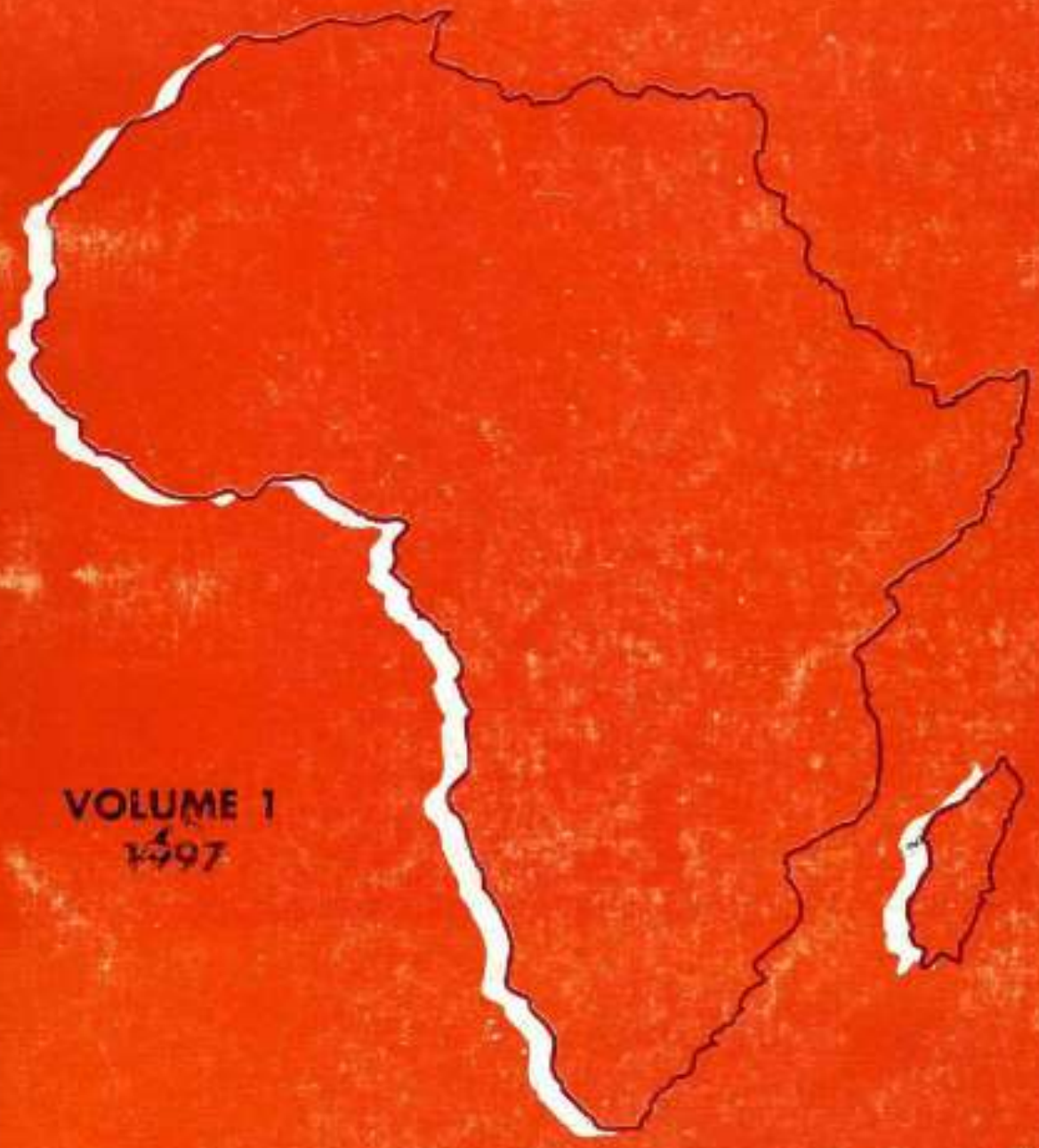
FOOD AND AGRICULTURAL
ORGANIZATION OF THE UNITED-NATIONS

LOCUSTOX PROJECT

ENVIRONMENTAL SIDE-EFFECTS OF LOCUST AND GRASSHOPPER CONTROL



VOLUME 1
1997

PLANT PROTECTION DIRECTORATE
MINISTRY OF AGRICULTURE
DAKAR, SENEGAL

ENVIRONMENTAL SIDE-EFFECTS OF LOCUST AND GRASSHOPPER CONTROL

VOLUME 1

1997

Editors:
James W. Everts
Djibril Mbaye
Oumar Barry

Food and Agriculture Organization
of the United Nations - FAO

Plant Protection Directorate,
Ministry of Agriculture - SENEGAL

This document is available in French and English versions. It can be ordered at the addresses below. The text can be cited with reference to the source.

The opinions expressed in this document represent those of the authors and do not necessarily reflect an official position of FAO or the Senegalese government. The insecticides studied are not necessarily those assessed by the Pesticide Referee Group for FAO.

LOCUSTOX Project - GCP/SEN/041/NET, FAO Representation in Senegal
P.O. Box 3300, Dakar - Senegal

FAO -Headquarters, Distribution Centre, Viale delle Terme di Caracalla
00100 Rome - ITALY

Addresse de visite : DPV, km Route de Rufisque, Dakar
Tel : (221) 834 42 94 - 834 12 83 Fax : 834 42 90
E-mail : locustox@metissacana.com

TABLE OF CONTENTS

Preface

CHAPTER		Page N°
CHAPTER 1		
	ENVIRONMENTAL EFFECTS OF CHEMICAL LOCUST CONTROL *JAMES EVERTS & LAMINE BÂ*	
	Introduction	1
	Risks Assessment	3
	Summary of results	7
	Ecosystem at risk?	10
	Conclusion	11
	References	12-14
CHAPTER 2		
	EFFECTS OF EXPERIMENTAL LOCUST CONTROL WITH FENITROTHION AND DIFLUBENZURON ON THE AQUATIC INVERTEBRATE FAUNA OF TEMPORARY PONDS IN CENTRAL SENEGAL *JOOST LAHR & ALPHA OUMAR DIALLO*	
	Summary	15
	Introduction	16
	Study area, material & methods	18
	Results	25
	Discussion	50
	Conclusion	55
	Recommendations	57
	References	59-60

CHAPTER 3

THE EFFECT OF FENITROTHION AND DIFLUBENZURON ON NATURAL ENEMIES OF MILLET PESTS IN SENEGAL. (THE 1991 STUDY).
HAROLD VAN DER VALK & OUSMANE KAMARA.

Summary	61
Introduction	62
Study setup & methodology	66
Results	71
Discussions	89
Conclusions	95
References	96-98
Annexes	99-106

CHAPTER 4

IMPACT OF FENITROTHION APPLICATIONS ON NATURAL MORTALITY OF GRASSHOPPER EGGPODS IN SENEGAL. (1991 TREATMENTS).
ABDOULAYE NIASSY, ALIOUNE BEYE & HAROLD VAN DER VALK.

Introduction	107
Materials and methods	108
Results	110
Discussion & Conclusions	118
References	119
Annexes	120-122

CHAPTER 5

A LABORATORY TOXICITY TEST WITH *BRACON HEBETOR* (SAY) (HYMENOPTERA, BRACONIDAE) -FIRST EVALUATION OF REARING AND TESTING METHODS.
HAROLD VAN DER VALK , JAN VAN DER STOEP , BABA FALL & ELOI DIEME.

Summary	123
Introduction	124
The rearing of *Bracon hebetor* on *Ephestia kuehniella*	125
The effect of Fenitrothion on *Bracon hebetor*	139
Discussions & Conclusions	145
References	148-150
Annexes	151-154

CHAPTER 6

TOXICITY TESTS WITH FENITROTHION ON *PIMELIA SENEGALENSIS* & *TRACHYDERMA HISPIDA*. (COLEOPTERA, TENEBRIONIDAE).
ABDOULAYE DANFA & HAROLD VAN DER VALK .

Summary	155
Introduction	156
Materials & Methods	157
Results	159
Discussions & Conclusion	162
References	165-166
Annexe	167

CHAPTER 7

TOXICITY TESTS WITH *METARHIZIUM FLAVOVIRIDE* (DEUTEROMYCETES, MONILIALES) ON *BRACON HEBETOR* (HYMENOPTERA, BRACONIDAE), *PIMELIA SENEGALENSIS* AND *TRACHYDERMA HISPIDA* (COLEOPTERA, TENEBRIONIDAE).
ABDOULAYE DANFA .

Summary	168
Introduction	169
Study Methodology	170
Results	173
Discussion & Conclusions	175
References	176
Annexes	177-181

CHAPTER 8

DEPOSITION AND DEGRADATION OF FENITROTHION AND DIFLUBENZURON ON VEGETATION AND IN TEMPORARY PONDS IN A SAHELIAN AREA (1991 CAMPAIGN).
BABA GADJI.

Summary	182
Introduction	183
General elements of the treatments	184
Sampling	186
Analysis of the samples	188
Results	190
Comparison with other results & standards	203
Conclusions	207
References	208
Annexe	209-210

CHAPTER 9

DEPOSITION AND DEGRADATION OF FENITROTHION AND DIFLUBENZURON ON VEGETATION AND IN THE SOIL IN SENEGAL & RESIDUE FOLLOW-UP IN MILLET STORAGE IN RURAL AREAS (1992 CAMPAIGN).
BABA GADJI .

Summary	211
Introduction	212
Materials & Methods	213
Results	220
Discussion	241
Conclusions	245
References	246
Annexe	247

PREFACE

The present volume is a compilation of technical reports of the Project ECLO/SEN/041/NET "LOCUSTOX" which have been issued in the years 1993 and 1994. The studies presented concern various aspects of the environmental side-effects of chemical locust and grasshopper control.

Chapter 1 is an original text. Chapters 2-9 have been issued earlier as separate reports, which were coded as follows:

Chapter 2 : Report 93/3

Chapter 3 : Report 93/2

Chapter 4 : Report 93/1

Chapter 5 : Report 94/1

Chapter 6 : Report 93/6

Chapter 7 : Report 94/2

Chapter 8 : Report 93/4

Chapter 9 : Report 93/5

Both the present volume and the reports are available in french and english versions.

The reports of the studies carried out in 1994-1997 will be issued in a second volume.

CHAPTER 1 :

Environmental Effects of Chemical Locust Control.

INTRODUCTION

Toxic chemicals are still an essential weapon in the war against locusts. Despite recent progress in non-chemical methods (Lomer & Prior 1991, CILSS 1994, Krall & Wilps 1994) insecticides provide the only solution for large scale infestations. The combination of large scale treatments and the relatively high dosage required for effective control results in large amounts pesticides being used (Table 1.1).

Table 1.1 : Areas treated with chemical pesticides for locust control from late 1992 to the end of December 1994. (Source : FAO , 1995c).

Country	Treated surface (ha)
Saudi Arabia	1,705,992
Mauritania	910,487
Pakistan	316,979
India	311,372
Sudan	281,065
Senegal	253,074
Yemen	156,650
Morocco	41,337
Eritrea	27,570
Egypt	20,400
Algeria	15,694
Oman	10,900
Other (9 countries)	1,549
Total	4,053,256

The chemicals are applied in a wide variety of landscapes. In recession areas, where the locusts are endemic, it concerns primarily "green islands" in a barren environment, such as wadis and around oases. Plagues, on the other hand, which arise after the gregarization of the otherwise solitary species, invade all possible habitats: cultivated and inhabited areas as well as pastures, forests and wetlands. Because of infrastructural limitations, the use of less discriminate spraying methods, e.g. fixed wing aircraft, is often unavoidable. Futhermore, in plague emergencies extra personnel may be employed that are poorly trained in pesticide application. The most selective pesticides may also not be available. In arid and semi-arid zones, the areas sprayed for locusts, and where environmental effects are likely to occur, are often those with high biological activity because rain has fallen there. They are also likely to be areas in which human activity is concentrated and crop protection is important. The environmental risk of chemical locust operations, therefore, is a matter of international concern (FAO 1989, USAID 1991). For that reason, FAO and the Senegalese Plant Protection Service started a progamme ("LOCUSTOX") of which the principal objectives are to describe the direct and indirect effects caused by the treatments; to

develop methods for the prediction of hazard in the ecosystems concerned; to monitor operations; to train scientific and field operations personnel and decision-makers in safe handling of pesticides and hazard assessment respectively. In this issue, the results are presented of the years 1991 to 1993. The studies carried out from 1994 to 1996 will be published in a following issue.
Other similar studies have been carried out by South African (Stewart *et al.* 1995), American (Dynamac 1988a,b ; Keith 1992), German (Müller 1988), Norwegian (Ottesen *et al.* 1989, 1990), French (Balança & De Visscher 1992, Rachadi *et al.* 1995) and Ethiopian (Muinamia & Megenasa 1995) research teams. Compared to these studies which have all been exclusively field-based, LOCUSTOX is the only one which involved both field and laboratory studies and which includes aquatic studies.

In locust control there are factors that mitigate the risk of environmental side-effects. Most communities of organisms in arid zones are adapted to highly variable physical conditions (Bourlière 1983, Polis 1991) and may therefore exhibit a remarkable resilience to disturbance. The fact that locust interventions are rarely repeated at the same place over consecutive seasons enhances the chance of recovery from damage, provided unaffected sources for recolonisation (refugia or non affected dormant stages) are available. High UV radiation and elevated temperatures also accelerate degradation of the pesticides used. Despite the limited training possibilities in an emergency situation, the more regular treatments especially in recession areas, are carried out by professionals who are well aware of the risks involved.

RISK ASSESSMENT

The pesticides discussed in this paper are to those which have been judged effective against locusts at a given field dose by an independent expert panel which advises FAO (Pesticide Referee Group, FAO, 1995a).

The hypothetical hazard of the pesticides used in locust control can be derived through various models. We summarize toxicity data available from the literature in Table 1.2. (With the exception of human hazard, the scaling used do not represent an internationally adopted classification). These data from laboratory based studies clearly indicate a general potential hazard for aquatic invertebrates. Fenitrothion is specifically toxic to bees and may also represent a hazard to birds, as is the case with bendiocarb. Chlorpyrifos and lambdacyhalothrin show the highest fish toxicity. On the other hand, the low vertebrate toxicity of diflubenzuron is striking. We will discuss below the toxic effects in these risk groups as observed under field conditions.

Table 1.2 : Indicative classification of insecticides used for locust control[1].
Humans* : 0 = Non-Hazardous[2], 1 = Slightly Hazardous, 2 = Moderately Hazardous, (following IPCS/WHO classification Class IV-II). Others** : 0 = Very Slightly Toxic, 1 = Slightly Toxic, 2 = Moderatly Toxic, 3 = Highly Toxic, 4 = Extremely Toxic (classification according to Canton et al. (1991).
- = insufficient data

Pesticide	Classification				
	man mammals	birds	fish	aquatic invertebrates	bees
bendiocarb	2	4	2	4	3
chlorpyrifos	2	2	3/4	3/4	2
deltamethrin	1	0/1	3	3/4	2
fenitrothion	2	3	2	3/4	4
lambdacyhalothrin	1	1	4	-	0/1
malathion	1	1/2	3	3/4	3/4
diflubenzuron	0	0	0	4	0/1

* = formulated product; ** = technical product

[1] These pesticides have been recognized by FAO to be effective against locusts at specific field dose rates.
[2] Unlikely to produce non-desired effect on human health when used according to good agricultural practice.

Human exposure

There are three principal human risk groups: the personnel handling the pesticides, persons exposed to the treatments and the consumers of contaminated food products. In locust control, the first is the most important. As early as 1976 the exposure of spray team members has been assessed by MacCuaig (1976) and recent studies have been carried out in Senegal. Toxic effects were monitored in operators of vehicle mounted devices (ULVA mast and airblast-cannons). The latter was found the most hazardous device for operators, even those trained in the safe handling of pesticides. (The details of this study will be published elsewhere). By comparison, ground teams for aerial operations appeared to work with utmost care, whenever witnessed by us.

For the second risk group, i.e. inhabitants or passersby in areas that are treated by air, a hazard may be present when they are directly exposed to spray.

If we estimate the relative risk for this group, using only dermal toxicity data (Walker & Keith 1994, Tomlin 1994) and taking the field dose into account, we may conclude that pyrethroids and diflubenzuron present a low risk but carbamates and organophosphates score higher.

Contaminated crops represent a risk to consumers. Although subject to rapid degradation, the residues may be unacceptably high shortly after spraying (Chapter 9).

During the campaings of 1988-1995 in Senegal and Mauritania, no cases of poisoning of inhabitants have been reported despite the fact that in both countries substantial amounts of the higher ranking compounds have been used. This is probably due to two factors: inhabitants are warned by radio not to enter the treatment zones and pilots take care not to overspray inhabitants (Source : Plant Protection Services and local press).

Animal husbandry

There are two risk groups in domestic animals: those directly exposed to a spray and those feeding on contaminated vegetation or fodder. Very often, herds are not removed from areas to be sprayed, even after extensive warning. The dosages to which the animals are maximally exposed may pose a hazard (ref: human exposure). Contamination of forage grass or fodder of fenitrothion and diflubenzuron may give rise to withdrawal periods of over two weeks (Chapter 9). Although complaints occasionally reach the authorities after treatments, no proven cases of intoxication of grazing animals have been reported, the reported symptoms (abortions) not being attributable to the pesticides used. (This observation is limited to Senegal and Mauritania, over the period 1988-1995. Source : veterinary services).

Wildife

Aquatic fauna

Although most operators (both aerial sprayers and ground teams) avoid spraying open water as much as possible, the risk of contamination is real, and is substantiated by reports on fish kills (Lahr pers. comm.). Fish kills are likely to be reported, although they reflect often the less sensitive part of the aquatic fauna (Table 2). Most sensitive is the invertebrate community: crustaceans and insects. Experimental treatments have demonstrated a devastating effects in these groups (Chapter 2; Lahr & Banister, in press). The risk of unacceptable damage largely depends on the type of water. Three main types have to be considered: temporary pools, perennial standing water, and running waters.

The fauna of temporary pools consists of organisms with a rapid lifecycle (mostly crustaceans), of immigrants (flying insects) and of a few vertebrates with a dormant stage in the dry period (toads, tortoises and (rarely) lungfish). Side-effects of locust treatments on invertebrates have been presented in Chapter 2 and Lahr *et al.* (1995) in experiments with fenitrothion, diflubenzuron, deltamethrin and bendiocarb. The primary concern for these waters is the risk of wiping out dormant or active stages of sedentary organisms, resulting in long-term disruption of the population. Despite some devastating acute effects, long-term effects have not been demonstrated. The latter observation may be explained by the fact that the populations concerned are adapted to erratic physical conditions. For instance, during the wet season, only part of a population may hatch or become active, conserving another part for a later opportunity. This strategy of risk spreading is known for many (semi-) arid zone species (Polis 1991).

In perennial stagnant waters often the presence of fish implicates an entirely different invertebrate community. Tests carried out in these waters (Lahr & Banister in press) demonstrated a certain risk for small fish species, added to effects on invertebrates. Of the recommended insecticides tested, i.e. fenitrothion, chlorpyrifos and diflubenzuron only chlorpyrifos proved toxic to fish.

Running waters generally harbour not only a rich fish fauna but also larger crustaceans, specifically shrimps, some of which may be of economic importance. Shrimps are known to be sensitive to

insecticides, in particular to pyrethroids (Hill 1989, Everts 1983). Lahr & Banister (in press) demonstrated that two common Sahelian species (i.e. *Palaemonetes africanus* and *Caridina africana*) are also at risk after treatments of fenitrothion and chlorpyrifos. Running water, however, is unlikely to be contaminated by locust treatments to an extent that the survival of populations is at risk. Locust treatments take place mostly in the wet season when waters are high, thus eliminating the risk of wiping out remnant communities in dry season pools (an important reservoir for recolonisation of the lotic fauna).

The field research referred to above, has been restricted to Senegal. Superficial observations were made during monitoring missions in Mauritania in 1995 (Diallo pers. comm.). No data are available from other countries. It is, therefore, difficult to estimate the risk of aquatic side effects of campaigns in the whole invasion area. The data allow us to compare the risk of pesticides and, to a limited extent, extrapolate to a given situation outside Senegal, because the species at risk occupy in general a large geographical range over the semi-arid area of sub-saharan Africa, north of the equator.

Birds

Birds are at risk in areas treated against locusts (Mullié & Keith 1993). The effects, however, are generally limited to individual cases of poisoning, rather than affecting populations. In many cases no effects were observed at all (Dynamac 1988a,b, Balança & De Visscher 1992, Keith 1992), although the methodology of these studies does not exclude false negatives. The risk for birds includes both direct intoxication and food deprivation. Exposure to the spray is supposed to be hazardous when toxic amounts are ingested through preening (Driver *et al.* 1991). Uptake of contaminated food is another important risk factor: debilitated or dead insects are an easy prey. Although not quantitatively proven, this route of exposure is considered most important. Lasting effects on bird populations have yet to be demonstrated. It has been suggested that the decline of white stork *Ciconia ciconia* in western Europe since the 1950s may be related to locust operations depriving migrant stork of an important source of food (Goriup & Schultz 1991), but the data presented do not support this conclusion (Mullié *et al.* in press).

Reptiles and amphibians

Although reptiles and amphibians play a key role in arid ecosystems (Cloudsley-Thompson 1991; Vitt 1991) studies including these taxa are rare. Stewart *et al.* (1995) studied the effect of deltamethrin on the activity of two lacertid species. He related the absence of an effect partly to the fact that sprays are usually applied during the hours of inactivity of the lizards. Direct toxic effects are unlikely to occur. On a few occasions, tadpoles exposed to experimental sprays did not appear sensitive (Lahr pers. comm.). Poisoning through uptake of contaminated food (the vast majority of species are insectivorous and/or carrion eaters) is a risk factor which has yet to be studied. The lack of information on this group is a serious omission which will be addressed in the near future (FAO 1995b).

Mammals

The insecticides used in locust control have been selected for low mammalian toxicity (Table 2). Side-effects, if any, are to be expected among the insectivores. Only in rodents have indications of toxicity been demonstrated (Keith 1992). The hazard to grazers, including animal husbandry, has been discussed above.

Terrestrial invertebrates

Insecticides are meant to kill insects though preferably only the target species. Specific acridicides have not yet been developed. The relatively high dosages of insecticides required for locust control represent a high potential risk for non-target insects. This has been confirmed in all field studies carried out thus far (Chapters 3 to 7, Müller 1988, Ottesen 1990, Dynamac 1988a,b, Keith 1992 , Everts 1990, Muimania & Megenasa 1995, Rachadi *et al.* 1995). Nevertheless, there is a sharp differentiation between the various pesticides with respect to the taxa and, more important, the functional groups that are affected.

