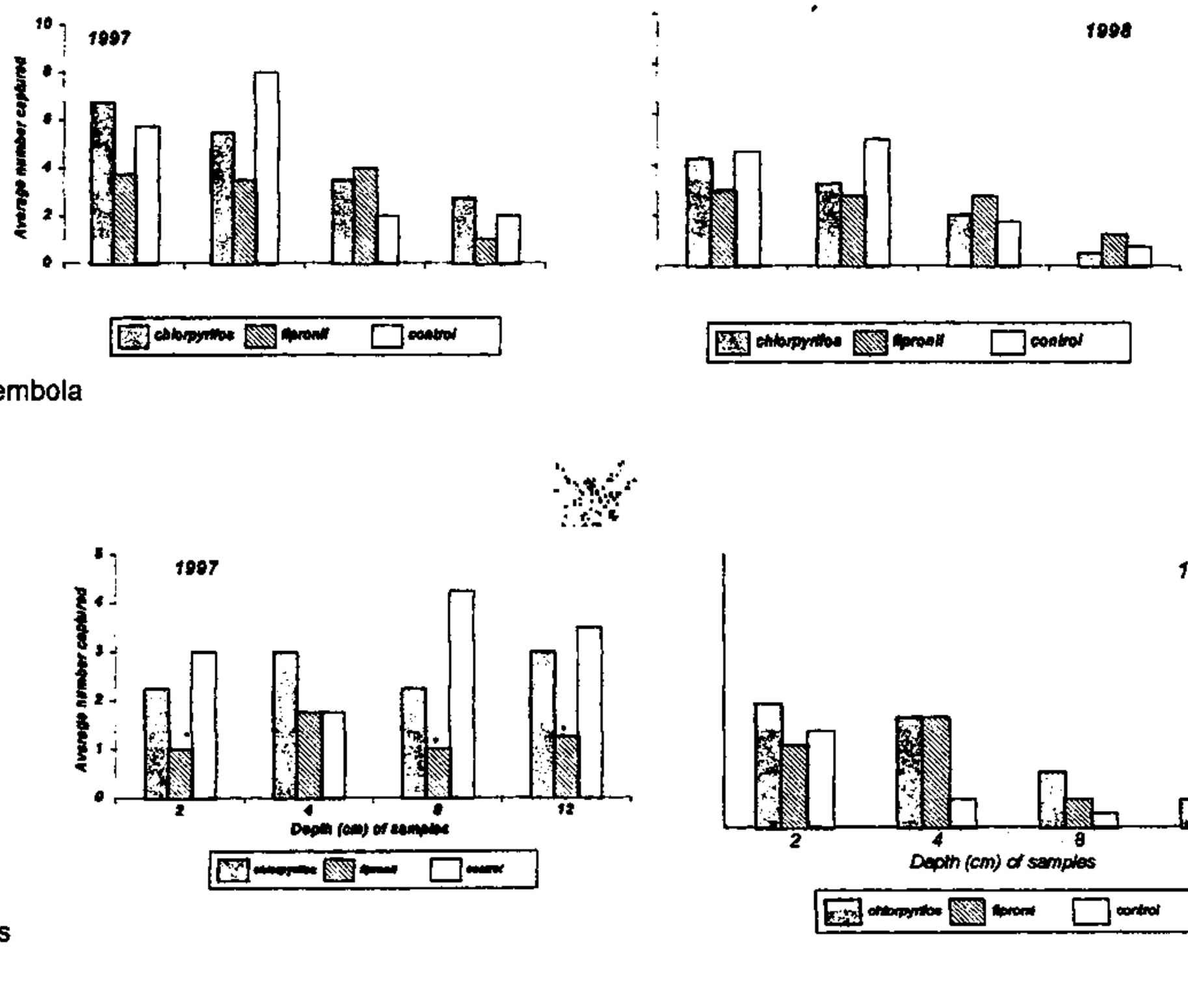


Figure 14. Average Collembola and mite catches from core soil samples taken at different depths, one and two years after treatment (* $p<0.05$).

Table 4. Average weekly number of galleries counted in 2 m wide transects in each plot a year before and three years after treatment.