The functional groups we are primarily interested in are : pollinators, natural enemies of locusts and other pests, and insects essential for maintaining soil functions.

Pollinators

In the most elaborate bee study carried out in relation with locust control, Keith (1992) showed that bees can be seriously affected by malathion. Dichlorvos, tested in the same study, did not have this effect (it should be noted that both pesticides were strongly underdosed). Van der Valk (1990) found a 95% reduction in halictid bees after an experimental treatment with chlorpyrifos, applied at 160% of the recommended dose.

Antagonists of pest species

Of all side-effects, those on the natural enemies of pest species have most extensively been studied (Chapters 3 to 7, Müller 1988, Ottesen 1990, Dynamac 1988a,b, Keith 1992, Everts 1990). In most cases, effects were described on family or order level, showing strong indications for potential risk to beneficial species. Serious effects are observed on species, which sometimes correlate with the upsurge of secondary pests (Chapter 3, Kamara & van der Valk 1995). The latter authors also demonstrated a striking difference in these effects between various recommended insecticides (Table 4).

Soil invertebrates

Ants and termites are essential for the productivity of tropical and semi-arid soils (Polis 1991). In many places tree growth is directly related to termite activity, aiding root penetration and transporting organic material into the subsoil. Side-effects on ants have been observed by various authors (Keith 1992, Ottesen & Sømme 1990, van der Valk 1990), malathion and fenitrothion being especially harmful. The few observations made on termites indicate a hazard of the latter insecticide to this group as well (Everts 1990, van der Valk pers comm.).

Key Soil Processes

Studies on key soil processes such as respiration (Müller 1988, Grant 1990), nitrification and chlorophyll production (both by Grant 1990) showed only marginal disturbances, even at high dosages. The insecticides tested (i.e. fenitrothion, chlorpyrifos, diflubenzuron and deltamethrin) may be considered not harmful for key soil processes, under the given circumstances.

SUMMARY OF RESULTS

In Table 1.3 we summarize the side-effects of chemical locust control presented in this paper. Of this summary and the literature reviewed above, a number of conclusions can be drawn.

1. All current insecticides present a risk to non-target organisms.
2. The groups of organisms at risk differ for each insecticide.
3. Long-term effects are rare and, as far as known, limited to disturbances of inter-species relationships within insect communites.
4. Terrestrial vertebrates are at risk as individuals but the survival of populations does not seem to be threatened.
5. Fish are at risk when chlorpyrifos is used near open water.
6. Aquatic invertebrates are sensitive to all recommended locust pesticides.
7. Terrestrial invertebrates are at risk in all cases, sometimes leading to undesired effects (i.e. upsurges of secondary pests).

Table 1.3 : Relative hazard to non-target organisms of pesticides used for locust control[1]

+ = effect observed/slightly toxic ; ++ = strong effect observed/highly toxic ; **bold** = observed in field and laboratory experiments ;
- = no effect observed/non-toxic ; blank = no observation ; underlined = non-experimental field observation ;
normal print = observed in either field or laboratory experiments.

Non-target Group or Species	Fenitrothion	Chlorpyrifos	Bendiocarb	Delta-methrin	Diflu-benzuron	Malathion
Birds	+	+				
Fish	-	+, ±			-	
Aquatic Invertebrates						
Zooplankton						
Cladocerans (water fleas)	++	+	++	++	++	
Copepods	-	-	-	-	-	
Ostracods (seed shrimp)	-	-	-	-	-	
Macrocrustaceans						
Anostraca (fairy shrimp)	-		-	++	++	-
Decapoda (shrimp)	++	++			-	
Hemipterans (water bugs)						
Notonectics (backswim-mers)	++	++	-	++	-	++
Corixids (waterboat men)	++	+	-	++	-	
Gerridae (water striders)	++	++	+	+	-	
Coleopterans						
Dytiscidae	++	++	-	++	-	
Hydrophilidae	++		+	++	-	
Odonata (dragonflies & damselflies)						
Larvae	+		+	+		
Diptera						
Chironomide larvae	-	+				

Table 1.3 : (continued)

Non-target Group or species		Fenitrothion	Chlorpyrifos	Bendiocarb	Deltamethrin	Diflubenzuron	Malathion
Terrestrial Invertebrates							
	Grasshopper egg pods, natural mortality	-					
Coleopterans							
	Tenebrionids	++	-	+	-	-	
	Carabids	-	+			+	
	Coccinellids	++	+	++	++		++
Hymenopterans							
	Braconids	++	+	+	++	++	
	Ichneumonids	+	-		++	++	
	Sphecids	+	-			+	
Beneficial Diplerans		+	-			-	
Termites		+		-		+	
	Millet Headminer (resurgence)	++	++		-	-	

ECOSYSTEMS AT RISK ?

In order to appraise the risk posed by chemical locust control to the ecosystems in the invasion area, we should compare the scale and intensity of treatments to the surface area covered by the ecosystems or communities concerned. It is clear that locally treatments may have a deleterious effect, specifically on invertebrates, sometimes resulting in long-lasting disturbances in numerical relationships. However serious these effects may be, they are often characterized by their limited scale when compared to the size of the ecosystem concerned. For example, in the desert the majority of wadis in a certain area may be treated and, thus, damaged. However, not all wadi's in the invasion area are likely to be treated. In an extremely serious upsurge not more than an estimated 10 % of the productive (green) area is infested. Recovery will always occur, albeit in some cases slowly.

This holds for the terrestrial and for temporary aquatic communities. Perennial aquatic communities in arid areas, on the other hand, may harbour entirely isolated relict populations (e.g. aquatic tortoises in oases). Local extinction in these cases could affect biodiversity in the Desert Locust area. Fortunately, this risk is limited. Local operators are in most cases very well aware of the risk of contaminating open waters. Open water is drinking water and its contamination may have serious consequences for local authorities. In the few reported cases of serious contamination, foreign applicators were involved. It is, therefore, essential that the selection of spray companies by donors be based on quality rather than cost.

Although recent research has revealed some of the hazards related to chemical locust control the larger part of potential risk is still unknown. We know for instance virtually nothing about the risk to reptiles and insectivorous mammals. The mechanisms of observed disturbed interspecies interactions in invertebrate communities are unknown, which hinders extrapolation to other situations. We know that ants are at risk. We do not know, however, which species are affected and more important, the ecosystem consequences of a temporary decrease in activity. Furthermore, in respect of resilience of communities, we still refer to qualitative or phenomenological observations. Unless the underlying processes have been studied, recovery rates cannot be predicted and the factor "recovery" cannot be used for risk assessment.

Wherever insecticides are used, humans are at risk. The most important risk group, the persons handling the chemicals, can be trained and provided with protective material at low cost. Nevertheless negligence remains a problem and consequently contamination and accidents will sometimes occur.

CONCLUSION

In the absence of selective acridicides, chemical control of locust still presents a hazard to human health and the environment. The risk of undesired side-effects can be mitigated by a proper choice of pesticides applied under specific circumstances (e.g. near open water, in cultivated and inhabited areas, or over uninhabited rangeland). Guidelines for such a differential use should be based on thorough risk assessment using data from literature combined with the field data reviewed in this paper. Furthermore, in the field, the hazard can be much reduced by more judicious spraying; i.e. treating only when economically justified and adhering strictly to the recommended dosages. This can only be achieved by proper training of decision-makers and operators, combined with monitoring of field operations.

REFERENCES

Balança G & De Visscher M-N (1992) Les effets sur l'environnement des traitements insecticides contre les criquets ravageurs. CIRAD-GERDAT-PRIFAS, Document 457, Montpellier, France.

Bourlière F (Ed) (1983) Ecosystems of the world. 13. Tropical Savannas. Elsevier, Amsterdam.

Canton JH, Linders JBHJ, Luttik R, Mensink BJWG, Panman E, Van de Plassche EJ, Sparenburg PM & Tuinstra J (1991) Catch-up operation on old pesticides : an integration. Report RIVM 678801002, Bilthoven, The Netherlands.

Carruthers GF, Hooper GHS & Walker PW (1993) Impact of fenitrothion on the relative abundance of diversity of non-target organisms. In : Corey SA et al. (Eds) 1993 "Pest control & Sustainable Agriculture" CSIRO, Canberra Australia.

CILSS (1994) Troisième séminaire international sur la lutte intégrée contre les ennemis des végétaux au Sahel, Dakar 1-9 Avril 1994. Résumés : Acridiens. (Comité Inter-Etats de lutte contre la Sécheresse au Sahel). Institut du Sahel, Bamako, Mali.

Cloudsley-Thompson JL (1991) Ecophysiology of Desert Arthropods and Reptiles. Springer Verlag, Berlin.

Danfa A (1994) Tests de toxicité de *Métarhizium flavoviride* (Deuteromycetes : Moniliales) sur *Bracon hebetor* (Hymenoptera : Braconidae) *Pimelia senegalensis* et *Trachyderma hispida* (Coleoptera : Tenebrionidae). Report FAO Project GCP/SEN/041/NET, FAO, BP 3300, Dakar, Senegal.

Driver CJ, Ligotke MW, van Voris P, McVeety BD & Brown DB (1991) Routes of uptake and their relative contribution to the toxicologic response of northern Bobwhite (*Colinus virginianus*) to an organophosphate pesticide. Environ Toxicol Chem 10 : 21-33.

Dynamac (1988a, 1988b) Results of the Mali Pesticide Testing Trials against the Senegalese grasshopper: Final Report, USAID, Washington DC.

Everts JW (1983) Animal indicators for side-effects of chemical vector control. Environ monitor Assess 3 : 229 - 235.

Everts JW (Ed) (1990) Environmental effects of chemical locust and grasshopper control. A pilot study. Project Report FAO, ECLO/RAF/001/NET. FAO, Rome.

FAO (1989) Report of the working group on environmental side-effects of Desert Locust Control, 14-16 February 1989. FAO, Rome.

FAO (1995a) Evaluation of field trial data on the Effectiveness of insecticide against locust & grasshopper. Rep. Pesticide Referee Group. FAO, Rome, 8 pp + app.

FAO (1995b) Plan d'Opération. Document de Projet GCP/SEN/041/NET, Food and Agricultural Organization, Rome, Italy.

FAO (1995c) Report of the thirty third meeting of the Desert Locust Control Committee, Rome, Italy, 16-20 January 1995. FAO, Rome.

Goriup PD & Schultz H (1991) Conservation management of the white stork : An international need and opportunity. In : Salathé T (Ed) Conserving Migratory Birds, ICBP Tech. Publ. n° 12, Cambridge, UK.

Grant IR (1990) Key Soil Processes. In : Everts Ed (1990) Environmental effects of chemical and grasshopper control. A pilot study. Project Report FAO, ECLO/RAF/001/NET. FAO, Rome.

Hill IR (1989) Aquatic organisms and pyrethroids. Pesticide Science 27 : 429-459.

Kamara O & Van der Valk H (1995) Side-effects of fenitrothion and diflubenzuron on beneficial arthropods in millet in Senegal. (The 1992 study). Report FAO Project GCP/SEN/041/NET. FAO, BP 3300, Dakar, Senegal.

Keith JO (1992) Effects of experimental applications of malathion and dichlorvos on populations of birds, mammals, and insects in Southern Morocco. Research Report. Consortium for International Crop Protection. Denver Wildlife Res. Centre, Denver, USA.

Krall S & Wilps H (Eds) (1994) New trends in locust control. Schriftenreihe der GTZ n° 245. Eschborn, Germany.

Lahr J & Banister K (in press) The impact of chemical desert locust control on the aquatic fauna of the Senegal River System. In: Remane K(Ed) African Inland Fisheries, Aquaculture and the Environment. Fishing News Books Publ.

Lomer CJ & Prior C (1991) Biological control of locusts and grasshoppers. CAB Int., Wallingford UK, 394 pp.

MacCuaig RD (1976). The occurence of insecticides in the blood of staff of a locust control organization. Bull Env Cont Toxicol 15 (2) 162-170.

Muinamia CK & Megenasa T (1995) Observations on the environmental impact of fipronil on non-target organisms on the Desert Locust habitats of Eastern Ethiopia. DLCO-EA, Nairobi.

Müller P (1988) Ökotoxikologische Wirkungen von chlorierten Kohlenwasserstoffen, Phosphorsäure-estern, Carbamaten und Pyrethroiden im nordöstlichen Sudan. Inst f Biogeographie, Univ des Saarlandes, Saarbrücken, Germany.

Mullié W, Browner J & Scholte P (in press) Numbers, distribution and habitat of wintering white storks Ciconia ciconia in the East-Central Sahel in relation to rainfall, food and arthropogenic influences. In: Biber O & Salathé T (Eds) Proceedings of the International Symposium on White Storks (Western Population). Basel, Switzerland 7 - 10 April 1994.

Mullié WC and Keith JO (1993) The effects of aerially applied fenitrothion and chlorpyrifos on birds in the savannah of northern Senegal. J Appl Ecol 30 : 536-550.

Ottesen P, Fossland S, Johannessen B & Simonsen JH (1989) Taux réduits de fénitrothion : l'effet sur *Oedaleus senegalensis* (Orthoptera) et sur les arthropodes non-visés au Mali, Afrique de l'Ouest. Rapport destiné au Ministère Royal Norvégien de la Coopération du Développement. Université de Oslo, Inst. de Biologie, Blindern, Norvège.

Ottesen PS & Sømme L (1990) The impact of three pesticides, fenitrothion, lambdacyhalothrin and teflubenzuron, on grasshoppers and non-target organisms in Mali, West Africa. Univ. of Oslo, Dept. of Biology, Blindern, Norway.

Polis GA (Ed) (1991) The ecology of desert communities. University of Arizona Press, Tucson, USA.

Rachadi T, Balança G, Duranton J-F & Foucart A (1995). Les effets du fipronil sur Schistocerca gregaria (Forskäl 1775), divers sauteriaux et la faune non-cible. CIRAD-GERDAT-PRIFAS, Document 513, Montpellier, France.

Stewart DAB, du Preez T & Price RE (1995) Environmental impact of deltamethrin on non-target organisms in the Karoo. Agricultural Research Council - PPRI, Repport 05: 7/95, Pretoria, SA.

Tomlin C (Ed) (1994). The pesticide manual 10th Ed. British Crop Protection Council, The Royal Soc. of Chem., UK.

USAID (1991) Environmental concerns in AID programs for Locust and grasshoppers Control. USAID-AFR TR/ANR. Washington DC 20523, USA.

Van der Valk HCHG (1990) Beneficial arthropods. In: Everts (Ed 1990) Environmental effects of chemical locust and grasshopper control. A pilot study. Project Report FAO, ECLO/SEN/003/NET. FAO, Rome.

Van der Valk H & Kamara O (1993) The effect of fenitrothion and diflubenzuron on natural enemies of millet pests in Senegal. Report FAO Project ECLO/SEN/003/NET, FAO, BP 3300, Dakar, Senegal.

Vitt LJ (1991) Desert Reptile Communities. In: Polis GA (Ed). The Ecology of Desert Communities. Univ of Arizona Press, Tucson, USA.

CHAPTER 2:

Effects of experimental locust control with fenitrothion and diflubenzuron on the aquatic invertebrate fauna of temporary ponds in central Senegal

SUMMARY

Sixteen temporary ponds in the area around Nioro du Rip in the semi-arid zone of central Senegal were selected for investigations on the side-effects of pesticides used in Desert Locust control. They were monitored during two consecutive rainy seasons in 1991 and 1992.

In September 1991 five ponds were treated with the Insect Growth Regulator diflubenzuron, a benzoyl-urea, at the suggested dosage against hopper bands. Five others were treated with the organophosphate fenitrothion, used for both hopper and swarm control, while the six remaining untreated ponds served as controls.

The average applied dosages for diflubenzuron and fenitrothion were 75 and 506 g a.i./ha respectively. Initial average concentrations of the products in the aqueous phase of the water were 10 µg/l for diflubenzuron and 80 µg/l for fenitrothion. Dissolved diflubenzuron was no longer detectable after 24 hours. Fenitrothion was more persistent in the water with an average half-life of 43 hours.

Diflubenzuron had a significant effect on populations of the branchiopods *Streptocephalus* spp. (Anostraca) and *Diaphanosoma* sp. (Cladocera). Both species were virtually eradicated and did not recover before dessication of the ponds in December 1991. However, they reappeared in the rainy season of 1992. A calanoid copepod, *Paradiaptomus rex*, considerably increased in number following the diflubenzuron treatments, possibly due to the elimination of the branchiopods. Other species of Cladocera, Copepoda, Ostracoda and different aquatic insects were not significantly affected.

Among the different crustaceans, only the cladoceran *Diaphanosoma* sp., was significantly reduced by fenitrothion, but the populations recovered after one month. *Paradiaptomus rex* also increased in number after these treatments. On the other hand, fenitrothion had a much more negative impact on aquatic insects, notably Hemiptera and Coleoptera. Many of these species were found dead floating on the surface of the ponds shortly after the applications. The very numerous *Anisops* species (Hemiptera, Notonectidae) recovered quickly (after one and three weeks for adults and nymphs respectively), probably by migration of flying adults from nearby untreated water bodies. Other affected, but less numerous species also reappeared after one to several weeks.

The possible ecological consequences of the impact of diflubenzuron and fenitrothion on temporary ponds are discussed and recommendations are given for the use of both products in Desert Locust control, improvements of the experimental methods used in this study, and possibilities for future research.

INTRODUCTION

Chemical locust and grasshopper control and the aquatic environment

Chemical locust and grasshopper control in West Africa takes place in arid and semi-arid areas. Control of the Desert Locust *Schistocerca gregaria* is carried out during recessions in the northern parts of this region, i.e. the Sahara desert, while in the case of a large outbreak treatments of swarms also take place in the Maghreb countries and the Sahel zone. Although large outbreaks occur irregularly, the areas treated and the amount of pesticides applied are considerable (Everts, 1990). Control of grasshopper species such as *Oedaleus senegalensis* is much more common and regular, especially in the Sahel region during the rainy season. Both groups can be treated with the same pesticides, but dose rates are usually higher for locust control than for grasshopper control. The products used include organophosphates, carbamates, pyrethroids and mixtures of these different compounds. The Food and Agricultural Organization of the United Nations (FAO, 1992) supplies a list of products and their advised dose rates to control Desert Locust. For grasshopper control indicative dosages are regularly updated by the "Organisation Commune de Lutte Antiacridienne et Anti-aviaire" (OCLALAV), prescribed by pesticide manufacturers or determined by the users themselves (e.g. the countries involved). Research on biological control alternatives is currently being carried out, but it seems unlikely that these methods could be used in the near future.

In dry environments such as the Sahara desert and the Sahel region the availability of water is of the utmost importance. Various types of waterbodies are used for transport, fishing, irrigation and as a source of drinking water for the population and their livestock. These aquatic habitats also attract a great deal of wildlife. Many wetlands in the region are considered to have a global importance (as nature preserves), especially for waterbirds.

Aquatic environments are never targeted purposely during locust and grasshopper control. However, when control measures take place in the vicinity of open waters there are several ways that contamination could occur. Larger waterbodies such as lakes, rivers, floodplains and estuaries might be exposed to downwind drift. In areas where lots of smaller waters are abundant, such as marshes, irrigation systems and small ponds, large scale operations might not be able to distinguish between the dry and wet parts of the target zone. There is a risk of direct deposition, especially in the rainy season when small temporary ponds are abundant in the savannah or cultivated areas and are aerially sprayed. Since aerial treatments are performed at a high speed over large surfaces it is virtually impossible for a pilot to locate ponds in advance or to interrupt the treatment when one is spotted ahead on the spray track. Another source of contamination of aquatic habitats by insecticides is the washing of application equipment in nearby waters, unfortunately still a rather common practice.

In general, aquatic organisms are very sensitive to insecticides. Although the actual aquatic surface area is small compared to the terrestrial environment, the effects of contamination can be more disastrous.

The 1989 LOCUSTOX pilot study

In 1989 the United Nations Food and Agricultural Organization (FAO) carried out a pilot study (Phase I) near Richard-Toll in the arid zone of northern Senegal. A preliminary investigation of the environmental impact of chemical locust and grasshopper control was made by teams representing many disciplines, including aquatic biology (Everts, 1990).

Large irrigation reservoirs received experimental aerial applications with the organophosphates chlorpyrifos and fenitrothion and with the insect growth regulator (IGR) diflubenzuron. The impact on aquatic invertebrates and fish were reported by Lahr (1990) and Banister (1990). Chlorpyrifos and fenitrothion had a significant harmful effect on two species of shrimp and some aquatic insects. Chlorpyrifos also eradicated one small species of fish. Effects on zooplankton seemed much less severe for these two compounds. Diflubenzuron, on the contrary, did not seem to affect macroinvertebrate populations or fish, but indications were obtained of effects on groups of zooplankton.

The current study

As a result of the evidence found during the pilot study, a consecutive three year phase of the project, financed by the Netherlands, was implemented by FAO and the Senegalese government in the beginning of 1991. By then the program had become known as the "LOCUSTOX" project. The project is based at the headquarters of the counterpart organization, the Senegalese Crop Protection Directorate (DPV) in the capital Dakar, but field studies are carried out at different locations in the country during the annual rainy season. The project currently includes a unit for aquatic ecotoxicological research which conducts principally invertebrate studies.

It was decided in 1991 to focus further research on a different type of aquatic environment: temporary or ephemeral ponds. These are abundant throughout the Sahel region during the rainy season between July and November, but dessicate in the early dry season. It was already mentioned that small ponds like these are likely to be hit by aerial treatments in the case of large scale operations against Desert Locust. Unfortunately these ponds have never been studied well in the past and very little background information is available on the biology, the ecology and the function of these ecosystems.

The 1991 and 1992 field study had the following objectives:

- identification of a suitable study area,
- measurements of invertebrate population densities during a pre-treatment period,
- experimental treatments with insecticides in several ponds including sufficient spatial repetitions,
- estimation of acute effects in the field by means of *in situ* bioassays with a suitable invertebrate and post-treatments searches for possible victims,
- measurement of residues of the applied insecticides and their persistence in the water,
- measurement of post-treatment densities of invertebrate populations to study possible longer term effects and the recovery rate, and
- accumulation of biological and ecological information on temporary pond ecosystems.

A suitable study area was found in the region of Nioro du Rip in the Sudano-Sahelian zone between Kaolack and the Gambian border. The rainy season in this area is more pronounced and prolonged than in the drier northern parts of the country, and therefore it presents better opportunities for detailed field research. The availability of a DPV field base and housing for the personnel was also an important consideration.