	1995 n = 8 weeks	1999 n = 3 weeks
Chlorpyrifos		
I D	82.3	68.0
II A	74.6	60.3
III C	88.3	30.7
IV 1	74.9	66.7
Average	80.0	56.4
Fipronil		
I C	88.5	19.3
II B	77.0	20.6
III B	70.3	10.3
IV D	74.8	11.3
Average	77.6	15.4
Control		
I A	97.9	77.7
II C	81.6	56.3
III D	87.3	52.3
IV B	69.6	48.7
Average	84.1	58.7

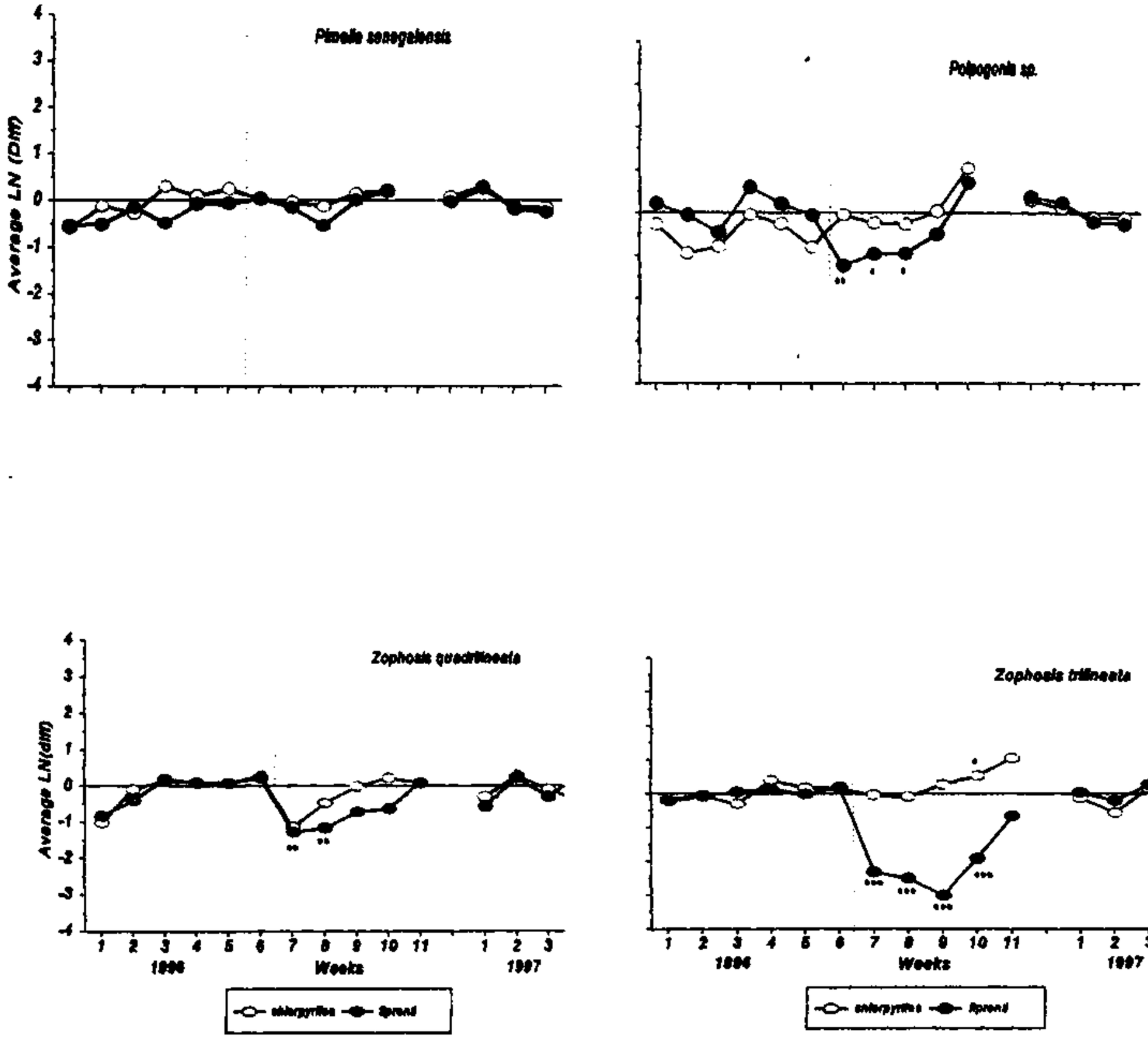


Figure 15. Average weekly beetle species catches (of 4 plots). The log-transformed date used are differences between the treated and control plots. The vertical broken line marks the moment of treatment (*p<0.05, ** p<0.01, *** p<0.01).

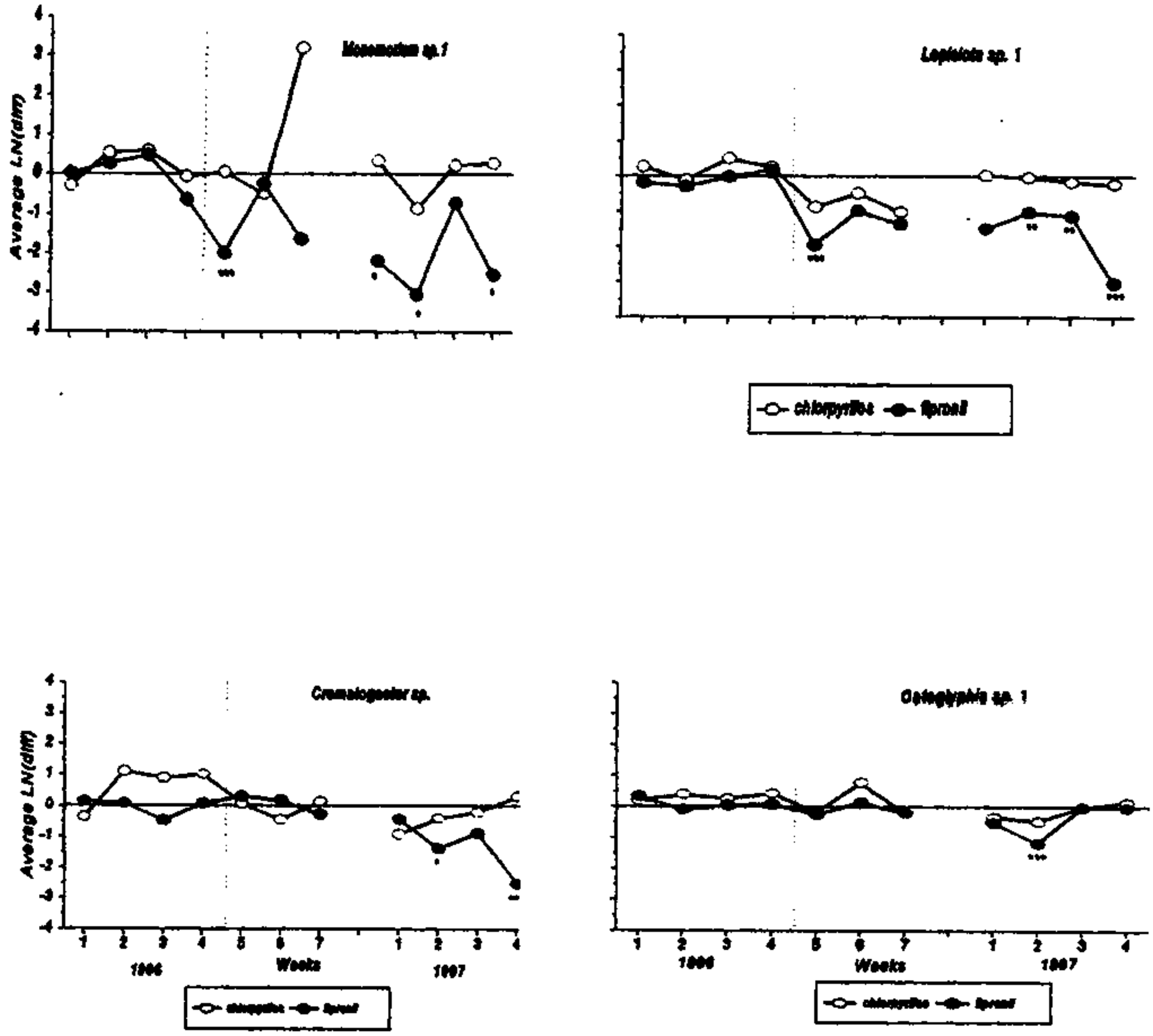


Figure 16. Average weekly pitfall ant species catches (of 4 plots). The log-transformed data used are differences between the treated and control plots. The vertical broken line marks the moment of treatment (* p<0.05, ** ^p <0.01, *** p<<0.001).