In the 1991 rainy season experimental treatments were carried out with fenitrothion and diflubenzuron. Fenitrothion is the most commonly applied product for chemical control of locusts and grasshoppers in the Sahel. It is also used against many other pests in different crops. Diflubenzuron seems a suitable and potentially less environmentally harmful insecticide to replace the internationally banned dieldrin in hopper band control. Both products had also been tested in the 1989 pilot study.

The 1992 rainy season was used for ecological and recovery studies in the same ponds. Although there will be occasional references to our ecological data, this report will only deal with the study of side-effects. An analysis of biological and ecological data from the ponds will be published separately.

STUDY AREA, MATERIAL AND METHODS

Study area

Nioro du Rip (13°45N, 15°46W) is situated in the center of a vast cultivated region in Senegal's "groundnut belt". Serer and Wolof farmers rely for their income on rainfed cultivation of peanuts, millet, maize, sorghum and watermelons, while Pular shepherds herd their cattle throughout the area. Bushes and uncultivated fields are scattered between the many fields and small villages. The landscape is very slightly sloping with higher grounds and depressions varying in size. The soil is usually sandy at the higher open parts, but in the depressions it is comprised of a very fine brown-red clay that turns into a thick and sticky mud after only the slightest rain.

Rain can be abundant from June until November, but the bulk usually falls in August, September and October. Rainfall is never very regular and drier periods alternate with rainy periods during this season.

It is in the depressions and flat parts of the region that temporary ponds can be found. Water is abundant during and shortly after the rainy season. In the early dry season all water evaporates and what remains are shallow bowl-shaped depressions filled with dried mud. Shrubs and taller trees provide some shade at the sides of the ponds during the rainy season when the vegetation is green. Most of the studied ponds were situated at some distance from fields. The aquatic vegetation in and surrounding the ponds is scarce, probably because of the temporary nature of the habitat. However, a few of the studied ponds developed a rich floating vegetation. Submerged waterplants were occasionaly found. Several of the ponds became wholly or partly covered with duckweeds of the genus *Lemna* after there had been water in them for some time. The effects this duckweed layer had on the abundance of some aquatic species will later be discussed in more detail. An inventory of the aquatic vegetation in temporary ponds in eastern Senegal can be found in Van den Berghen (1990).

After two weeks of surveys in the area sixteen ponds were selected for the study. Figure 2.1 shows their position, name and experimental number. The chosen ponds were large enough to prevent them from drying out during drier periods in the rainy season. They ranged in size between approximately 0.2 and 1 ha at the time of the pesticide treatments in mid-September.

Meteorology

During the field studies of 1991 and 1992 weather data were obtained from the meteorological station of the Senegalese Ministery of Rural Development and Hydrology (MDRH) in Nioro du Rip. Daily observations included rainfall, evaporation, relative air humidity and temperatures. However, it must be taken into consideration that data such as rainfall can vary considerably from one location to another in the study area.

Physical and chemical water parameters

The physical and chemical properties of the ponds were monitored every time biological samples were taken. However, on many occasions data were not available because of problems with the availability of equipment or malfunctions.

Water temperatures were measured with the devices on the pH-meter, the oxymeter or with a thermometer (accuracy 0.1°C).

In each pond one specific site was marked. On these sites the fluctuations of the water level were measured (1991 only).

Visibility through the water was assessed with a Secchi disk. Data are available for late 1991 and all of the 1992 sampling cycle.

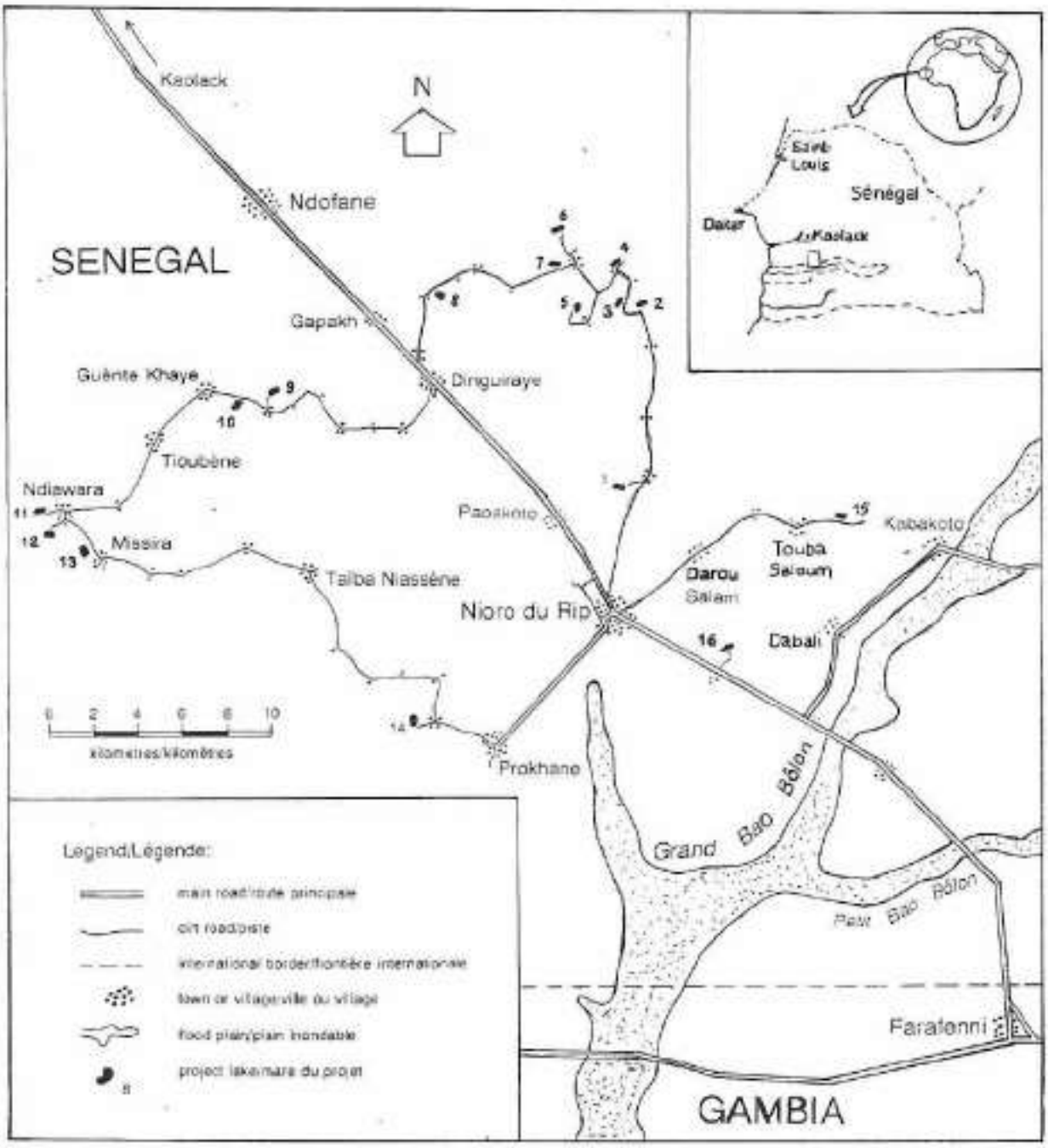


Figure 2.1: Map of the study area and location of monitored ponds. The ponds had the following local names.

1. Barkével	5. Daladam	9. Kourène	13.Alkaly
2. Koudote	6. Wimbody	10.Passoula	14.Sam
3. Sadiowar	7. Palagne	11.Mbambah	15.Gandiang
4. Counène	8. Kouthioum	12.Fana awa	16.Débreye

During the latter part of the 1992 field study a portable meter became available for water conductivity measurements.

The acidity of the surface water was usually measured with a portable pH-meter in the field, but on some occasions water samples were taken to the field station and analyzed on the same day with a laboratory pH-meter.

Dissolved oxygen (concentration and percentage saturation) was analyzed on site with portable O_2-meters.

The portable pH- and O_2-meters that were available during the early field work in 1991 started to malfunction after some time and had to be replaced. Therefore pH and O_2 data were not collected for some of the 1991 sampling period.

Pesticide treatments

Six of the selected ponds were chosen as controls and were not treated. Five ponds were sprayed on 15 September 1991 with the IGR diflubenzuron at a nominal dose rate of 60 g a.i./ha and five others on 17 September 1991 with the organophosphate fenitrothion at 450 g a.i./ha. Ponds that were treated with the same pesticide were evenly distributed among different parts of the study area. In 1992 no treatments took place.

The formulated products used were Dimilin® 450 ODC (an oil dispersable concentrate of 450 g a.i./l diflubenzuron, manufacturer Duphar) and Sumithion® 500 ULV (an ultra low volume formulation of 500 g a.i./l fenitrothion, manufacturer Sumitomo). Dimilin was diluted in diesel fuel prior to the treatments at a ratio of 1:6.5. The concentration of the applied formulation therefore was 60 g a.i./l.

The products were applied with a portable Micro-ULVA® rotary atomizer, according to the drift spray technique used in locust control recommended by the FAO (1992). The applicator kept the atomizer 1 m above the water surface and proceeded through the water at a speed of approximately 30 m/min. Swath widths varied between 17.5 and 18.0 m for fenitrothion treatments and between 9 and 18 m for diflubenzuron treatments. The rotation speed of the atomizer disk was approximately 7,000 r.p.m. and the droplet size 70-90 µm.

Residue sampling and analysis

Surface water samples of each treated pond for the analysis of pesticide residues were taken 1, 24 and 72 hours and 1 week after the treatments in the diflubenzuron ponds and after 1, 24 and 48 hours and 1 week in the fenitrothion ponds. Five subsamples of the surface water, taken at equal distances of 10 m from the center of the ponds, were mixed in a bucket. From these mixtures a 1 liter sample was taken, transferred to the field station and preserved with acid. Purification, extraction and analyses by GLC (fenitrothion) and HPLC (diflubenzuron) were done at the DPV analytical laboratory and at GTZ in Darmstadt, Germany. Methods and materials are described by Gadji in chapter 8.

***In situ* bioassays and collection of victims**

In situ bioassays were carried out with one of the most abundant macroinvertebrates in the ponds: *Streptocephalus* spp. (Branchiopoda, Anostraca). For these tests anostracans, captured on the spot, were transferred to floating cages made of stiff mosquito netting (length x width 20 x 20 cm, height 40 cm, mesh width 1 mm). Ten specimens were put in each cage. Per pond five cages were connected to each other at distances of 1 m. The number of survivors in all of the connected cages was counted after 24 hours. Control bioassays to assess handling and cage mortality in the ponds that had to be treated were carried out before treatment from 7 to 8 September. On spray-days tests were started one hour before treatment.

One hour and 24 hours after each treatment the water surface and the sides of each pond were searched thoroughly for approximately one half hour. Any dead and floating macroinvertebrates were collected and

taken to the field station for identification.

Biological sampling procedures

For this study it was not necessary to make reliable estimates of the absolute numbers of aquatic organisms in the ponds. Ecotoxicological field studies often consider relative changes in time rather than absolute abundances. All parameters described below therefore only serve as suitable measures to investigate changes in relative abundance of different species in time.

All biological samples were taken in the center of the ponds to avoid possible interference with surrounding aquatic and terrestrial vegetation. No investigation was made of the epifytical fauna (the aquatic fauna around water plants).

Pelagic (free-swimming) macroinvertebrates were sampled with a circular scoop-net (diameter 35 cm, mesh width 1mm). One or more swaths of 10 m, dependent on the species abundance, were made with the upper part of the net approximately 5 cm below the water surface. One 10 m swath is equal to a sample of 1 m^3 water. When very low water levels occurred, samples were taken with the net partly submerged and the number of animals captured was extrapolated to 1 m^3. Captured specimens were put into bottles containing a 5% formaldehyde solution in the field for preservation. They were stored this way at the field station until the end of the rainy season. Prior to identification and counting, different groups of animals were separated and transferred into 70% ethanol.

Zooplankton samples were collected in the same way as pelagic macroinvertebrate samples, except a zooplankton net (diameter 28 cm, mesh width 250 µm) was used for this purpose. Samples of 10 m length correspond with 0.62 m^3 surface water. All zooplankton were preserved in a 5% formol solution.

The inbenthic (living in the sediment) and epibenthic (living on the sediment) macroinvertebrate fauna was sampled with a square scoop-net (32x32 cm, mesh width 1 mm) by dragging it through the top layer of the sediment (depth approximately. 5 cm). Depending on the densities of the benthic fauna, especially chironomid larvae, different swath lengths were used. Clay was washed out of the net and animals were collected in the field from the remaining detritus. A sample of 10 m corresponds with 1 m^3 of water above the sediment and 3.2 m^2 of sediment surface layer. Animal samples were preserved in the same way as the pelagic samples.

The different assessment methods are shown in Figure 2.2.

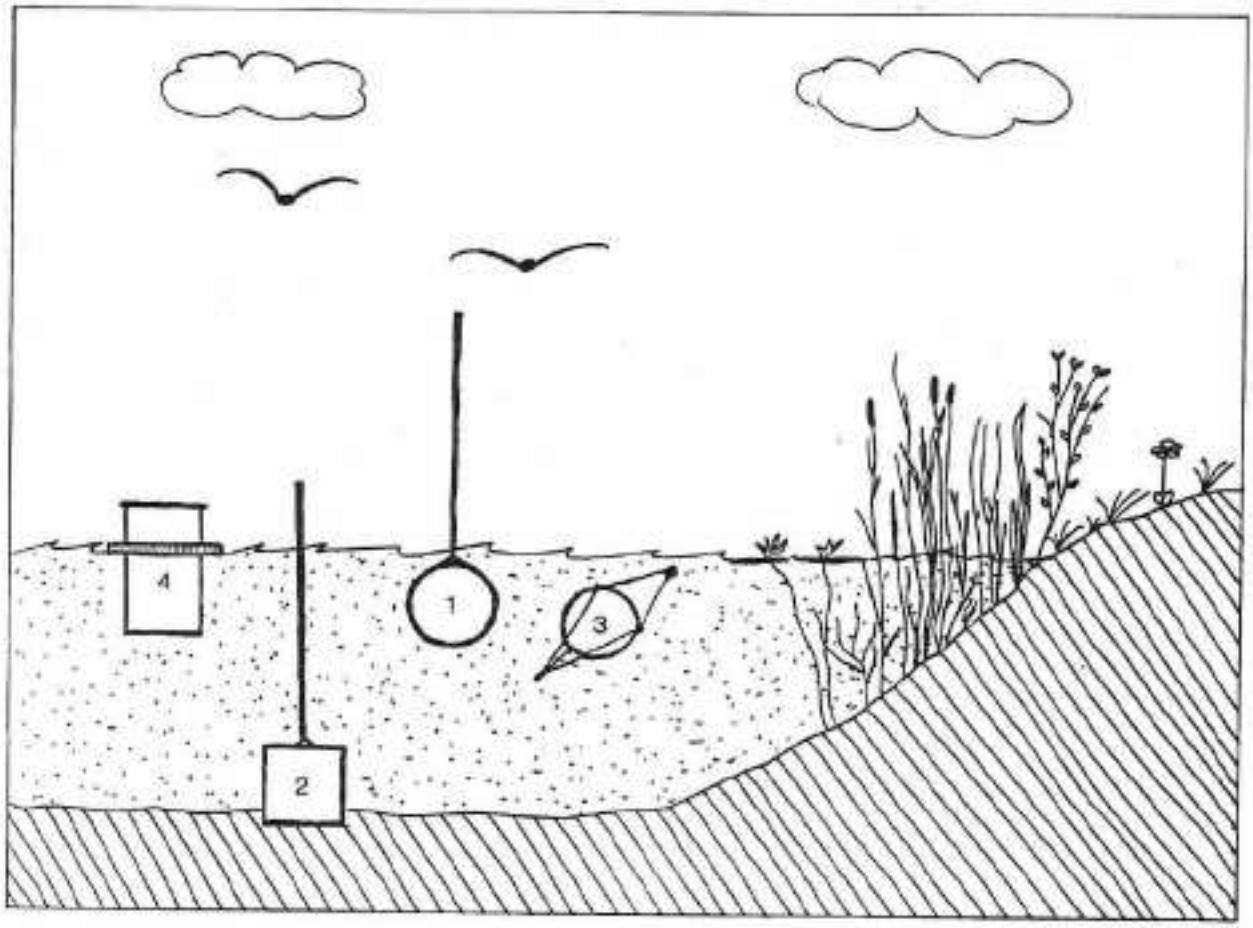


Figure 2.2 Assessment methods used for biological monitoring of the ponds: pelagic, sediment and zooplankton sampling (1,2 and 3 resp.) and a bioassay cage (4).

All densities of zooplankton and pelagic and epibenthic fauna presented in this report are expressed as numbers/m^3. Numbers of inbenthic fauna (chironomid larvae) will be expressed as numbers per m^2.

In order to improve the comparison between samples from each pond taken at different dates, sampling rounds from pond to pond were always undertaken in the same order. Consequently, all samples from the same pond were taken approximately at the same time of the day.

Biological sampling took place at bi-weekly (pelagic fauna and zooplankton) or weekly (sediment) intervals in each of the ponds during part of August, September and most of October of 1991. Two sampling rounds were undertaken in November '91, but by then many of the ponds were drying out. The results of these last two samples will not be taken into account in this study because different stages in the process of dessication caused large differences in species abundance between the ponds.

In 1992, sampling was done for a longer period. The recovery and ecological studies were started 5 days after the first rains occurred in the beginning of June and were continued until the end of November. Pelagic and zooplankton samples were taken at two-weekly intervals. Since few obvious effects of either of the two pesticides on the densities of the benthic and epibenthic fauna had been found the year before, it was decided not to carry out a strenuous and time consuming sampling of this fauna during 1992.

Statistical procedures

Residues

It was assumed that the disappearance of residues from the aqueous phase was a first order process. Therefore all measured concentrations were ln-transformed and linear regression of these data against sampling time was used where possible to calculate half-lives.

In situ bioassays

The variances of the average mortalities before and after treatment in the bioassays in each pond were compared using a two-tailed F-test described by Sokal and Rohlf (1981, p.189). When the observed variances were not significantly different, the differences between the mean mortalities before and after treatment were compared using the two-tailed t-test procedure given by Sokal and Rohlf (1981, p.228).

Population densities

A decision on the most appropriate statistical method to analyze the effects of the pesticide treatments on population densities of different species had not been made when this report was written. The difficulties in deciding upon the appropriate statistical method are discussed later.

In this report only the results of a preliminary analysis of the data will be presented.

The experiment was set up according to BACI-design (Before After Control Impact). All ponds were monitored for four and a half weeks before pesticides were applied. Each treatment was replicated five-fold while six ponds served as controls.

The spatial interspersion of the different treatments was chosen arbitrarily, but it approached a systematic design (Hurlbert, 1981). For the allocation of treatments to different ponds, factors such as accessibility, geographical situation and distances from villages were taken into account.

For each treatment group (control, diflubenzuron and fenitrothion) and each organism, the average number per cubic or square meter plus one (AVG#+1), called C, D or F for the different the treatment groups respectively, was calculated for each sampling date. These values were transformed as LN(C), LN(D) and LN(F). Then for each transformed set of data from a treatment group, LN(D) or LN(F), the transformed average numbers of the control group, LN(C), were substracted. Stewart-Oaten *et al.* (1986) proved that the values of these effect-parameters (P^E), obtained during several observations before an environmental disturbance, can be treated as statistically independant replicates. Data from before and after the disturbance can thus be compared using a t-test.

Summarizing, the effect-parameters (P^E) tested here can be expressed as:

LN(D)-LN(C) or LN(F)-LN(C),

in which

C= AVG#+1 for the control group,
D= AVG#+1 for the diflubenzuron group, and

F= AVG#+1 for the fenitrothion group.

The way of testing the data can be scheduled as follows:

	Before treatment observation				After treatment Observation		
	1	2...	k		k + 1	k + 2	k + n
Test parameter							
Diflubenzuron	P^E_1	P^E_2	P^E_k	$AVG(P^E_{1..k})$	P^E_{k+1}	P^E_{k+2}	P^E_{k+n}
Fenitrothion	P^F_1	P^F_2	P^F_k	$AVG(P^F_{1..k})$	P^F_{k+1}	P^F_{k+2}	P^F_{k+n}

Each individual post-treatment effect-parameter per treatment group, P^E_{k+n}, was then compared to the average pre-treatment effect-parameter, $AVG(P^E_{1..k})$, using a two-tailed t-test for the comparison of a single observation with the mean of a sample (Sokal and Rohlf, 1981, p.231) at α=0.05, 0.01 and 0.001.

It must be emphasized that average densities of ponds from the same treatment group were tested. Therefore when effects are mentioned here, they refer to the average of all ponds in a group. In the same way recovery time only refers to the average recovery time. Any variation between different ponds in response to the treatments is not considered in this report.

RESULTS

Meteorology and water levels

Some meteorological measurements, such as relative air humidity and air temperatures will not be discussed in depth. In general, the humidity starts rising at the end of the dry season (May/June) and is at its highest during rainy days (up to 100%). In the early dry season days are hot and nights are relatively colder. During the rainy season maximum temperatures drop and minimum temperatures rise.

1991

In 1991 the first rains in Nioro du Rip fell in early July. During the rainy season rains were irregular, but some fell approximately once a week. The rainy season was over by mid-October and the total amount of rain measured was 506 mm.

The average evaporation from mid-August until mid-November, the period that the ponds were sampled, was 4.7 mm per day. The total amount that evaporated during this time was 430 mm, while 273 mm rain fell.

When sampling started the average depth at the measuring sites in the ponds was approx. 50 cm. In August and the beginning of September water levels fluctuated because of consecutive periods of heavy rainfall and days without rain (Figure 3.1). By mid-September the water levels started to decrease steadily and in mid-November the average depth was 3.5 cm. The average decrease over this period was 5.8 mm a day, a little higher than the average evaporation, which probably means that water soaked into the ground.

1992

In 1992 the rains started much earlier than in 1991. The first significant rainfall occurred on May 30. For the month of June and the first part of July little rain fell until the rainy season actually started in mid-July. The overall rainfall in Nioro du Rip for the 1992 season was 722 mm, a larger amount than in 1991. Rainfall was also measured at Prokhane (see Figure 2.1), except for the month of May. Total rainfall was slightly less than in Nioro and on several occasions rainfall occurred only at one of the two locations. In 1991, while the sampling team was in the field much of the time, it was observed that the occurrence of rainstorms could vary tremendously from one locality to another.