DISCUSSION AND CONCLUSIONS

Chlorpyrifos applied on vegetation at an average rate of 207 g a.i./ha (under-dose as compared to the target dose of 240 g a.i./ha) did not have a major effect on epigeal beetles. Only the Carabidae and Elateridae were slightly affected in most plots, but there was a resurgence in their activity before the monitoring ended, five weeks after treatment. In the Tenebrionidae, a gradual and significant increase of activity became apparent in the catches after treatment. This could mainly be attributed to an increase in catches of *Zophosis trilineata*, the dominant tenebrionid beetle. This increase could possibly be due to a treatment related decrease of their main parasitoids or predators, which were not studied. Van der Valk & Kamara (1997) and Sarr (1998) have shown that experimental insecticide treatments in Senegal in millet against grasshoppers using fenitrothion, reduced parasitoid and predator densities caused an increase in the Millet Head Miner (*Heliocheilus albipunctella*) population. Our results differ slightly from those of Van der Valk (1990), where there was no significant effect of chlorpyrifos applied at 270 g a.i./ha on the carabid and tenebrionid species (*P. senegalensis* and *V. senegalensis*). Also in a detailed study, using field bio-assays, Van der Valk *et al.* (1998) did not find any significant increase in mortality of *P. senegalensis* after chlorpyrifos application at the rates of 248 and 386 g a.i./ha respectively. Reed *et al.* (1992) remarked that the mortality of the Carabidae was significantly higher in plots treated with fonofos and terbufos than chlorpyrifos-treated and control plots.

According to Racke *et al.* (1994), typical agricultural soil applications of chlorpyrifos (0.56 - 5.6 kg a.i./ha) result in initial soil surface residues of 0.3 to 32 mg a.i./kg, corresponding to a residue/application rate ratio of 0.57. In our study, initial residues were on the average 0.10 mg a.i./kg, or a ratio of 0.48, which is in the same order as that mentioned by Racke *et al.* (1994). Although chlorpyrifos is considered a termiticide (Forschler & Townsend 1996), initial soil residues after termicidal soil barrier treatments are usually in the order of 1000 mg a.i./kg or higher (Racke *et al.* 1994). Termite LC50s for chlorpyrifos are in the range of 0.2-2 mg/kg of soil (Racke *et al.* 1994), and it is therefore not surprising that no significant effect was observed on termites. Also in ants, no effect of chlorpyrifos could be found. This is in agreement with the results of Barbour & Brandenburg (1995) where there was no effect of chlorpyrifos (260 g a.i./ha) on the Formicidae in general. Chlorpyrifos did not cause any significant effect on the Collembola and mites a year after treatment, but the immediate effects on these taxa following treatment have not been studied. Bellows & Gaston (1992), however, have shown in toxicity studies of five insecticides, including chlorpyrifos, that they caused little or no mortality to mites three days after treatment.

Fipronil applied at an average rate of 11 g a.i./ha caused a significant but transient reduction, two to three weeks after treatment, in catches of various coleopteran taxa, with the exception of the Scarabaeidae. However, there was a highly marked effect in the Tenebrionidae from the first to the fourth week after treatment. This was mainly due to a decrease in the dominant species (*Z. trilineata*), representing almost 80% of the numbers trapped. Among the five tenebrionid species studied, only two were not significantly affected by the product. One year after treatment, there was still some measurable effect on the carabids (increase) and elaterids (decrease). Contrary to what was expected, there was no effect on *P. senegalensis* . In a field bio-assay with *Trachyderma hispida*, treatment with fipronil at the rate of 10 g a.i./ha caused 90-100% mortality within 15 days, even when the beetles were introduced in the arenas 35 days after treatment (Van der Valk *et al.* 1998). Both LD50 and LC50 values for fipronil on *T. hispida* are in the same range as those for *P. senegalensis* (unpublished data Locustox). The impact on epigeal beetles has already been cited in literature. Balança & de Visscher (1997) observed more than 99% mortality in non-target insects, particularly the Carabidae (90 - 98%) and the Tenebrionidae (80 - 99%) following fipronil treatment at the rates of 4.2 and 13.4 g a.i./ha. Johnsen *et al.* (1997) observed a reduction in beetle predators on plants as well as in water in fipronil-treated rice experimental plots.

The social insects (ants and termites) were particularly affected by fipronil. Sampling of ants and termites for two and four years after treatment respectively (1996-2000) showed a strong reduction after treatment. A year later and even two for termites, the ant and termite populations had not yet reached the normal level. In other taxa, resurgence was usually observed a few weeks after treatment. It was only three years after treatment that the termite activity started to recover, when visual observations

made in March 1999 as well as the October to November catches indicated *P. hybostoma* activity in the fipronil-treated plots. Toxicity studies in the laboratory with *P. hybostoma*, exposed to fipronil on soil substrate (Nodjikouman 1996), indicated that this compound acted slowly, but after 4-10 days LC50 values attained levels which were in the same range as the residues found in this study (4-5 µg a.i/kg of soil). Modelling of the data, using DEBtox (Luger *et al.* 1998) showed that at this soil concentration, it would take about a week to attain 50% reduction in the exposed population in so far as all the termites are exposed simultaneously. Since under natural conditions this is unlikely to occur, significant reductions could become apparent only after a few weeks. This is in agreement with our field data. Further modelling of the data with DEBtox showed that LC50 values continued to decrease with time. Therefore, it is possible that even at lower rates, significant mortality of *P. hybostoma* may occur. Since this is based on laboratory tests, field tests with lower rates are required to confirm this.

The persistent decrease in population of social insects could result in the destruction of all individuals in the colonies in the treated plots. In this case, resurgence of the insects can only occur if there is establishment of new colonies. Whereas, new colonies are only formed by dispersal which occurs once per year at the beginning of the rainy season, that is, before the treatment period. Therefore, the establishment of new populations can only take place one year, at the minimum, after treatment and workers can be recorded only a year later. This hypothesis corroborates the 1999 field observations, which showed the emergence of a single termite caste of small workers undoubtedly from young colonies. Our observations show the possible long term effect of a locust control treatment with fipronil on useful epigeal termites.

It is interesting to compare the effects caused by the two products. Fipronil had more severe effects than chlorpyrifos, but in a few taxa the effects were parallel. Both products caused a reduction in carabid beetle activity after treatment and in plots treated with either product, carabid activity increased a year following treatment. Both products also affected termite populations, but in chlorpyrifos-treated plots this effect was no longer apparent in the second year after treatment, whereas in the fipronil-treated plots, full recovery was only achieved in the fourth year. This indicates that recovery in the chorpyrifos-treated plots probably occurred from within the plots themselves, whereas in the in the fipronil plots, as explained above, this was not the case. On the contrary, in the Tenebrionidae, chlorpyrifos generally induced an increase while fipronil caused a decrease in population.

The sampling method used for termites (Pearce trap) allowed observations to be made on only the sand termite, *P. hybostoma*. Although this species has some characteristics of an ecological indicator (abundance, homogenous distribution, can be kept in the laboratory), it is not necessarily a key species in the Sahelian ecosystem. Therefore, more detailed studies using different sampling methods are needed to draw conclusions on the toxicity of fipronil on useful soil termites in general. The importance of long-term monitoring has been shown in this study and therefore, should be applied to other taxa, particularly ants, that are sensitive to fipronil. These studies have to be made with dosages currently recommended by the manufacturer. It is recommended that the effect of 2-5 g a.i./ha in blanket treatment against locusts should be monitored.