During the 1992 sampling period from June until the end of November the total evaporation was 1,001 mm and the average was 5.4 mm/day. Water levels were not measured in 1992. After the first significant rainfall in May all ponds contained some water, but most of them became dry again during the following period with little precipitation. On July 16 (62 mm of rainfall) they refilled. They finally dried out in November or December.

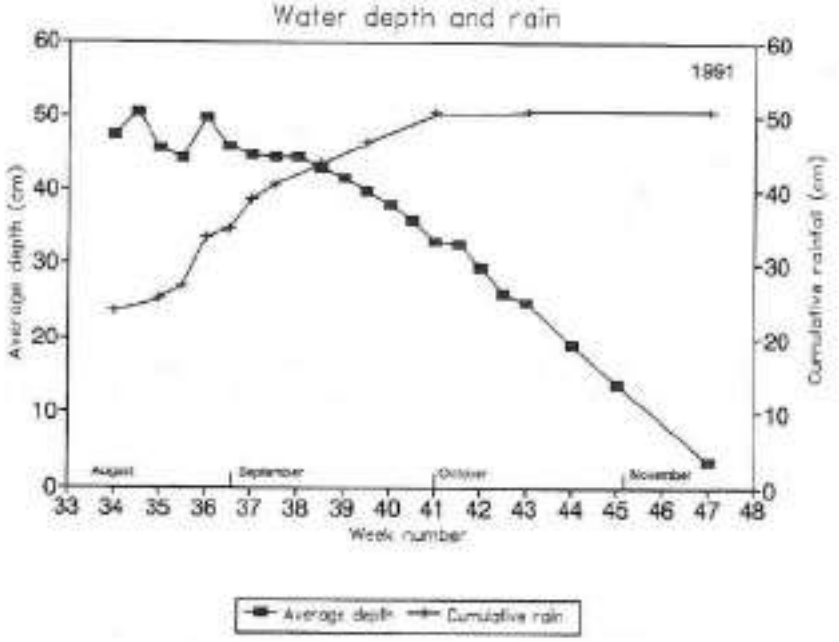


Figure 2.3: Cumulative rainfall in Nioro du Rip and average water depth of the ponds during the sampling period in 1991.

Physical and chemical water parameters

To illustrate general trends in water physical and chemical parameters in this chapter, only a selection of data from 1991 and 1992 was used.

Water temperatures will of course fluctuate between night and day. Therefore the temperatures that were measured in the ponds more or less reflect the time of the day they were usually sampled. In the early morning temperatures could be as low as 25°C, while in the afternoon occasionally 35°C was measured in the more shallow ponds. It is assumed that the water temperature in the ponds fluctuates daily somewhere between these two extremes. In November the colder weather results in lower average water temperatures (Figure 2.4).

In 1992 the visibility through the water of the ponds was followed throughout the rainy season. The average Secchi depth is shown in Figure 2.5. After the first series of rains in May, the visibility dropped again during the following period when ponds were drying out. When new rains fell in mid-July the visibility increased. Maximum average visibility was observed in early October. During the process of dessication in late October and November, visibility decreased rapidly again.

We think that three main processes determine the turbidity in the ponds: abundance of algae, disturbance by cattle, and the process of dessication in which suspended particles are concentrated. At the end of the rainy season the last two processes occur at the same time. Particles concentrate and the cattle in the region will more frequently come to drink from the larger ponds (studied by the project), because the smallest ones are already dry. The effect of this can be seen clearly in Figure 2.5.

Dissolved oxygen (DO) was rarely measured in 1991, but for 1992 data are available from June until the end of November. The averages are shown in Figure 2.6. The development of the average DO-concentrations in time resembles the development of the visibility. The higher the water visibility, the higher the DO-saturation.

In 1991 pH-values were measured throughout the sampling period, but during 1992 data for two months are missing. The observations of 1991 are shown in Figure 2.7. The values for ponds that developed a layer of duckweed and one pond that showed unusual fluctuations (nr.7, Palagne) are shown separately. It can be seen that the average pH for most ponds fluctuated slightly around the neutral value of 7 during most of the period. At the end of the rainy season the acidity increased. Pond 7 showed large fluctuations with some very high values. The DO-concentrations and the visibility through the water in the same pond were also higher than in other ponds during both years of observations. The pH in the ponds with duckweeds dropped when these layers developed in mid-September.

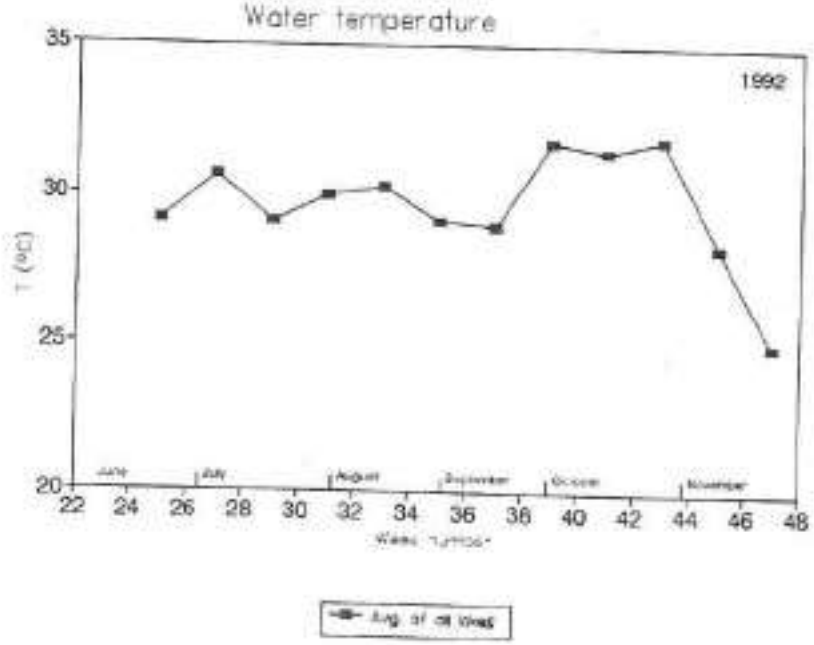


Figure 2.4: Average water temperature of the ponds at sampling times during 1992 (n=16 ponds).

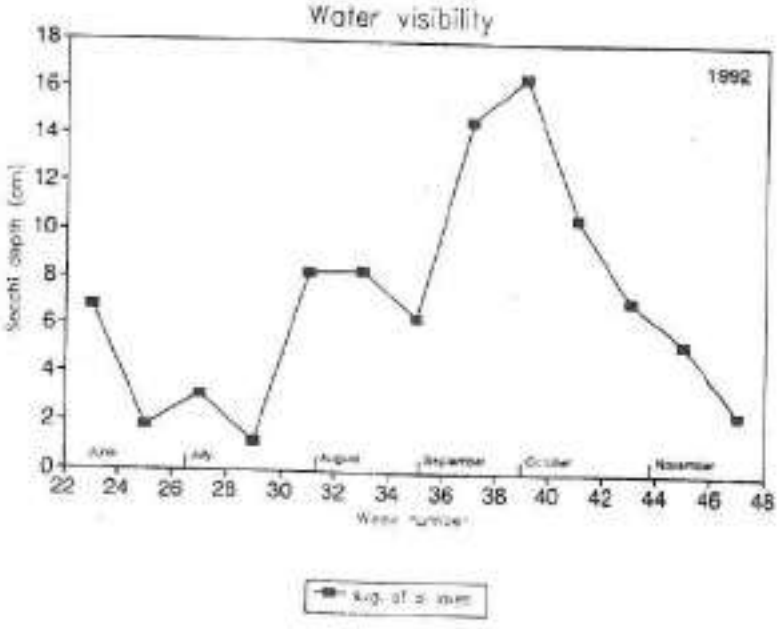


Figure 2.5: Average water visibility in the ponds during 1992 (n= 16 ponds).

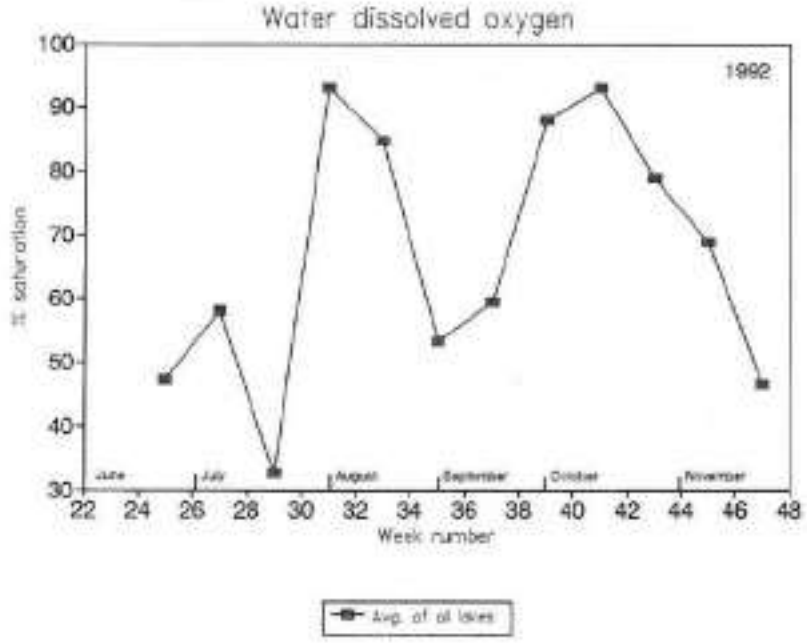


Figure 2.6 Average percentage saturation with dissolved oxygen in the ponds during 1992 (n= 16 ponds).

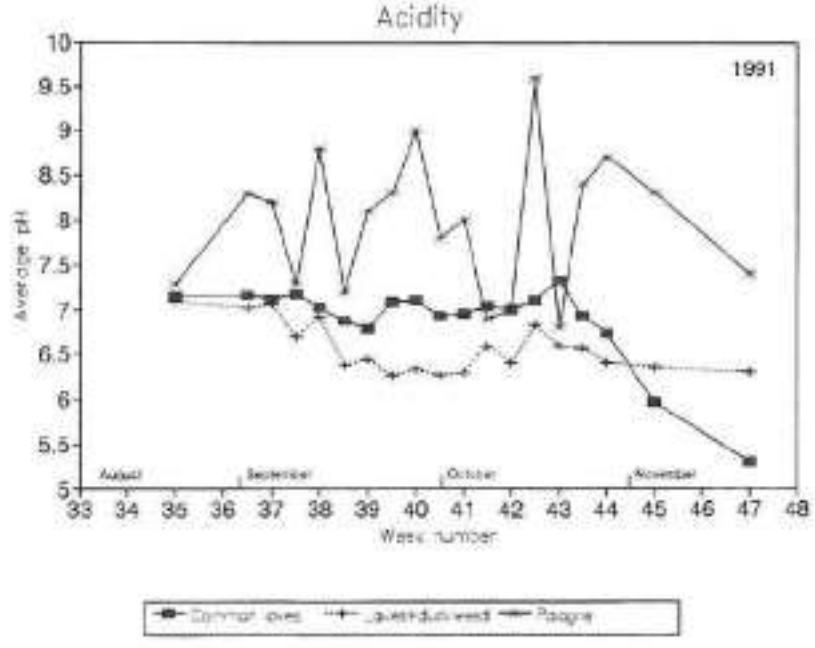


Figure 2.7 Average acidity in Pond 7 (Palagna) and ponds with or without duckweed layers during 1991 (n= 1, 3 and 12 ponds

From the DO and pH data we now have strong indications that oxygen-producing algae are abundant and active in the ponds when enough light is permitted to penetrate the water. When the turbidity is low, as in Pond 7, more oxygen is produced and pH-values do correspondingly increase. When light can not penetrate the water because of the abundance of duckweeds or because the water is opaque, DO and pH-values decrease. From the relationship between the DO and the pH it can also be deduced that the water (from rain) in the ponds must have a low buffer capacity.

Water conductivity was only measured during the last week of October and the first half of November 1992. The observations showed an increase in conductivity during this period, probably due to the concentration of dissolved matter when the ponds are drying.

Abundance of different organisms

The ponds contained a rich community of aquatic invertebrates consisting of crustaceans and aquatic insects. Every group of organisms has its own strategy to deal with the temporary character of the ponds and the rapidly changing circumstances. Macrocrustaceans and zooplankton species produce resting eggs (also called cysts) that survive in the dry mud until the next rainy season. Adults of aquatic insects such as hemipterans and coleopterans often have the capacity to fly and can migrate to permanent waters when temporary waters dry out. Other insects, dragonflies for instance, only use the ponds during the larval stage in their life cycle.

The proper identification of the biological material still poses a considerable problem. With the assistance of different international experts in taxonomy some progress has been made, especially for larger Branchiopoda, Copepoda, Ostracoda and Hemiptera. A special key for the different *Anisops* species (Hemiptera, Notonectidae) was composed for the project by Nieser (1993). Coleopterans were identified to some extent, but they are probably the most difficult group of organisms to identify at the species level. Cladoceran species could be separated, but our identifications still need confirmation by an expert.

Table 2.1 presents a list of the most abundant species and groups of species whose densities could be monitored quantitatively with our sampling methods. However, not all of these groups were suitable for the detection of pesticide effects. Several among them were only found in a limited number of ponds, some of them were only abundant before or after the treatments, and the numbers of various species were too variable in time. Therefore Table 2.1 also indicates if densities and their development in time permitted application of the statistical analysis described previously. Only the population densities of the most suitable groups and species will be treated in this report. However, some of the organisms, whose densities could not be properly measured, or that were not even caught by scoop-netting, will figure here if they were found as victims shortly after treatment of the ponds.

A few remarks on some of the groups must be included.

- *Streptocephalus* spp. consisted mainly of *Streptocephalus sudanicus*, but one of the samples sent abroad contained also some *S. zeltneri* (Pond 7, Palagne).
- *Anisops* spp. consists of *A. varius*, *A. jaczewski*, *A. debilis perplexus* and *A. sardeus*.
- Small coleopterans include all coleopterans smaller than 5 mm. Many of these species could barely be identified at the genus level. They include Dytiscidae, Hydrophiilidae and probably some Haliplidae. Species that were larger were identifiable at the species or genus level and will be referred to individually.

For adult *Streptocephalus* and *Anisops* spp. males and females have been counted separately. For *Streptocephalus* the average lengths of the organisms and the numbers of females with resting eggs in their ovisac were determined as well. However, since no apparent differences in pesticide-induced effects

were observed for each of these groups, the sexes were grouped together and the lengths of *Streptocephalus* will not be reported. Data on adults and nymphs of aquatic insects (with or without wings) will be treated separately.

It is likely that in the future the project will possess more taxonomical information and better identification keys. At that time some taxons may be further divided to determine possible differences in response to the pesticides (e.g. among *Anisops* species).

Although this study was mainly preoccupied with aquatic invertebrates, vertebrates were also observed in many ponds.

Almost all ponds contained considerable populations of aquatic turtles, possibly *Pelomedusa subrufa* (Pleurodira, suborder Chelonia). Local residents noted that they can be captured by digging in the dry mud of the pond beds during the periods when water is absent. In 1992 we observed many swimming adult turtles in the ponds on June 4, only five days after the first rains.

Different species of frogs and toads are abundant in or near the water. Tadpoles are very numerous in the early rainy season. Their numbers decrease and their size increases as time passes.

Many species of birds were observed in or near the ponds. Some of them, such as kingfishers and waders, were clearly feeding on organisms from the ponds.

Table 2.1: Most abundant species and groups of species in temporary ponds in the area around Nioro du Rip. P,S and Z indicate the kind of scoop-net samples in which they were principally captured and it is shown if statistical analyses could be applied on the data.

Taxon		Samples	Statistics
Branchiopoda			
	Conchostraca		
	Caenestheriella sp.	S	no
	Leptestheria cf. *mayeti*	S	no
	Cyclestheria hislopi	S	no
	Anostraca		
	Streptocephalus spp.	P	yes
	Cladocera		
	Moinodaphnia sp.	Z	no
	Moina sp.	Z	yes
	Ceriodaphnia sp.	Z	no
	Diaphanosoma sp.	Z	yes
Copepoda			
	Cyclopoida		
	Thermocyclops decipiens	Z	yes
	Mesocyclops kieferi	Z	no
	Calanoida		
	Tropodiaptomus banforanus	Z	no
	Paradiaptomus rex	Z	yes
Ostracoda			
	Cyprídae		
	Strandesia evae	Z	no
	Heterocypris symmetrica	Z	yes
Hemiptera			
	Notonectidae		
	Anisops spp.	P	yes
	Anisops spp.nymphs	P	yes
	Corixidae		
	Agraptocorixa senegalensis	P,S	yes (for S)
	A. senegalensis nymphs	P,S	yes (for S)
	Micronecta sp.	P	no
Coleoptera			
	Dytiscidae		
	Eretes sticticus	P,S	yes (for P)
	E. sticticus larvae	P,S	no
	various small coleopterans (<5 mm)	P,S	no
Odonata			
	Lestidae larvae	P	no
Diptera			
	Chironomidae larvae	S	no

Samples: P pelagic; S in and just above sediment; Z zooplankton

Effects of duckweeds

In some of the ponds a layer of duckweeds, *Lemna* sp., developed in the beginning of September 1991. One week before the pesticide treatments Pond 2 (fenitrothion), Pond 5 (fenitrothion) and Pond 13 (control) became wholly covered. In Pond 8 (control) duckweeds became abundant as well, but this pond was never covered entirely. Depending on the wind the layers would sometimes wash aside, but most of the time they remained closed and did not disappear until the end of the rainy season.

The abundance of these duckweed covers interfered with the abundance of some species. Fortunately the layers developed one week before the treatments, so their effect could be separated from the effects of the

applied pesticides. Species that disappeared from pond 2, 5 and 13 were *Streptocephalus* spp. (Branchiopoda, Anostraca), *Paradiaptomus rex* (Copepoda), and *Moina* sp., *Ceriodaphnia* sp. and *Diaphanosoma* sp. (Branchiopoda, Cladocera). All species that were affected were pelagic crustaceans. Sediment-dwelling organisms and pelagic insects seemed unaffected. The partial duckweed layer in Pond 8 did not interfere with the abundance of species.

One ostracod, *Strandesia evae*, was only captured in ponds where duckweeds developed. It is not known if they really became more abundant or if they changed their behaviour so they were more easily captured by our zooplankton sampling method (for example if their lifestyle became more pelagic instead of benthic).

For the species that were affected by duckweeds, the data of Ponds 2, 5 and 13 are not included in the statistical calculations or the graphic presentations that were used to determine the impacts of the two pesticides.

Pesticide treatments

Some treatment data are summarized in Table 2.2.

The average applied dosages were 75.1 and 506.1 g a.i./ha for diflubenzuron and fenitrothion respectively. If we compare these to the nominal rates of 60 and 500 g a.i./ha it must be concluded that some overdosing occurred in the case of diflubenzuron. The most extreme overdosing took place in Pond 7, Palagne, which received 74% too much diflubenzuron. Pond 2, Koudote, treated with fenitrothion, was 21% underdosed. The overall variation between the treatments was not too large. The coefficient of variation was 23% for the diflubenzuron treatments and 19% for fenitrothion.

Deposition papers that were attached to the bioassay cages before the treatments revealed that deposition of the sprays was good and droplet sizes reflected the spectrum usually found in drift spraying. All ponds were sprayed at windspeeds higher than 0.5 m/s.

Table 2.2: Summary of treatment characteristics of ponds treated with pesticides.

Pond	time of treatment	min.-max. windspeed (m/s)	min.-max. air temperature (°C)	surface treated (ha)	swath width (m)	number of swaths	quantity applied (l)	actual dose g a.i. / ha
Diflubenzuron [1]								
September 15, 1991								
3. Sadiower	10.45	1-2.5	27-31	0.61	9	9	0.700	68.9
7. Palagne	13.20	0.5-1.5	29-31	0.53	10	9	0.920	104.2
9. Kourême	10.15	1-2	31-31	0.65	18	4	0.645	59.5
12.Fana awa	13.45	1-3.5	33-34	0.39	18	4	0.495	76.2
16.Debreye	16.30	1-2	29-29	0.36	10	8	0.400	66.7
Average applied dose								75.1
s.d.								17.3
C.V.								23%
Fenitrothion [2]								
September 17, 1991								
2. Koudofe	9.30	0.5-2	30-30	0.97	17.5	6	0.690	355.7
5. Daladiam	12.05	0.5-4.5	33-33	0.29	17.5	3	0.295	508.6
6. Wimbody	14.30	0.5-3.5	34-34	0.75	18	7	0.735	490.0
11.Mbambah	9.50	1.5-3	27-28	0.79	18	4	0.950	601.3
15.Gandiang	13.40	2-3	30-30	0.20	18	2	0.230	575.0
Average applied dose								506.1
s.d.								95.6
C.V.								19%

[1] Formulation: 450 ODC diluted with diesel at 1:6.5 = 60 g a.i./l [2] Formulation: 500 ULV = 500 g a.i./l

Pesticide residues in pond water

Table 2.3 summarizes the measured concentrations after one hour, the coefficients of correlation of the regression lines and the half-lives for both applied pesticides in the aqueous phase of the surface water.

Diflubenzuron was only detected in water samples taken one hour after each treatment. After 24 hours levels had decreased below the detection limit of 0.6 µg/l. The average concentration in the ponds one hour after treatment was 10 µg/l. In Ponds 3 and 7 much higher concentrations were measured. A satisfactory explanation for this phenomenon can not be given, but the applied sampling method will be discussed in more detail in the discussion section. It can only be concluded here that dissolved diflubenzuron virtually disappears within 24 hours after treatment.

Fenitrothion remained measurable in the aqueous phase during a longer time. Concentrations after one hour averaged 80 µg/l. Again two ponds, Ponds 5 and 11, showed much higher concentrations. For four ponds (2, 6, 11 and 15) regression analysis could be applied to calculate half-lives. The correlation of the regression lines was generally good, except for Pond 11.

If we divide the average applied doses by the average initial concentration the factor would be 7.5 for diflubenzuron and 6.3 for fenitrothion. These values are very similar, which probably means that the initial concentrations after one hour are mainly determined by the applied dose and have not yet been under much influence of processes like evaporation, absorption, etc.

The average half-life of fenitrothion in the ponds was 43 hours, but there were considerable differences among the ponds. The disappearance of the dissolved compound was especially rapid in Pond 5 and Pond 6.

Table 2.3: Initial concentrations and half-lives of pesticide residues in the aqueous phase of temporary pond water. Correlation coefficients for regression lines established to calculate half-lives on the basis of first order removal.