To what extent the elimination of termite activity for a number of years, as we have found in this study, contributes to an increased soil degradation or prevents already degraded soils from recovery, remains essentially unanswered. Environmental degradation, particularly in the semi-arid Sahelian zone is proceeding at an unprecedented rate. As mentioned by Stroosnijder (1992), the combined effect of soil organic matter depletion, primary production decrease, and the harsh climatic conditions have resulted in an extension of crusted soils. Termites were found to play a crucial role in the rehabilitation of these soils, when a mulching layer was present (Mando 1997). Nearly all arid and semi-arid environments are characterized by extreme year-to-year weather fluctuations that cause vast changes in the ecosystem. Consequently, it is difficult and sometimes nearly impossible to distinguish between short-term and long-term trends, as well as between temporary and permanent changes (Dodd 1994). Therefore, the added effect of soil arthropod depletion may be difficult to assess. In addition, soil variability even within short distances, is very pronounced. This is partially related to microtopography and thereby to hydrology, but also to former termite activity such as that of *Macrotermes* sp. (Brouwer & Bouma 1997). At the ICRISAT Sahelian Center in Niger, it was found that up to 20% more production could be attained on areas where termites had improved soil physical properties (J Brouwer, *pers. comm*). The number of replications needed in an experiment, to allow for statistical inferences, might be prohibitive and new methods may be required to study the environmental consequences of partial depletion of social insect populations, which form an important component of the soil ecosystem.

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APPENDICES

Appendix 1: List of various taxa captured atFété-Olé.

Code	Taxa	Authors	Families	Observations
				same species with different codes
4	*Abecetus gagates*	*Dej.*	*Carabidae*	*same for codes 13, 16 & 17*
22	*Abecetus gagatinus*	*Chd.*	*Carabidae*	
23	*Acupalpus* sp.		Carabidae	
50	*Acupalpus* sp.		Carabidae	
67	*Aulacoryssus pulchellus*		*Dej.* *Carabidae*	*same for codes 71, 73 & 83*
95	*Brachinus humeralis*	*Dej.*	*Carabidae*	
26	*Brachinus* sp.		Carabidae	
34	*Bradybaenus scalaris*	*OP.*	*Carabidae*	*same for codes 76, 77*
2	Carabidae sp. n° 13		Carabidae	
3	Carabidae sp. n° 30		Carabidae	
9	Carabidae sp. n° 14		Carabidae	
28	Carabidae sp. n° 16		Carabidae	
32	Carabidae sp. n° 36		Carabidae	
33	Carabidae sp. n° 15		Carabidae	
35	Carabidae sp. n° 31		Carabidae	
45	Carabidae sp. n° 32		Carabidae	
48	Carabidae sp. n° 33		Carabidae	
49	Carabidae sp. n° 17		Carabidae	
54	Carabidae sp. n° 23		Carabidae	
57	Carabidae sp. n° 24		Carabidae	
61	Carabidae sp. n° 25		Carabidae	
64	Carabidae sp. n° 26		Carabidae	
65	Carabidae sp. n° 27		Carabidae	
74	Carabidae sp. n° 34		Carabidae	
78	Carabidae sp. n° 35		Carabidae	
80	Carabidae sp. n° 28		Carabidae	
82	Carabidae sp. n° 29		Carabidae	

The LOCUSTOX Project ECLO/SEN/003/NET, GCP/SEN/041/NET, GCP/SEN/053/NET was set up in 1989 by the governments of Senegal and the Netherlands. It was executed in collaboration with the Food and Agriculture Organization of the United Nations (FAO).

The objective of the project is to study the environmental side - effects of pesticide applications against locust and grasshoppers and to inform and train decision makers and applicators on selective and safe treatment methods.

The project became a public foundation in 1999 with the same objectives as the project as well as an extension. The foundation is not limited to pesticides used in locusts and grasshoppers campaigns, it works also on other pesticides.

From 1996 to 2002 studies were carried out on the following subjects :

- The risk in temporary aquatic ecosystems in central Senegal to human health of anti-locust and anti-grasshoppers campaigns.

- The side-effects on temporary aquatic fauna and beneficial insects.

- The synthesis of research and literature data for advise to the Government and FAO on selectivity and safety of chemical treatments.

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