Pond	measured conc.(1h) (µg/l)	coeff.of correlation	half life T½ (h)
Diflubenzuron			
3.Sadiowar	16	-	-
7.Palagne	18	-	-
9.Kouréne	7	-	-
12.Fana awa	5	-	-
16.Debroye	4	-	-
Average	10		
s.d.	7		
C.V.	70%		
Fenitrothion			
2.Koudote	34	0.91	64
5.Daladiam	163	-	-
6.Wimbody	27	0.92	14
11.Mbambah	145	0.62	46
15.Gandiang	31	0.99	49
Average	80		43
s.d.	68		21
C.V.	85%		49%

Acute toxicity

The results of the *in situ* bioassays with *Streptocephalus* spp. are shown in Table 2.4. It can be seen that mortalities in the pre-treatment assays were very variable. In half of the ponds the mortalities were lower than 15%, but in the others they ranged from 26 to 90%. If either the control-mortality or the after-treatment mortality was zero, the difference between a pair of data could not be tested statistically.

because a division by zero was taking place.

After-treatment mortalities in the ponds treated with diflubenzuron seemed unrelated to any effect of the pesticide. Both increases and decreases were observed compared to the control tests. Diflubenzuron is of course an insecticide with a relatively slow action and will usually cause little mortality in 24 hours.

The post-treatment mortalities in the ponds that were treated with fenitrothion were surprisingly low compared to the controls. The cause of this is not known, but it is evident that there was no acute adverse effect on *Streptocephalus*.

Because of the high mortalities in the controls the results of the *in situ* bioassays had no toxicological value. Before these tests can be used any further it will be necessary to find the causes for the high and variable control mortalities.

Table 2.4: Results of *in situ* bioassays with the anostracan *Streptocephalus* before and after treatments of temporary ponds with pesticides (n= 6 cages per pond with 10 specimens each).

Pond	% mort.before treatment (s.d.)		% mort.after treatment (s.d.)		significance of difference
Diflubenzuron[1]					
3.Sadiowar	0	(0)	4	(8)	-
7.Palagne	62	(19)	58	(16)	0
		59[2]	(27)		0
9.Kourène	42	(22)	14	(12)	0
		74[3]	(11)		***
12.Fana swa	0	(0)	23	(8)	***
			(n = 4)		
16.Debreye	56	(23)	10	(6)	**
Fenitrothion[4]					
2.Koudole	26	(29)	-		-
5.Delediam	14	(15)	0	(0)	-
6.Wimbody	90	(15)	0	(0)	-
11.Mbambah	0	(0)	0	(0)	-
15.Gandiang	6	(12)	4	(9)	0

[1] before treatment 7-8 Sept.
after treatment 15-16 Sept.

[2] extra before treatment 5-6 Sept.

[3] extra before treatment 2-3 Sept.

[4] before treatment 7-8 Sept.
after treatment 17-18 Sept.

Significance:

- not calculable
0 not sign.
* sign. for $0.01<\alpha<0.05$
** sign. for $0.001<\alpha<0.01$
*** sign. for $\alpha<0.001$

Searches for floating victims on the water surface revealed more about the acute toxicity although the ecological impact can not be quantified.

After diflubenzuron treatments few dead macroinvertebrates were found. The only dead species observed were some adult *Anisops* spp. and some *Agraptacorixa senegalensis* adults and nymphs. However, there was no proof that the treatments caused their death. Adults are not likely to be affected by the IGR, and the nymphs found showed no deformations.

The results of the searches in the ponds treated with fenitrothion are shown in Table 2.5. It can be seen

that many species were affected, especially hemipterans and coleopterans. Many victims found belong to the larger species that were rarely captured by our biological sampling methods. Most of these species are also thought to be important predators. It is therefore possible that the predation on their prey in the ponds was considerably reduced.

Table 2.5 Dead aquatic invertebrates found floating on the water surface of ponds treated with fenitrothion.

Taxon	Pond nr.	1	5	6	11	15
Branchiopoda						
Conchostraca						
Caenestheriella sp.					X	
Hemiptera						
Notonectidae						
Anisops spp.		X	X	X	X	X
Anisops spp. nymphs				X	X	X
Corbidae						
Agraptacorixa senegalensis			X	X	X	X
Agraptacorixa senegalensis nymphs			X	X	X	X
Micronecta sp.			X		X	
Pleidae						
Plea pullula			X			
Gerridae						
Limnogonus sp.		X	X		X	
Mesoveliidae						
Mesovelia vittigera					X	
Belostomatidae						
Diplonichus nepoides			X		X	X
Diplonichus nepoides nymphs					X	
Nepidae						
Laccotrephes fabrici					X	X
Laccotrephes fabrici nymphs				X		X
Lethocerus cordofanus				X	X	
Lethocerus cordofanus nymphs					X	
Coleoptera						
Dytiscidae						
Eretes sticticus		X	X	X	X	X
Eretes sticticus larvae						X
Rhantaticus congestus			X			X
Cybister sp.			X	X	X	X
Cybister sp. larvae					X	X
Hydrophilidae						
Hydrophilus cf. *senegalensis*		X	X		X	X
various small coleopterans (<5 mm)			X	X	X	X
Odonata						
Libellulidae larvae					X	X
Aeshnidae larvae					X	
Lestidae larvae						X
Hirudinoidea						X

Effects on population densities

In this section the development of population densities of the most abundant species and the effects of the two pesticides on them will be described. The most relevant species or groups of species and the samples in which they were captured are given in Table 3.1. The classification and nomenclature for the different groups and species were already described.

During the 1992 season only pelagic and zooplankton samples were taken for logistical reasons. Where 1992 data are available, they are presented for roughly the same period as for 1991 to facilitate comparison. The authors feel that data from the early and late 1992 rainy season were less suitable for any judgement on recovery because these periods were characterized by rapid and less predictable physical and chemical changes in the water.

When effects are called significant here, this means that average post-treatment densities of the treated ponds were statistically different from the average of the control ponds.

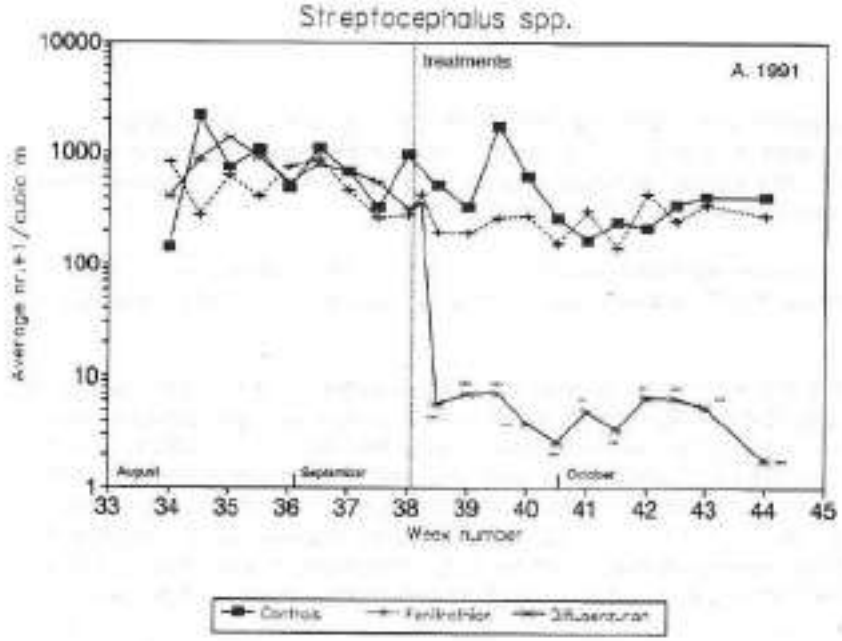
For 1992 differences in average densities between the same groups of ponds were not tested statistically.

Macrocrustaceans

The only macrocrustaceans that were found suitable for density monitoring were *Streptocephalus* spp., pelagic anostracans that dominated in all ponds. The development of their densities in the different groups of ponds during 1991 and 1992 is shown in Figure 2.8a and 2.8b respectively. Average numbers in the control group fluctuate roughly between 100/m^3 and 1000/m^3.

Fenitrothion treatments did not cause any significant reduction or increase of the average density. During the same period in 1992 the numbers in the treated ponds are slightly lower than in the control group, but not radically depressed.

For diflubenzuron no significant effect of the treatments could be detected on the day following the treatments. However, after four days the average density in the treated ponds had significantly dropped to 5/m^3, while the average for the control group was somewhere between 600/m^3 and 1000/m^3. During the rest of the season living specimens of *Streptocephalus* spp. were found occasionally, but the average density remained close to 100% reduced compared to the controls until the ponds dried out (Figure 2.8a). In Figure 2.8b it can be seen that in 1992 considerable numbers of the species were found again. The development of the average density of the diflubenzuron ponds closely resembles that of the fenitrothion ponds. They both are somewhat lower than the control group, but probably not significantly so.



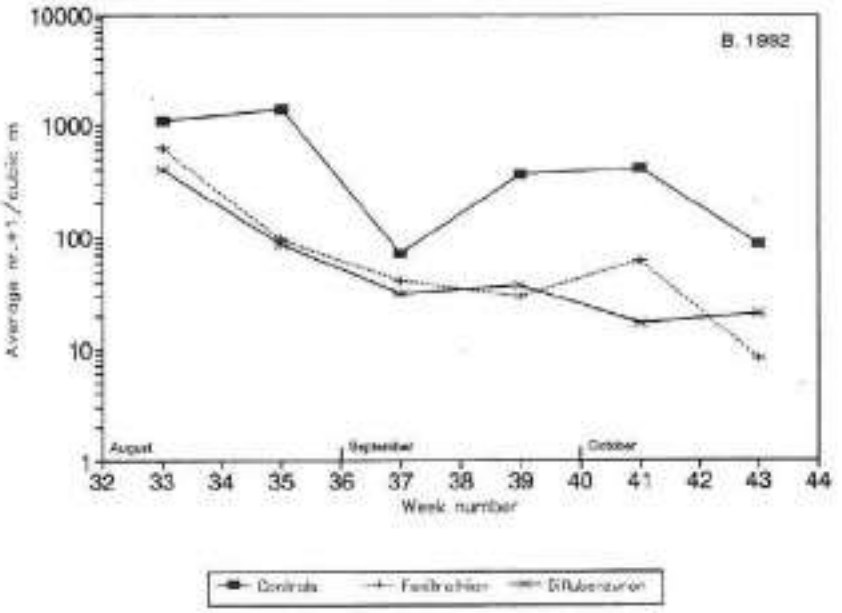


Figure 2.8: Average population densities of *Streptocephalus* spp. (Branchiopoda, Anostraca) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control and diflubenzuron: n= 5 ponds; fenitrothion: n= 3 ponds.

Zooplankton

The densities of the copepods *Thermocyclops decipiens* and *Paradiaptomus rex* for 1991 and 1992 are shown in Figures 2.9a, 2.9b, 2.10a and 2.10b.

Fenitrothion did not cause a significant reduction of *T. decipiens*. From two to four weeks after the diflubenzuron treatments numbers are lower compared to the controls, but the difference is only significant for week 41 (a reduction of 90%). Although it looks like there is a considerable effect, its statistical significance is small, probably because the average number in the ponds before the treatment was already low compared to the controls. During 1992 the average densities of the different groups showed approximately the same development.

Paradiaptomus rex showed a different effect. After both fenitrothion and diflubenzuron treatments the average densities steadily increased and remained significantly higher for the rest of the season. Compared to the controls the increases vary from 9 to 65 times for fenitrothion and from 8 to 129 times for diflubenzuron. In the ponds treated with diflubenzuron the increase is larger and more significant: average densities of almost 10,000/m^3 were reached. The year after the treatments, numbers still seem somewhat elevated for the diflubenzuron ponds, but much less than after the treatments in 1991.

The cladoceran *Moina* sp. was already decreasing in number before the pesticide applications (Figure 2.11a). In the ponds that were to be treated with fenitrothion the average density even declined to zero just before the treatments. Therefore the density reductions that were observed after the treatments could not be attributed to pesticide effects. Few average densities were significantly reduced compared to the control group. Whether there were any effects of the pesticides or not, populations of both treated groups became more similar to the control group again approximately two weeks after the treatments. During the early 1992 rainy season the average densities of the three groups are very different and show large fluctuations, but later in the season they converge (Figure 2.11b). The earlier differences in 1992 do not seem treatment related.

For the other cladoceran, *Diaphanosoma* sp., the same kind of development was observed as for *Moina*. Particularly in the ponds to be treated with fenitrothion, the average density was decreasing the week before the pesticide application. Although the average density remained zero during four weeks following the treatment, this 100% reduction is only significant for two out of eight measurements (at α=0.05), as can be seen in Figure 2.12a. After the diflubenzuron treatments the average density remained significantly reduced at 100% during the rest of the season, except for one day in week 41, when one high average density was observed. This increase could be attributed to one single sample from Pond 3 (Sadiowar). The reason for this sudden local abundance is not known, but it might have to do with shoal formation. During 1992 average densities look reasonably comparable to the controls for both the fenitrothion and the diflubenzuron groups that were treated in 1991 (Figure 2.12b).

No significant effect of any of the treatments was observed on the ostracod *Heterocypris symmetrica* (Figure 2.13a) and numbers also developed similarly during 1992 (Figure 2.13b).

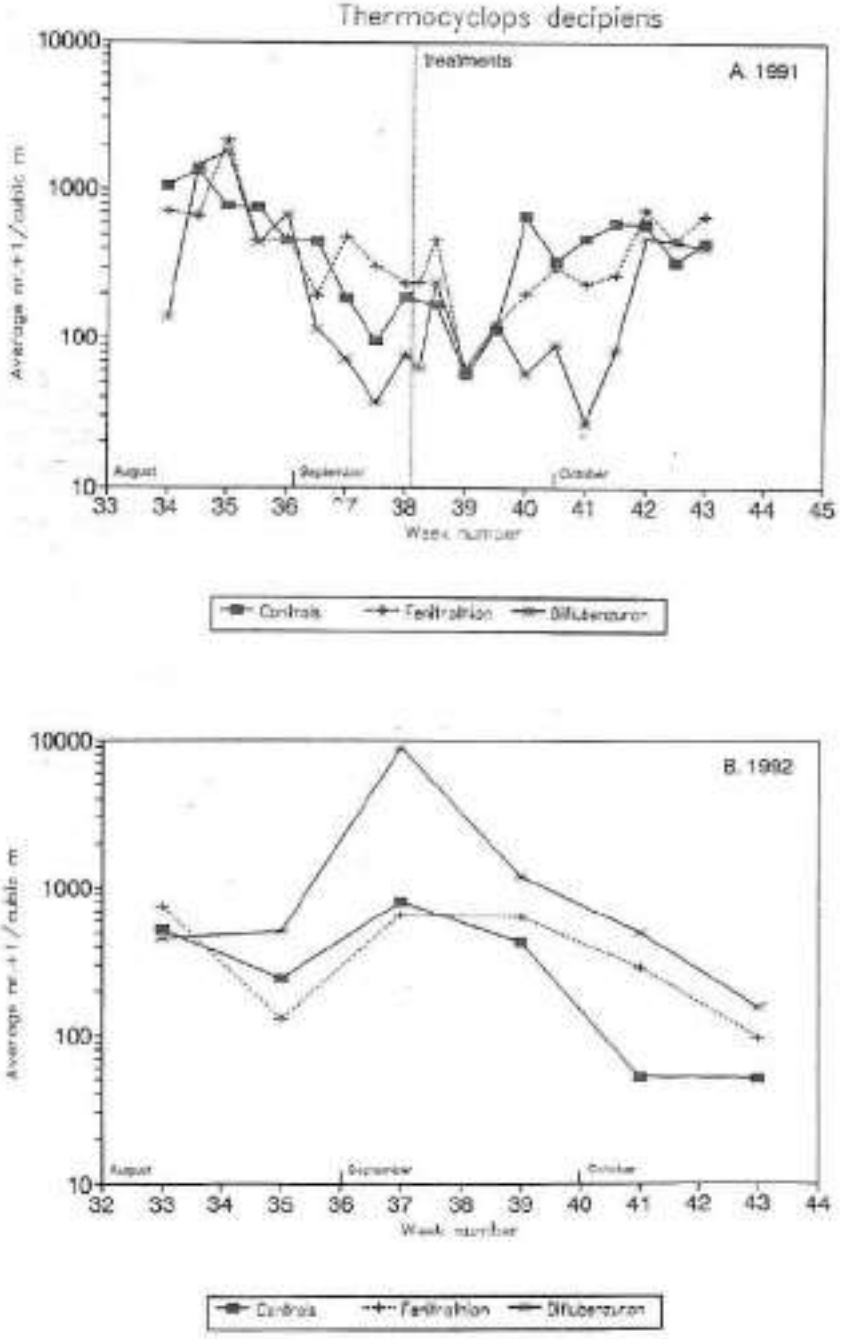
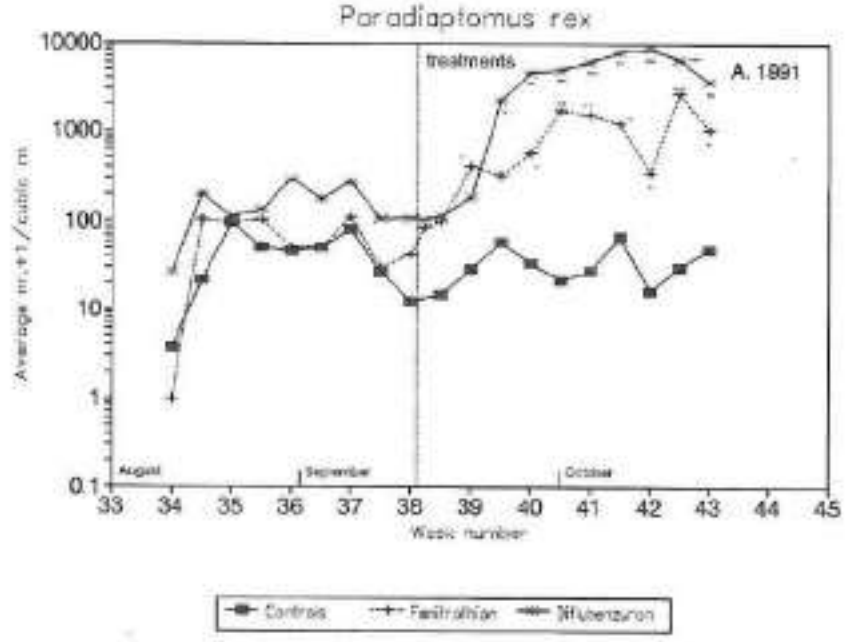


Figure 2.9. Average population densities of *Thermocyclops decipiens* (Copepoda, Cyclopoida) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds.



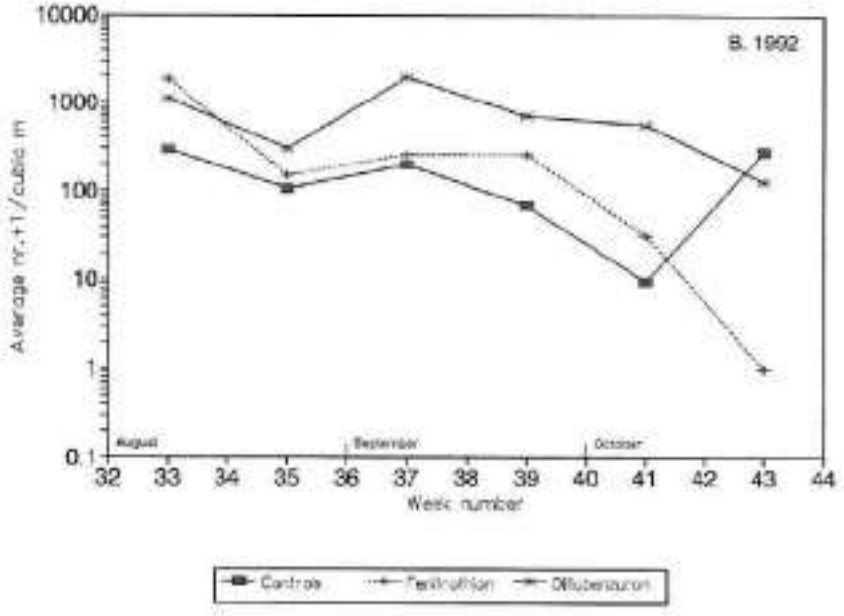


Figure 2.10: Average population densities of *Paradiaptomus rex* (Copepoda, Calanoida) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control and diflubenzuron: n= 5 ponds; fenitrothion: n= 3 ponds.

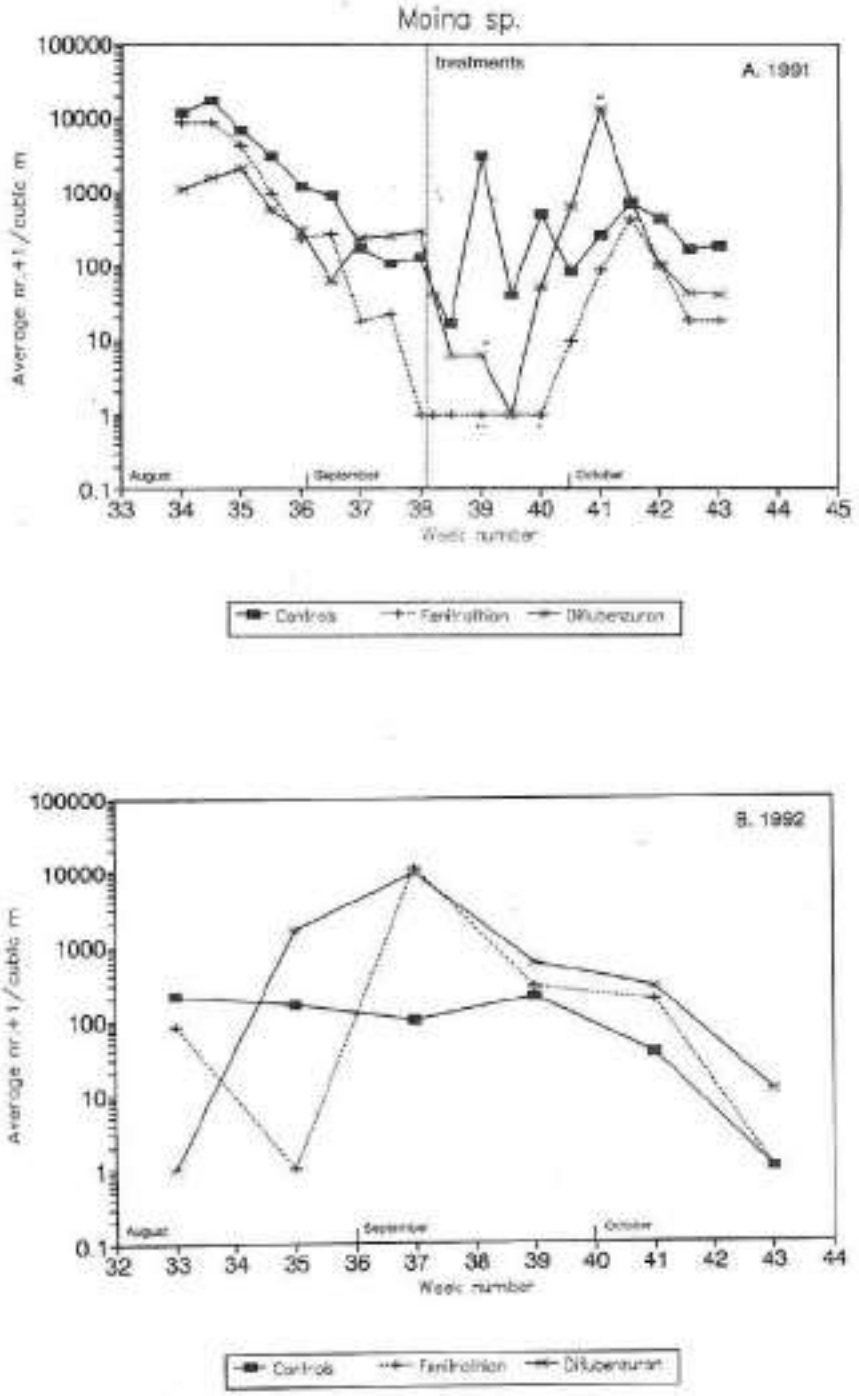
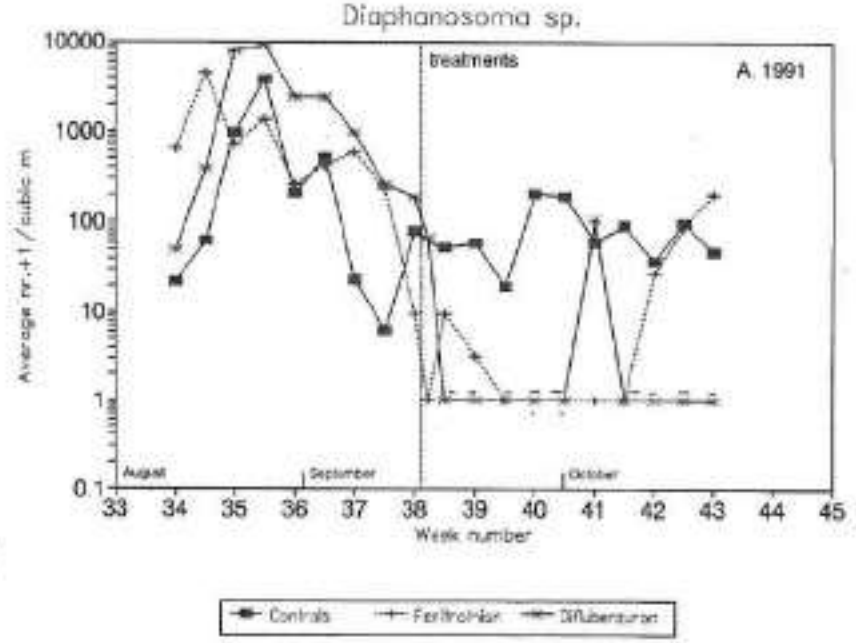


Figure 2.11 Average population densities of *Moina* sp. (Branchiopoda, Cladocera) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control and diflubenzuron: n= 5 ponds; fenitrothion: n= 3 ponds.



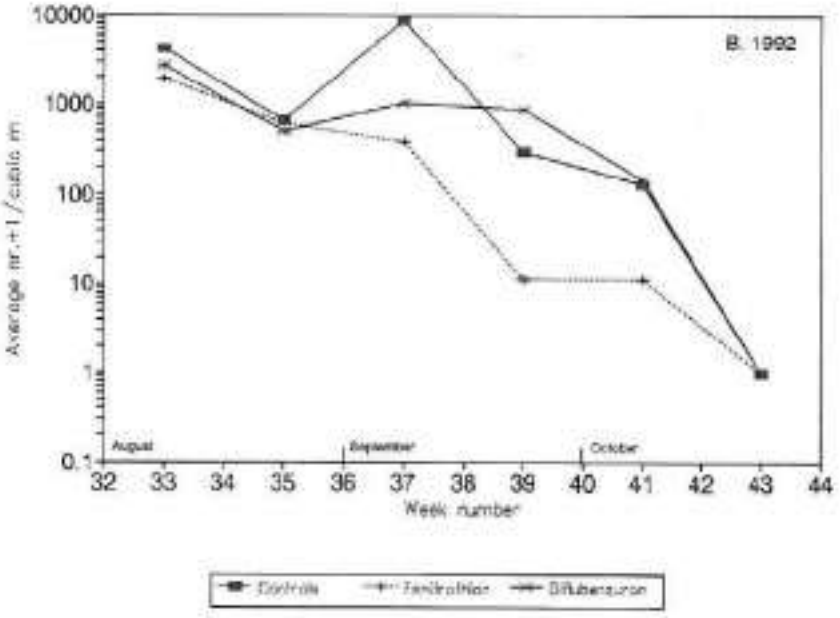
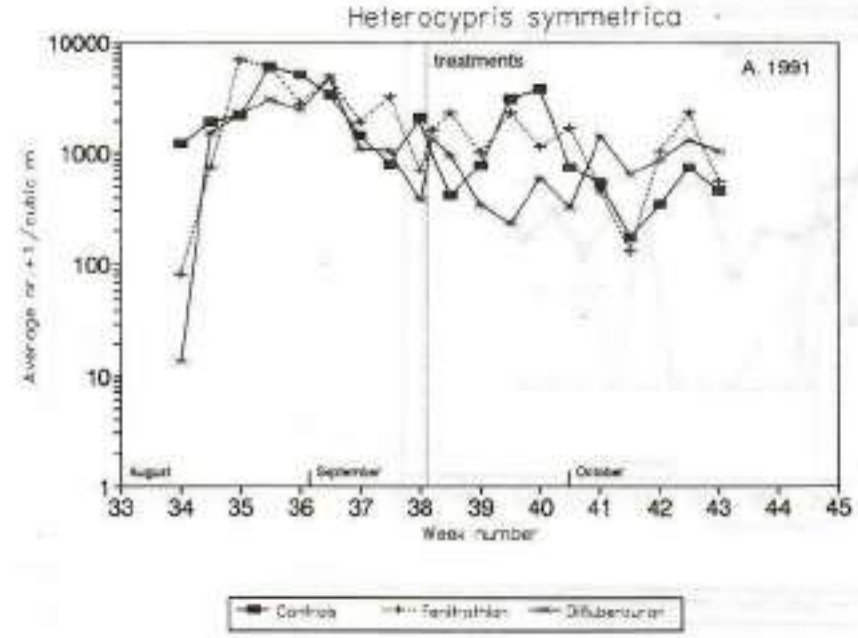


Figure 2.12. Average population densities of *Diaphanosoma* sp. (Branchiopoda, Cladocera) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control and diflubenzuron: n= 5 ponds; fenitrothion: n= 3 ponds.



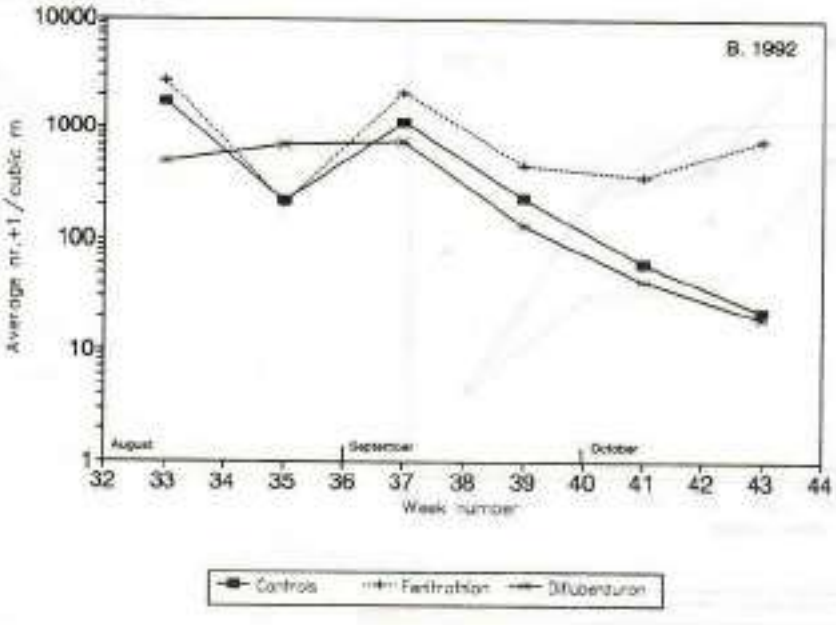


Figure 2.13: Average population densities of *Heterocypris symmetrica* (Ostracoda) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds.

Aquatic insects

The most abundant aquatic insects were *Anisops* spp. (Hemiptera, Notonectidae), a complex of at least four species. As can be seen in Figure 2.14a fenitrothion caused a reduction of some 85% in the abundance of adults. Recovery was complete after one week. The effect of fenitrothion on the nymphs (Figure 2.15a) was more drastic and prolonged: significant reductions of almost 100% were observed for three weeks following treatment.

Diflubenzuron had no immediate adverse effect on *Anisops* adults (Figure 2.14a), but in October densities were significantly higher than those in the controls on several occasions. It is not clear if this was a pesticide related effect. No immediate effect of diflubenzuron was found on the larvae either (Figure 2.15a), but after two weeks one single significant reduction of 95% was measured. In October the average number of nymphs in these ponds was found to be significantly increased during one week.

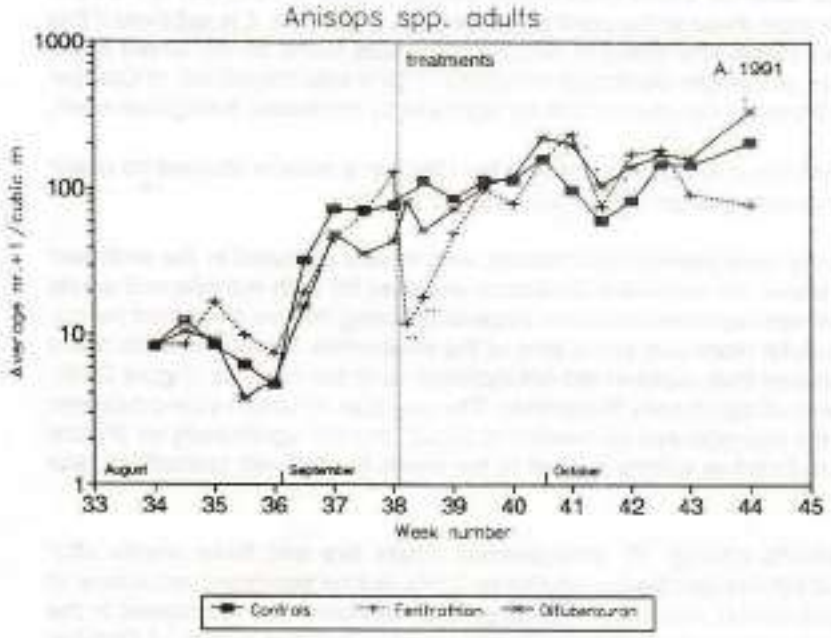
Development of both adults and nymphs of *Anisops* spp. during the 1992 rainy season showed no major differences between treated and control groups (Figures 2.14b and 2.15b).

Another hemipteran, *Agraptocorixa senegalensis* (Corixidae), was mostly captured in the sediment samples and probably lives just above the sediment. Statistical analyses for both nymphs and adults were problematic because the numbers captured were low, especially during the pre-treatment period. Molting of the species seemed to take place just at the time of the treatments. Fenitrothion still had a measurable effect on adults, because their number did not increase as in the controls (Figure 2.16). This effect lasted until sampling was stopped early November. The average reduction varied between 90% and 100%. The density of the nymphs was somewhat reduced, but not significantly so (Figure 2.17). Nymphs and adults were both found as victims in most of the ponds treated with fenitrothion (see Table 2.5).

Diflubenzuron showed some effects among *A. senegalensis* adults two and three weeks after treatment (reductions of 95% and 68% respectively, see Figure 2.16), but no significant reductions of nymphs were observed during this period, most likely because their numbers also decreased in the control group. Instead the number of nymphs significantly increased during the month of October (Figure 2.17).

Since sediment samples were not taken in 1992 there are no data of *A. senegalensis* for this year.

The last aquatic insect that could be monitored to some extent was the adult of *Eretes sticticus* (Coleoptera, Dytiscidae), a predatory beetle. Its densities during 1991 and 1992 are shown in figures 2.18a and 2.18b. The measured average densities are almost as low as for *A. senegalensis*, but *E. sticticus* was more abundant before the treatments. Hardly any effects could be proven statistically. A significant decrease in the average density for fenitrothion was only found three weeks after treatments and one significant increase for diflubenzuron was observed two weeks after treatment. Large numbers of dead *Eretes sticticus* were found immediately after each fenitrothion treatment, which is not in agreement with the apparent absence of an impact on its densities. The sampling method used might not be appropriate for this species. The average densities of *E. sticticus* in ponds treated with fenitrothion in 1991 are similar to the control group during 1992.



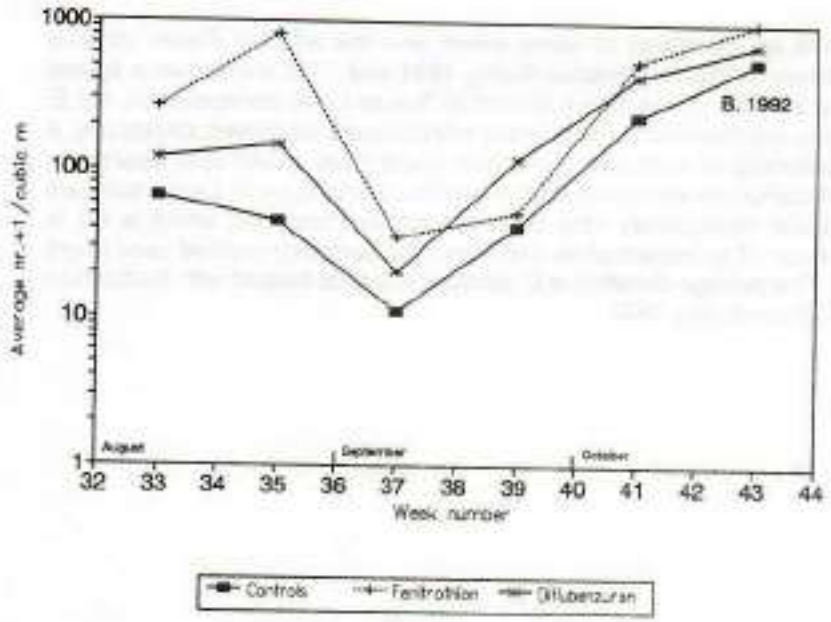


Figure 2.14: Average population densities of *Anisops* spp. adults (Hemiptera, Notonectidae) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds.

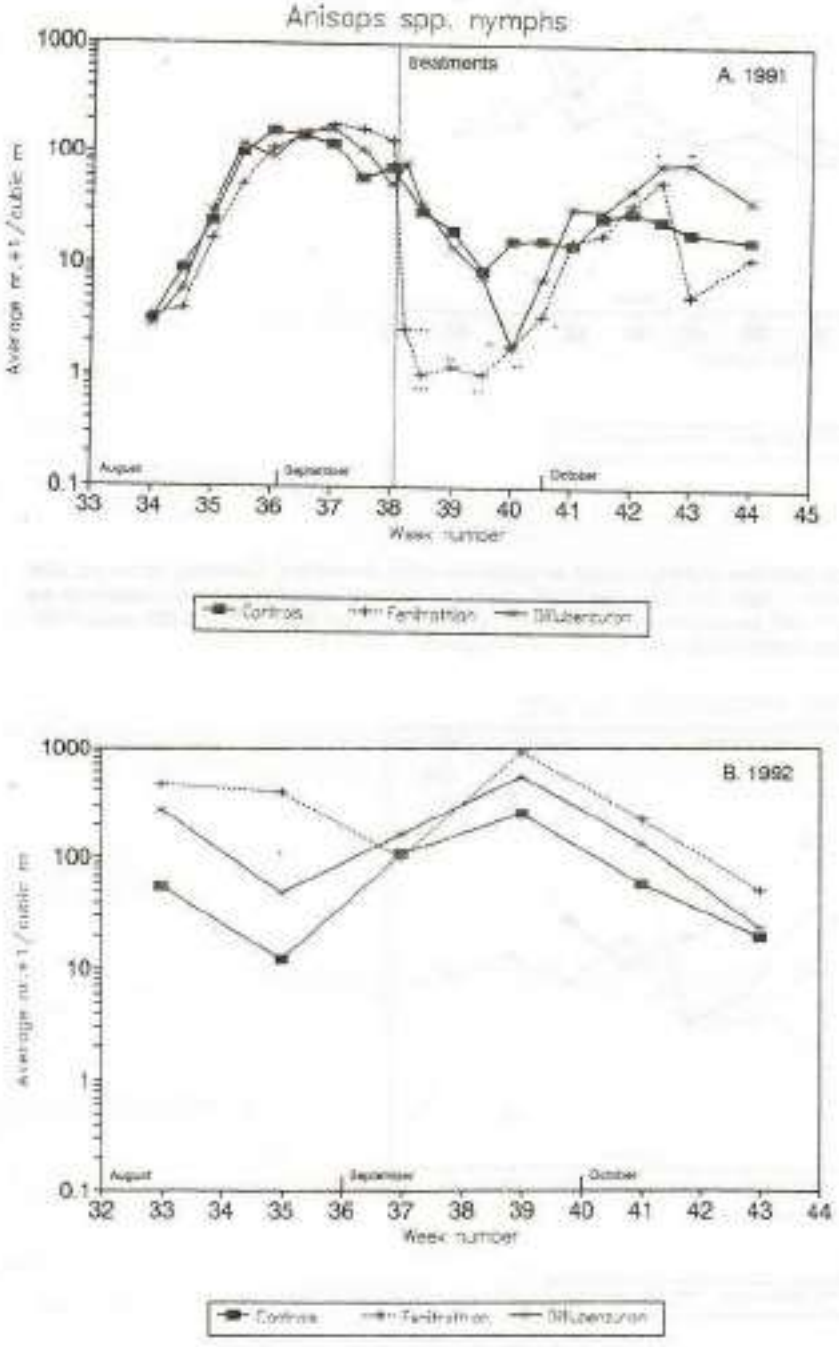


Figure 2.15 Average population densities of *Anisops* spp. nymphs (Hemiptera, Notonectidae) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds.

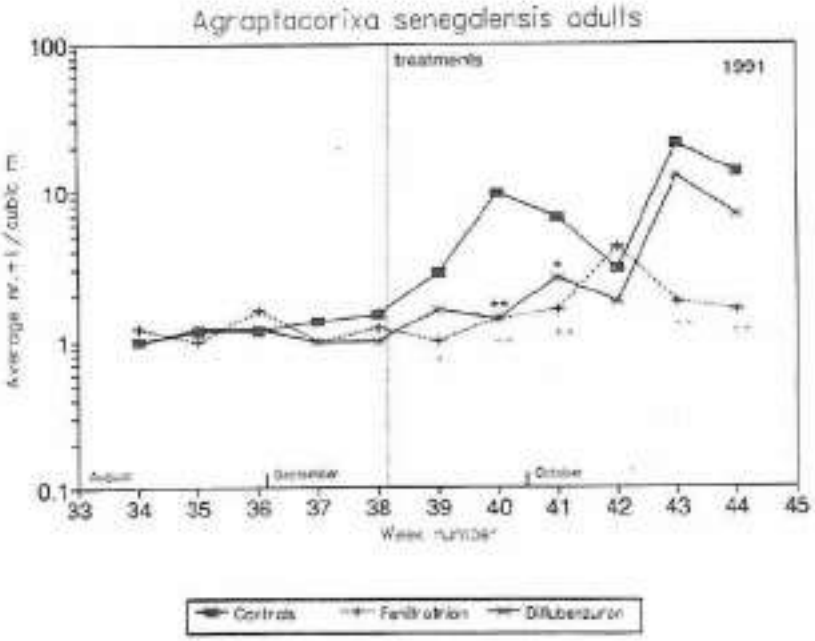


Figure 3.14: Average population densities of *Agraptacorixa senegalensis* adults (Hemiptera, Corixidae) before and after pesticide treatments in 1991. For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds

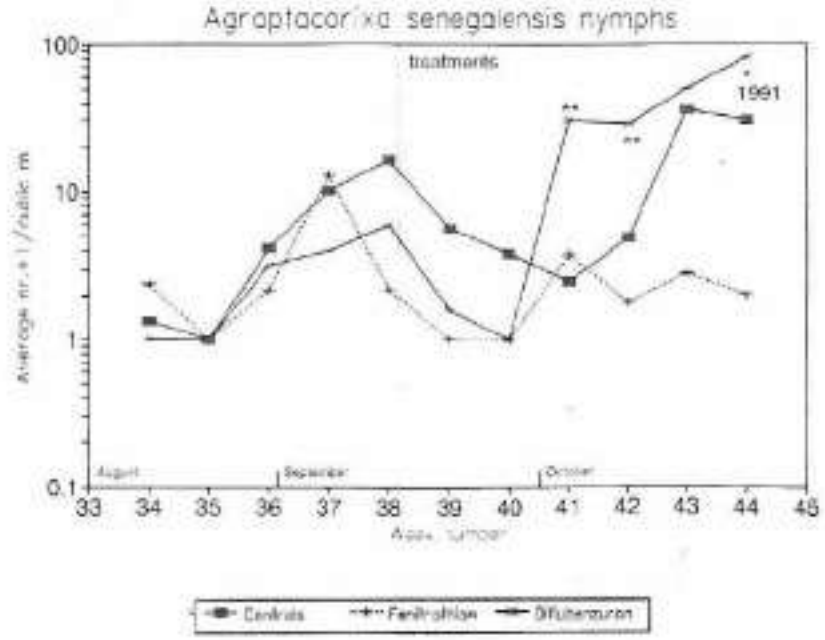
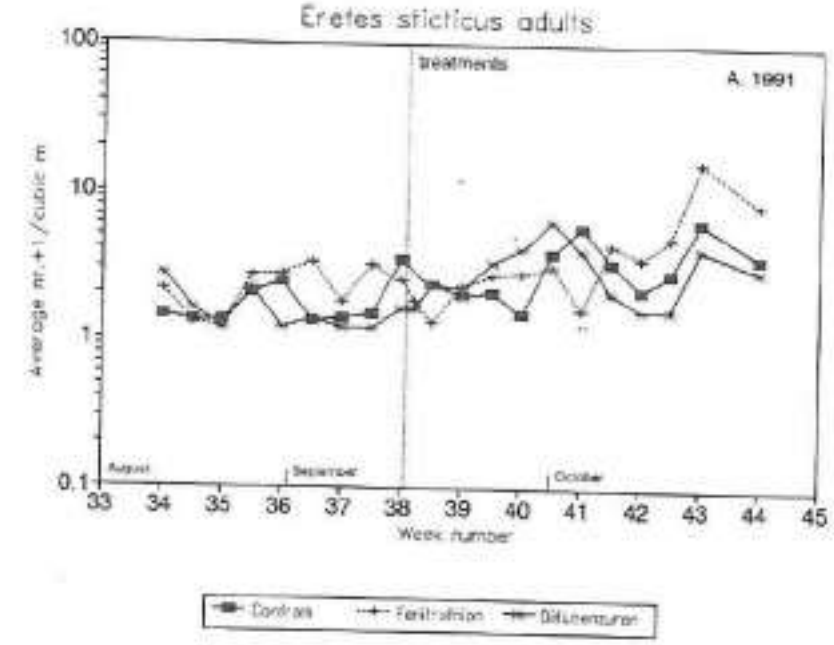


Figure 2.17 Average population densities of *Agraptacorixa senegalensis* nymphs(Hemiptera, Corixidae) before and after pesticide treatments in 1991. For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds



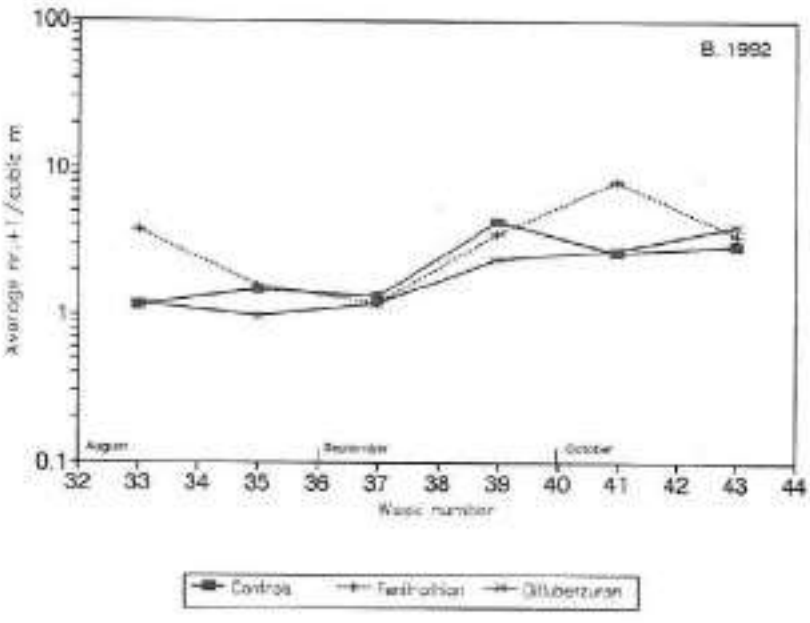


Figure 2.18: Average population densities of *Eretes sticticus* adults (Coleoptera, Dytiscidae) before and after pesticide treatments in 1991 (a) and during 1992 (b). For 1991 significant differences from the control groups after treatments are marked *, ** or *** (diflubenzuron) and +, ++ or +++ (fenitrothion) for α= 0.05, 0.01 or 0.001 respectively. Control: n= 6 ponds; diflubenzuron and fenitrothion: n= 5 ponds.

DISCUSSION

A major problem writing this discussion is the lack of biological and ecological information on temporary pond ecosystems in the Sahel and Africa. Ponds like these have been investigated in the past, but to our knowledge never over longer periods. Most of the invertebrate species encountered in this study can probably be identified if specialized taxonomists can be found, but much information that would be needed to explain the observed effects and recovery speeds, such as migration patterns, foodweb relationships, individual life cycles, etcetera, is not available. Adverse effects on different species were observed for both applied products, but we are hardly able to relate reductions of individual species to the structure and functioning of the ecosystems as a whole. However, the data that were gathered from control ponds combined with observations in the field and the laboratory might serve as a first attempt to fill this information gap. In-depth studies of disturbances of the systems by pesticides could also reveal more about species interactions and behaviour.

Diflubenzuron

The average applied dose of 75 g a.i./ha diflubenzuron on the five treated ponds resulted in an average surface water concentration of 10 µg/l after one hour, but one day later the compound could no longer be detected. The most probable process for this rapid disappearance is adsorption to suspended matter and the sediment (Cunningham, 1986; Cunningham and Myers, 1986).

The applications of diflubenzuron caused significant reductions of several abundant macro- and microcrustaceans. Less affected species were the cladoceran *Moina* sp. (Branchiopoda) and the copepod *Thermocyclops decipiens*. The most affected species, the anostracan *Streptocephalus* spp. and the cladoceran *Diaphanosoma* sp., are both branchiopods. The results suggest that branchiopods may be particularly susceptible to diflubenzuron. This can be caused by either a high intrinsic susceptibility or a high exposure because of a specific micro-habitat (pelagic) or feeding pattern (filter feeding). Branchiopods go through a large number of different molts in which an iGR like diflubenzuron might interfere. Miura and Takahashi (1974) report an LC-50 of diflubenzuron for *Triops longicaudatus* (Notostraca), a larger branchiopod, of 0.75 µg/l. The LC-50's for *Daphnia* (Branchiopoda, Cladocera) are 1.5-2 µg/l (Miura and Takahashi ,1974; Nebeker *et al.*, 1983). Both values are considerably lower than the measured initial concentrations in the ponds. A high intrinsic toxicity for the taxons mentioned above therefore seems very likely, but confirmation by laboratory testing will be needed.

Other known sensitive groups of macrocrustaceans are amphipods, notably *Hyalella azteca* (Nebeker *et al.*, 1983; Farlow *et al.*,1978; Ali and Mulla, 1978a and 1978b), and young stages of decapod shrimp *Palaemonetes pugio* (Touart and Ranga Rao, 1987; Wilson and Costlow, 1986; McAlonan, 1976).

Thermocyclops decipiens and *Moina* sp. recovered fairly quickly after the observed effects (within one week), but *Streptocephalus* spp. and *Diaphanosoma* sp. did not recover until the next rainy season, one year later. The first two species were only partly reduced, while the latter two were almost completely eradicated. Therefore an explanation for the difference in recovery might be given considering individual reproduction strategies. We can safely assume that reproduction occurs during the rainy season for all four species. However, if resting eggs of the first two species hatch during the same season and if those of the latter two do not hatch until the next time the ponds fill with rain, this would result in large differences in their ability to recover. Deposited resting eggs of *Streptocephalus* spp. do not seem to hatch during the same rainy season because younger and smaller stages are only found in the beginning of the season. However, it can not be excluded that resting eggs do hatch during the same season, but that effective predation of very young, small stages by insects prevents maturation. These predatory insects become more abundant later in the season.

Indications of considerable effects of diflubenzuron on zooplankton at the suggested dose for locust control were also obtained during the pilot study in 1989 (Lahr, 1990). Cladocerans, ostracods and cyclopoid and calanoid copepods in the studied irrigation reservoirs all were affected and recovery times varied from 2-3 weeks for ostracods and cyclopoid copepods to more than one month for cladocerans and calanoid copepods.

The effects of diflubenzuron on zooplankton observed in this study also reflect the results of other studies where comparable doses were used. Miura and Takahashi (1974) applied 5 µg/l diflubenzuron to outdoor aquaria. Populations of cladocerans and cyclopoid- and calanoid copepods all declined, but cladocerans recovered more slowly (three weeks) than the copepods. Kingsbury *et al.* (1987) treated two lakes with diflubenzuron. The initial concentrations were 5.9 and 13.8 µg/l. Cladocerans and copepods were reduced considerably, but recovery occurred within three weeks to two months with copepods recovering earlier than cladocerans.

Higher dose rates were applied by Ali and Mulla (1978a) to natural lakes. After doses of 110 and 220 g a.i./ha densities of *Daphnia* sp. (Branchiopoda, Cladocera) and *Diaptomus* sp. (Copepoda, Calanoida) were reduced, but another cladoceran, *Bosmina longistris* and a cyclopoid copepod, *Cyclops* sp., tolerated the treatments. Recovery of the affected species occurred within 1-4 weeks. After a different treatment by Ali and Mulla (1978b) at 156 g a.i./ha effects were much more severe. All groups of zooplankton were eliminated and the recovery was much slower (11 weeks to 6 months depending on the group).

Especially striking was the very large increase in the numbers of *Paradiaptomus rex* after the diflubenzuron treatments in this study. It may be that this species of calanoid copepod became so numerous because two other dominating organisms, *Streptocephalus* spp. and *Diaphanosoma* sp. were eradicted, a possible indication of interspecific competition.

The diflubenzuron treatments had a slight and temporary adverse effect on nymphs of *Anisops* sp. (Hemiptera, Notonectidae), but the number of adults was never reduced significantly. Nymphs of *Agraptacorixa senegalensis* (Hemiptera, Corixidae) became significantly more numerous from three weeks after the applications until sampling was stopped. The average number of adults on the contrary was depressed during the third week after treatment. No adverse effects were observed on the adults of *Eretes sticticus* (Coleoptera, Dytiscidae). Summarizing, the effects on aquatic insects in these temporary ponds seem much less severe than those on crustaceans. However, for several insect species the amount of material collected with the present sampling methods was probably too small for a proper analysis.

Larvae of other aquatic insects are known to be sensitive to diflubenzuron, both under laboratory conditions (Miura and Takahashi, 1974; Nebeker *et al.*, 1983) and in the field (Farlow *et al.*, 1978). Diflubenzuron treatments especially affect different mosquito larvae (Ali and Mulla, 1977 and 1978a). A related group, larvae of Chironomidae, were sometimes very numerous in the ponds studied here (up to 3,000/m^2), but they appeared and disappeared rapidly at different times in individual ponds. When the treatments were conducted there was not a single pond where they were abundant.

The groups most affected by diflubenzuron in this study are all pelagic branchiopod crustaceans. Their exact function in the observed temporary ecosystems is not known, but in general these groups feed on algae and probably also on bacteria and yeasts. Since these same groups, notably *Streptocephalus* spp., were very numerous before the treatments, the pesticide applications may temporarily have reduced the total consumption of algae, bacteria and yeasts in the ponds. If an algal bloom would have resulted from this it might have been detected by an increase in pH- and DO-values. The pH, however, seemed unaffected and DO-measurements were not taken at the time. The increase of the numbers of *Paradiaptomus rex* after the treatments was already mentioned. Possibly this species took over the functions of the affected species and was able to keep algal densities at a normal level.

Fenitrothion

The average applied dose of 506 g a.i./ha fenitrothion resulted in an average initial concentration of 80 µg/l. The average half-life of fenitrothion in the aqueous phase was 43 hours. The relative contributions of the different removal processes in the ponds are not known. In other experiments hydrolysis, photolysis and adsorption to sediments were of different importance, depending on the environmental conditions (Greenalgh *et al.*, 1980; Weinberger *et al.*, 1982; Maguire and Hale, 1980; Durand *et al.*, 1991; WHO, 1986). However, it is doubtful if photolysis plays an important role in the ponds because of their opaqueness. In river water the half-life of fenitrothion was 50 hours (information brochure of manufacturer Sumitomo).

The treatments affected a vast range of different aquatic insects (Table 2.5 and Figures 2.14-2.16). Given the rapid appearance of victims on the water surface the effect was very acute. Most of the victims were aquatic hemipterans or coleopterans and both young stages and adults were killed. Pelagic and more benthic species were equally affected. We have no indication that any of the abundant species of Hemiptera or Coleoptera were not affected. However, for most species, except *Anisops* spp. and *Agraptocorixa senegalensis* adults, the effects could not be quantified. Many species found as victims of the treatments were simply not or rarely captured with the applied sampling methods. Others were captured in such a low numbers that statistical analysis was unsatisfactory.

Anisops spp. nymphs were reduced more severely and recovered more slowly than adults (three weeks vs. one week respectively). Therefore the quick recovery observed for the adults could not have occurred through reproduction, because high numbers of nymphs would have appeared before the adult populations recovered. This leaves only migration as the mechanism for initial adult recovery, followed by reproduction and reappearance of nymphs. For other species the mechanism for recovery was not as evident, but individuals of virtually all affected species, including some of the rarely captured ones, were found within days or a few weeks after the treatments. Adults of these species are capable of flying as well.

Another group that was affected by fenitrothion were Odonata larvae. In Pond 6 and 11 larvae of *Libellulidae*, *Aeshnidae* and *Lestidae* were found as victims. From these three only *Lestidae* were captured more regularly in the scoop nets, but their numbers were still too low to determine the effects on the populations. Most larvae of Odonata are benthic predators that live on the sediment surface.

Probably all insect species in this study affected by fenitrothion, except hydrophilid beetles, are predators although their exact prey remains unknown.

In field experiments where rivers or the vicinity of rivers were treated with fenitrothion downstream drift and mortality of aquatic insect larvae increased (Yasuno *et al.*, 1981; Eidt, 1981; Morrison and Wells, 1981). In laboratory tests with different Plecoptera larvae LC50-values were 4-17 µg/l (Mayer and Ellersieck, 1986; Woodward and Mauck, 1980). The LC50's for different dipteran larvae varied between 2.6 and 11 µg/l (Mayer and Ellersieck, 1986; Sanders *et al.*, 1983; Parsons and Surgeoner, 1991). These data indicate that fenitrothion is generally highly toxic to larvae of different groups of aquatic insects.

Fenitrothion had no effect on *Streptocephalus* spp. The cladocerans *Moina* sp. and *Diaphanosoma* sp., however, were significantly reduced. The maximum reductions were 100% for both species, but the first recovered after three weeks and the second after four weeks. While *Diaphanosoma* sp. was not able to recover during the same rainy season from the diflubenzuron treatments, it did in the case of fenitrothion. This suggests that recovery in the diflubenzuron ponds was not prevented by limited seasonal reproduction of the species, but possibly by prolonged harmful concentrations of diflubenzuron in the suspended matter and/or the sediment of the ponds.

Different EC50- and LC50-values for the cladoceran branchiopod *Daphnia magna* range from 1.6 to 11 µl (LeBlanc, 1984; Takimoto *et al.*, 1987; Sanders *et al.*, 1983) considerably lower than the average initial concentration in the ponds (80 µg/l). However, these values are not in agreement with the observed absence of effects on the anostracan branchiopod *Streptocephalus* spp. For *Gammarus limnaeus* (Amphipoda) 24h-LC50's were 53-56 µg/l (Mayer and Ellersieck, 1986) and 96h-LC50's were 4.3-8.8 µg/l (Woodward and Mauck, 1980; Sanders *et al.*, 1983). A 24h-EC50 of 2.2 µg/l for *Palaemon paucidens* (Takimoto *et al.*, 1987) and a 24h-LC50 of of 0.07 µg/l for larvae of *Macrobrachium lamerii* (Sarojini *et al.*, 1986) indicate that decapod shrimps are also very sensitive to fenitrothion.

During the pilot-study of the LOCUSTOX project the decapod shrimps *Caridina africana* and *Palaemonetes africanus* in irrigation ponds were virtually eradicated by 338 g a.i./ha chlorpyrifos and 550 g a.i./ha fenitrothion, while diflubenzuron at 35 g a.i./ha caused no effects on the two species.

Methodology

Residue analyses

The initial concentrations measured in the aqeous phase of the water were quite variable. These differences are most likely due to environmental factors. The samples for the analyses of the initial concentrations were taken one hour after each treatment from the top of the water surface. Differences in physical circumstances such as wind turbulence and different amounts of suspended matter may have caused different concentrations in the water's top layer. Other sampling methods, such as the pipe device described by Boltovskoy (1990) may give more reliable results.

For fenitrothion half-lives could be calculated, but diflubenzuron disappeared from the aqueous phase within one day. The only way to determine the half-life of dissolved diflubenzuron would be to sample several times shortly after treatment. However, this would be logistically difficult and ecologically not very useful.

Pesticide residues should be measured not only in the aqueous phase, but also in suspended matter and the sediment. The adsorption process and its relative importance for different pesticides in the ponds (and under tropical circumstances) is unknown and would be more clearly defined.

In situ bioassays

The mortalities of *Streptocephalus* in the control tests were unpredictable and often much too high. No explanation for this was found. Factors that could have contributed to the low survival rate in the controls are the small space of the cages, the high densities of the animals in them, high surface water temperatures or an increased effect of bacteria and parasites that can penetrate the mesh. Survival rates however, were very variable from one pond to another and in time. The high survival rates after the fenitrothion treatments compared to the controls are very difficult to explain unless we assume the existence of an unfavorable factor in some ponds that disappears after the applications.

Anisops spp. might be a more suitable group of organisms for *in situ* bioassays because they are very abundant and suffer a more acute mortality from fenitrothion and possibly also from other insecticides with a fast mode of action.

However, it is questionable if bioassays on site with either of these two organisms do in fact provide any additional information, because effects on their population densities are so easily detected by scoop-net sampling.

Density sampling

The method of quantitative scoop-netting proved to be satisfactory for a number of organisms from the pelagic zooplankton, the pelagic macroinvertebrates and the epibenthic macroinvertebrates, but only when their densities were sufficiently high. Species with lower relative densities, for instance large predatory beetles, were not captured regularly enough to permit density estimations. Other methods, such as trap devices, might be more suitable for monitoring densities of these groups, but if they are too efficient they may interfere with those densities.

To our knowledge no easy quantitative sampling method is known for benthic zooplankton. The pond sediments are full of detritus and mud that make it very time consuming to find these small animals in a sediment sample.

For larger macroinvertebrates, such as *Eretes sticticus* and *Agraptocorixa senegalensis*, whose densities are low (1-10/m^2), the solution might be to increase the sample size of the scoop net samples. Since their dimensions allow them to be recognized by eye they could be separated from the other more numerous species in the field. To avoid having too much material of the most abundant species, separate samples of different lengths could be taken for groups of organisms with different densities.

The way the benthic and epibenthic fauna was sampled in 1991 was very exhaustive and time consuming. The reason for also taking samples of the sediment itself was the abundance of chironomid larvae. However, these were not suitable for the investigation of side-effects in the ponds (Table 2.1). During a subsequent field trial only the epibenthic fauna above the sediment should be sampled, probably by scratching the square scoop-net over the top of the sediment, thus avoiding pollution of the sample by mud and detritus. This will save a great deal of work in the field.

The numbers of some zooplankton species fluctuated greatly. We think that movement in shoals could have contributed to this phenomenon. To study this hypothesis and the spatio-temporal distribution of other species a separate study was carried out in August 1992 (Lahr and Diallo, in prep.). In the case of a very irregular distribution of important species it is advisable to take more, but smaller pelagic samples at different stations in one pond.

Statistical analysis of population densities

As pointed out earlier, it is felt that the currently applied method is not satisfactory. Although general, average patterns of side-effects can be described, information on the variability of the response of populations in different ponds is not optimally used. Some sort of analysis of variance (ANOVA) should be ultimately applied, but there are several problems. The study was set up according to BACI-design and each treatment was replicated five or six times. The treatments were evenly but not randomly distributed over different ponds in the study area to correct for possible differences caused by geographical factors. The question is whether and how an ANOVA can be applied correctly when such a systematic design is used.

Even if the treatments are considered to be replicates, it is difficult from a biological point of view to equate the populations of the same species in different ponds. Physical circumstances are different from pond to pond. Some ponds are larger or deeper than others, some of them are more frequented by cattle. Some of them are close to other temporary ponds, while others are rather isolated. Local differences in rainfall cause many ponds to be filled at different times during the early rainy season. The aquatic vegetation was equally variable. Organisms interact with their environment and different circumstances will result in different population densities, which was the case for the observed ponds. Why are some species abundant in only a few ponds? How does the unique abundance of several conchostracan species in Pond 15 affect the densities of other species that were of greater importance in this study on side-effects? How does the relative isolation of some ponds interfere with the recovery speed when the recovery mechanism is aerial migration from other ponds?

The questions are too numerous to be addressed immediately. It might be decided eventually to apply ANOVA-techniques and different results for individual ponds may be related to differences in local circumstances. Even when an ANOVA could not be strictly applied to these pond systems, the results of the analysis would reveal more about the importance of variability between ponds.

CONCLUSIONS

The effects of diflubenzuron and fenitrothion sprayed at dosages used for controlling Desert Locust on densities of aquatic invertebrates in temporary ponds are summarized in Table 5.1. A list of victims of the fenitrothion treatments was already given in Table 3.5.

Diflubenzuron caused considerable effects on pelagic anostracans of the genus *Streptocephalus* and one cladoceran, *Diaphanosoma* sp. Populations of these species did not recover until the next rainy season, one year later, when the ponds were filled again. One species of calanoid copepod, *Paradiaptomus rex*, increased significantly following the applications, probably because of reduced competition by the eradicated species. Diflubenzuron had little or no effect on other cladocerans and copepods, ostracods, and different aquatic insects.

Fenitrothion did not affect anostracans and ostracods. The cladoceran *Diaphanosoma* sp. was reduced significantly, but recovered after one month. As with the diflubenzuron treatments, numbers of *P. rex* increased compared to the controls. Other cladoceran and copepod species showed no significant effects. The most vulnerable groups of invertebrates were the aquatic insects, notably hemipterans and coleopterans. Almost all species that were found in the ponds before treatment appeared floating dead on the water surface shortly after the treatments. Most of the affected populations recovered after one to several weeks. For *Anisops* spp. (Hemiptera, Notonectidae) evidence was found that recovery took place by aerial migration. Since many adult aquatic insects are capable of flying, this might be the most important recovery mechanism for most affected insect species.

The products seem to interfere with organisms at different trophic levels of the temporary pond ecosystems. Diflubenzuron especially reduces the numbers of abundant herbivores (also grazers or filter feeders), while fenitrothion affects most invertebrate predators. Both products could therefore significantly change interactions in the ecosystem as a whole, but via different pathways.

Table 2.6: Summary of effects of diflubenzuron and fenitrothion on population densities of aquatic invertebrates in temporary ponds. When effects lasted longer than 6 weeks, no recovery was observed until the next rainy season.

Species		Diflubenzuron				Fenitrothion			
		effect	start effect after treatm.	duration of effect	maximum % change	effect	start effect after treatm.	duration of effect	maximum % change
Branchiopoda	Anostraca								
	Streptocephalus spp.	-	1/2 wk.	> 6 wks.	100	0			
	Cladocera								
	Moina sp.	-	1	1/2 wk.	100	-	1 wk.	1/2 - $1_{1/2}$ wk.	100
	Diaphanosoma sp.	-	1/2 wk.	> 6 wks	100	-	2 wks.	1 wk.	100
Copepoda	Cyclopoida								
	Thermocyclops decipiens	-	3 wks.	1/2 wk.	90	0			
	Calanoida								
	Paradiaptomus rex	+	1 wk.	> 6 wks.	12,90	+	1 wk.	> 6 wks.	6,565
Ostracod	Cypridae								
	Heterocypris symmetrica	0				0			
Hemiptera	Notonectidae								
	Anisops spp. adults	+ +	3 wks. 6 wks.	$1_{1/2}$ wk. > 1 wk.	180	-	1 day	1/2 wk.	87
	Anisops spp. nymphs	- +	2 wks. $4_{1/2}$ wks.	1/2 wk 1 wk.	95 337	-	1 day	$2_{1/2}$ wks.	100
	Corixidae								
	Agraptocorixa senegalensis adults	-	2 wks.	2 wks.	95	-	1 wk.	> 6 wks.	100
	A. senegalensis nymphs	+	3 wks.	2-4 wks.	3,150	0			
Coleoptera	Dytiscidae								
	Eretes sticticus	+	2 wks.	1/2 wk.	594	-	3 wks.	1/2 wk.	90

Effects: 0 no significant effect
- significant reduction of average density
+ significant increase in average density

RECOMMENDATIONS

The use of diflubenzuron and fenitrothion in Desert Locust control

Blanket spraying of diflubenzuron for the control of Desert Locust hopper bands should be avoided in areas where temporary pools and ponds are abundant. Even when no temporary water bodies are situated in the area to be treated, buffer zones of considerable distance from temporary waters in nearby areas should be employed to avoid any possibility of downwind drift deposits.

Care should also be taken to avoid contamination of temporary ponds with fenitrothion. Especially when large surfaces are treated, affected populations of aquatic insects will recover more slowly through aerial migration than in this study, because distances to untreated areas from which migration occurs will be longer.

Washing spraying equipment in waterbodies should be avoided altogether.

Methodology

The method for sampling the water surface for pesticide residues should be improved to give better, more representative results. Pipe sampling devices seem a good solution that need testing. Before field treatments with any other products the available data on their fate and behaviour in the aquatic environment should be studied in more detail to allow for a better estimation of the sampling frequency needed to calculate half-lives and other parameters properly.

The efficiency of *in situ* bioassays with anostracans should be improved by reducing mortalities in the controls. Deeper and larger cages may be a solution to the problems encountered. In addition tests with the pelagic notonectid *Anisops* sp. might be carried out.

The size and distribution of scoop net samples should be adapted to become more efficient for animals with low population densities or swimming in shoals. Sampling the sediment itself can be suspended, because the densities of the only organisms captured this way, chironomid larvae, were too variable. Instead it is suggested to take "scratch"-samples on top of the sediment to catch the epibenthic fauna.

A proper statistical method must be found to analyse the data on population densities following pesticide treatments in different ponds. Variation in densities from pond to pond should be an integrated part of this statistical evaluation.

Future research

In order to get a better idea of the fate of applied pesticides in temporary ponds, residues and relevant degradation products should also be measured in suspended matter and the sediment. When sampling in different ponds at short intervals in a large area like the region of Nioro du Rip poses too many logistical problems, the use of artificial test systems like mesocosms may be taken into consideration. These allow tests with different products under more controlled circumstances.

Streptocephalus spp., *Anisops spp.* and *Diaphanosoma* sp. could be used for the development of laboratory toxicity tests that will provide initial screening and contribute to the decisions on the acceptability of certain uses of pesticides in the region. Studies should therefore be undertaken to determine the right hatching and testing circumstances. Preliminary tests with *Streptocephalus* spp. and *Anisops* spp. have already been carried out at the laboratory in Nioro du Rip with specimens captured in the field. The results of these tests confirmed the effects of diflubenzuron and fenitrothion observed in the field. The tests also revealed that the pyrethroid deltamethrin may have effects on both species in temporary ponds at its operational dose against Desert Locust, while the carbamate bendiocarb would probably affect neither of them.

A better knowledge of the taxonomy of different groups of species will allow more accurate identifications in the future. When species from the currently distinguished groups can be further separated, some of the material collected for this study should be re-identified to study effects on individual species and their recovery times.

Future research should include field tests with other pesticides currently used in Desert Locust- and grasshopper control. Tests should especially be carried out with pesticides belonging to other chemical groups such as synthetic pyrethroids and carbamates. When the field trial methodology becomes more developed, alternative pesticides such as biological products may be tested for side-effects following preliminary screening for toxicity in the laboratory.

Of great importance is the acquisition of better information on the organisms in the ponds, their biology and their ecology. Foodweb relationships, spatio-temporal distributions, reproduction, migration patterns and abundance, among others, should be studied whenever possible.

REFERENCES

Ali A. & M.S. Mulla (1977) The IGR diflubenzuron and organophosphorus insecticides against nuisance midges in man-made residential recreational lakes. J. Econ. Entom. 70 (5): 571-577.

Ali A. & M.S. Mulla (1978a) Impact of the insect growth regulator diflubenzuron on invertebrates in a residential-recreational lake. Arch. Environm. Contam. Toxicol. 7: 483-491.

Ali A. & M.S. Mulla (1978b) Effects of chironomid larvicides and diflubenzuron on nontarget invertebrates in residential-recreational lakes. Ann. Entom. Soc. America 7 (1): 21-27.

Banister K. (1990) Chapter V: Fish. In: Everts, J.W. ,ed. Environmental effects of chemical locust and grasshopper control. A pilot study. Report project ECLO/SEN/003/NET, FAO Rome.

Berghen C. Van den (1990) La végétation des mares temporaires au Sénégal oriental. Rev. Hydrobiol. trop. 23 (2): 95-104.

Boltovskoy A. (1990) Muestreador de agua tubular con valvula de cierre autonomo. Rev. Hydrobiol. trop. 23 (2): 171-174.

Cunningham P.A. (1986) A review of toxicity testing and degradation studies used to predict the effects of diflubenzuron (Dimilin®) on estuarine crustaceans. Environm. Poll. (ser.A) 40: 63-86.

Cunningham P.A. and L.E. Myers (1986) Dynamics of diflubenzuron (Dimilin®) concentrations in water and sediment of a supratidal saltmarsh site following repetitive aerial applications for mosquito control. Environm. Poll. (ser.A) 41: 63-88.

Durand, G. D. Barcelo and J. Albaiges (1991) On the photolysis of selected pesticides in the aquatic environment. Toxicol. Environm. Chem. 31-32: 55-62.

Eidt D.C. (1981) Recovery of aquatic arthropod populations in a woodland stream after depletion by fenitrothion treatment. Can. Entom. 113: 303-313.

Everts J.W. ,editor (1990) Environmental effects of chemical locust and grasshopper control. A pilot study. Report project ECLO/SEN/003/NET, FAO Rome.

FAO (1992) The Desert Locust guidelines. IV. Control. FAO, Rome.

Farlow J.E., T.P. Breaud, C.D. Steelman & P.E. Schilling (1978) Effects of the insect growth regulator diflubenzuron on non-target aquatic populations in a Louisiana intermediate marsh. Environm. Entom. 7 (2): 199-204.

Greenalgh R., K.L. Dhawan and P. Weinberger (1980) Hydrolysis of fenitrothion in model and natural aquatic systems. J. Agric. Food Chem. 28: 102-105.

Hurlbert S.H. (1984) Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187-211.

Kingsbury P., K.M.S. Sundaram, S. Holmes, R. Nott & D. Kreutzweiser (1987) Aquatic fate and impact studies with Dimilin. File rep. nr. 78. Forest Pest Managem. Inst., Can. Forestry Service, Sault Ste. Marie, Ontario, Canada.

Lahr (1990) Chapter IV: Aquatic invertebrates. In: Everts, J.W. ,ed. Environmental effects of chemical locust and grasshopper control. A pilot study. Report project ECLO/SEN/003/NET, FAO Rome.

LeBlanc G.A. (1984) Interspecies relationships in acute toxicity of chemicals to aquatic organisms. Environm. Toxicol. Chem. 3: 47-60.

Maguire R.J. and E.J. Hale (1980) Fenitrothion sprayed on a pond: kinetics of its distribution and transformation

in water and sediment. J. Agric. Food Chem. 28: 372-378.

Mayer F.L. and M.R. Ellersieck (1986) Manual of acute toxicity: interpretation and data base for 410 chemicals and 66 species of freshwater animals. Resource Publication 160. U.S. Dept. Interior, Fish and Wildlife Service, Washington, D.C.

McAlonan W.G. (1976) Effects of two insect growth regulators on some selected saltmarsh non-target organisms. MSc Thesis, University of Delaware, USA.

Miura T. & R.M. Takahashi (1974) Insect developmental inhibitors. Effects of candidate mosquito control agents on nontarget aquatic organisms. Environm. Entom. 3 (4): 631-636.

Morrison B.R.S. and D.E. Wells (1981) The fate of fenitrothion in a stream environment and its effect on the fauna, following the spraying of a Scottish forest. Sci. Tot. Environm. 19: 223-252.

Nebeker A.V., Ph. McKinney & M.A. Cairns (1983) Acute and chronic effects of diflubenzuron (Dimilin) on freshwater fish and invertebrates. Environm. Toxicol. Chem. 2: 329-336.

Nieser (1993) Clé des Notonectides de Nioro du Rip et Richard-Toll (Sénégal). Internal document of Project ECLO/SEN/003/NET, FAO, Senegal.

Parsons J.T. and G.A. Surgeoner (1991) Effect of exposure time on the acute toxicities of permethrin, fenitrothion, carbaryl and carbofuran to mosquito larvae. Environm. Toxicol. Chem. 10: 1219-1227.

Sanders H.O., M.T. Finley and J.B. Hunn (1983) Acute toxicity of six forest insecticides to three aquatic invertebrates and four fishes. Technical Paper nr. 110. U.S. Dept. Interior, Fish and Wildlife Service, Washington, D.C.

Sarojini R., R. Nagabhushanam and Sr. Avelyn Mary (1986) Effect of fenitrothion on reproduction of the freshwater prawn *Macrobrachium lamerii*. Ecotoxicol. Environm. Safety 11: 243-250.

Sokal R.R. & F.J. Rohlf (1981) Biometry. The principles and practice of statistics in biological research. Second edition. W.H. Freeman and Company, New York.

Stewart-Oaten A., W.W. Murdoch & K.R. Parker (1986) Environmental impact assessment: "Pseudoreplication" in time? Ecology 67 (4): 929-940.

Takimoto Y., M. Oshima & J. Miyamoto (1987) Comparative metabolism of fenitrothion in aquatic organisms. III. Metabolism in the crustaceans *Daphnia pulex* and *Palaemon paucidens*. Ecotoxicol. Environm. Safety 13: 126-134.

Touart L.W. & K.R. Rao (1987) Influence of diflubenzuron on survival, molting, and limb generation in the grass shrimp, *Palaemonetes pugio*. In: Pollution ecology of estuarine organisms. W.B. Vernberg, A. Calabrese, F.P. Thurberg and F.J. Vernberg eds., Univ. of South Carolina Press.

Weinberger P., R. Greenalgh, R.P. Moody and B. Boulton (1982) Fate of fenitrothion in aquatic microcosms and the role of aquatic plants. Environm. Sci. Technol. 16: 470-473.

W.H.O. (1986) Organophosphorus insecticides: a general introduction. Environmental Health Criteria nr. 63. World Health Organization, Geneva.

Wilson J.E.H. & J.P. Costlow (1986) Comparative toxicity of two Dimilin formulations to the grass shrimp, *Palaemonetes pugio*. Bull. Environm. Contam. Toxicol. 36: 858-865.

Woodward D.F. and W.L. Mauck (1980) Toxicity of five forest insecticides to cutthroat trout and two species of aquatic invertebrates. Bull. Environm. Contam. Toxicol. 25: 846-853.

Yasuno M., J. Okata, K. Saito, Y. Nakamura, S. Hatakeyama and S. Kasuga (1981) Effects of fenitrothion on benthic fauna in small streams of Mt. Tsukuba, Japan. Jap. J. Ecol. 31: 237-245.

CHAPTER 3 :

The effect of fenitrothion and diflubenzuron on natural enemies of millet pests in Senegal (the 1991 study).

SUMMARY

Fields planted with pearl millet (*Pennisetum typhoides*) in central Senegal were experimentally treated with insecticides for locust control during the rainy season of 1991. This was done to study their impact on natural enemies of millet pests and the likelihood of pest resurgence after locust control operations in millet.

Four fields of 1-2 ha's each were treated with fenitrothion at around 450 g a.i./ha, four were sprayed with diflubenzuron at around 60 g a.i./ha, while four others functioned as untreated controls. Presence of natural enemies was assessed using flight interception traps and direct counts. Different life stages of the millet head miner (*Heliocheilus albipunctella*), a primary candidate for resurgence, were sampled throughout the millet growing period. Effects of the treatments on the degree of parasitism of this pest was assessed using various techniques.

Fenitrothion considerably reduced populations of Hymenoptera, in particular the parasitoids *Cardiochiles spp.* (Braconidae) and *Mesa sp.* (Tiphiidae). Recovery was not complete at the time of harvest, the end of the study. Coccinellidae and dipteran natural enemies were not affected. No reduction was found in natural mortality of *H. albipunctella* eggs. Probably the most important observation was a temporary resurgence of *H. albipunctella* larvae, amounting to a 75% increase in larval peak density, in all fenitrothion treated fields. No effect was found on emergence rates of diapausing millet head miner chrysalids nor of *Copidosoma* parasitoids the year after treatment.

Diflubenzuron did not cause any reductions in natural enemy taxa monitored by flight interception traps, except for the braconid parasitoid *Cardiochiles spp.* The latter was affected at the very end of the growing season. This insecticide did not appear to cause a reduction in natural mortality of *H. albipunctella* eggs. A 50% reduction in peak density of *Heliocheilus* larvae was observed in diflubenzuron treated fields. However, a 110% increase in *Heliocheilus* chrysalid emergence as well as a 50% reduction in *Copidosoma* parasitoid emergence was found the year after treatment. These two effects combined would result in a 75% reduction in the number of *Copidosoma* parasitoids available per emerged millet head miner the year after treatment, when compared to the unreated fields.

A number of taxa of beneficial arthropods has been identified which may be affected by treatments carried out earlier in the season.

INTRODUCTION

The crop which is probably most treated with insecticides during locust or grasshopper control campaigns in Africa south of the Sahara is pearl millet (*Pennisetum typhoides* (Burm.) Stapf and Hubt.). The use of insecticides may have certain side-effects which ultimately increase rather than decrease pest pressure on these crops. This involves the development of resistance of the target pest to the insecticide used, the resurgence of the target pest, or the development of secondary pests. The risk of resistance development in migratory locusts is generally considered to be remote. The combination of the relatively long generation time, and the high mobility and subsequent mixing of the sprayed and unsprayed populations, would effectively counteract the appearance of resistant populations. Pest resurgence and secondary pest development mostly occurs through the reduction of natural enemy populations which allows the pest population to "escape" regulation by the natural enemy. Waage (1989) lists the following ways through which insecticides may reduce natural enemy effectiveness:

1. insecticide kills natural enemies
2. insecticide has a sublethal effect on natural enemies, reducing survival or foraging activity
3. insecticide enhances pest reproduction, allowing pests to escape control by natural enemies
4. insecticide changes local pest distribution, causing temporary emigration of natural enemies or reduced foraging efficiency
5. insecticide synchronyzes pest population, causing the local extinction of natural enemy populations

Often several of the above processes will interact.

Waage (1989) suggests that the pest species posing the largest risk of resurgence are those which are substantially reduced by their natural enemies and which are less affected by the insecticide than their natural enemies .

A large number of insects attack pearl millet. In an Africa-wide review Geddes (1990) considers the millet earhead miner (*Heliocheilus albipunctella* De Joannis) as being the most damaging crop pest in the Sahelian climate zone, followed by grasshoppers such as *Oedaleus senegalensis* (Krauss, 1877) and *Kraussaria angulifera* (Krauss, 1877). However, the threat posed by different pests varies from year to year and other pests than the above may locally be more damaging (Jago 1992).

Heliocheilus albipunctella mines the earheads of millet plants. Its larvae will therefore be fairly well protected from treatments with contact insecticides. Soon after eclosion, which takes place on the more exposed outer side of the earhead, the millet earhead miner moves underneath the flowers. A large number of parasitoids and predators have been found to attack *H. albipunctella* (table3. 1). The degree of parasitism quoted in this table has limited value, however. Most of these figures have been obtained during natural enemy survey studies which are not necessarily well adapted to assessing actual parasitism rates. It is unlikely that any of these figures represent generational mortality caused by the given natural enemy (Van Driesche 1983). Most if not all of the natural enemies are highly active on the millet plant to search for their host or prey and are thus potentially highly exposed to insecticidal treatments.

For the above reasons the millet earhead miner almost ideally fulfills Waage's (1989) criteria of a species with a high chance of resurgence: the pest species is protected most of its life and the natural enemies play a potentially important role in pest regulation while being highly exposed to insecticides if treatments take place. Therefore, we have concentrated in this study specifically on the effects of locust control treatments on natural mortality factors of *H. albipunctella*. In addition we have studied effects of the treatments on a number of natural enemies of other crop pests, including grasshoppers, as well as on the presence of *Coniesta ignefusalis* Hmps., another stemborer which in some parts of the Sahel may cause important crop damage. A short description of the phenology of *Heliocheilus albipunctella* is given in fig.3.1.

Two insecticides were included in the study: fenitrothion, an organophosphate insecticide which acts mainly by contact (the most used insecticide against desert locust and grasshoppers in Africa) and

the phenyl-benzoyl-urea insect growth regulator diflubenzuron. The latter compound seems to work mainly by ingestion and disturbs chitin synthesis in the arthropod. Therefore it is especially toxic to the larval stages of arthropods which still need to moult. Diflubenzuron is not yet used in desert locust control but, together with other compounds within the same group of IGR's, it is considered a promising candidate for desert locust hopper band control. It was included in this study because of its principally different mode of action from the more classical contact insecticides.

Table 3.1: Arthropod natural enemies of *Heliocheilus albipunctella*, the millet earhead miner, in Senegal

Family	species	type	importance
COLEOPTERA			
Carabidae	*Chlaenius spp.* *Graphipterus obsoletus* (Olivier) *Harpaglossus laevigatus* (Dejean)	predator	not known
DIPTERA			
Asilidae	*Promachus sp.*	predator	not known
Bombyliidae	*Thyridanthrax sp. nr. kappa* (Bowden)	parasitoid	up to 4% of chrysalids paras.
Tachinidae	*Goniophthalmus hali* Mesnil	parasitoid	rarely found
	Palexorista quadrizonula Thomson	parasitoid	up to 16% of larvae paras.
HEMIPTERA			
Anthocoridae	*Orius sp.*	predator	not known
Reduviidae	*Eclomocoris fenestratus* (Klug) *Katanga olivrei* Schouteden	predator	rarely found
HYMENOPTERA			
Braconidae	*Bracon hebetor* Say	parasitoid	up to 14% of larvae paras.
	Cardiochiles sahelensis sp.n.	parasitoid	up to 30% of larvae paras.
Encyrtidae	*Copidosoma sp. nr. truncatellum* (Dalman)	parasitoid	up to 28% of late instar larvae paras.
Eumenidae	*Delta emarginatum emarginatum* L. and *Delta sp.*	predator	not known
Sphecidae	*Ammophila sp.*	predator	not known
Trichogrammatoidea	*Trichogrammatoidea ? lutea* Girault	parasitoid	up to 75% of eggs paras.
Vespidae	*Polistes sp.*	predator	not known
NEUROPTERA			
Chrysopidae	*Brinckochrysa sp.* *Chrysopa sp.* *Chrysoperla pudica* (Navás) *Mallada sp.*	predator	rarely found

Source: Bhatnagar (1987)

		H. albipunctella	natural enemies
PERIOD (LOCATION)	APPROXIMATE DURATION OF DEVELOPMENT	STAGE	
flowering (on/between flowers)	3-4 days	**EGG**	*Trichogrammatoidea sp.* *Copidosoma sp. nr. truncatellum (kills larvae only just before pupation)*
grain maturation (bores underneath grains	25-39 days (mostly 30-35 days)	**LARVAE**	*Braconidae* *Cardiochiles sahelensis* *Palexorista quadrizonula* *various predators*
end of grain maturation (pupation in soil)	diapause during dry season	**CHRYSALID**	*Thynnidanthrax sp.*
emergence generaly in period around millet flowering	5-7 days	**ADULT**	

Fig. 3.1: Life cycle of *Helocheilus albipunctella*, the millet earhead miner linked with some of its principal natural enemies. (Sources: Bhatnagar (1967), Ndoye (1991), Gahukar et al. (1986)).

STUDY SETUP AND METHODOLOGY

General study lay-out

Twelve farmer fields planted with with pearl millet (*Pennisetum typhoides*, variety Souna III) were selected for the study in late July 1991 around the village of Prokhane, in central Senegal (fig. 3.2). Growth stage in all fields at the time varied from emergence to early tillering (10-20 cm height). Most fields were chosen such that at least 60% of the directly neighbouring fields had crops other than millet, to reduce the possibility of reinvasion of the treated plots from neighbouring unsprayed millet fields. This was done to avoid underestimation of the impact of the insecticide which generally is applied to series of adjoining millet fields at a time.

Field size varied from 1 to 5 hectares. They were cultivated according to standard farmer practice, most of them not receiving any fertilizers. The Project staff did not intervene in cultivation apart from the insecticide treatments. No other pesticide applications were made by the farmer. All fields carried peanuts the preceding year.

Fields were assigned to four blocks of three. Each block contained a control field and two different treatments. Distances between neighbouring plots within a block was at least 100m but never more than 300m. Blocks, however, were spaced up to three kilometers apart.

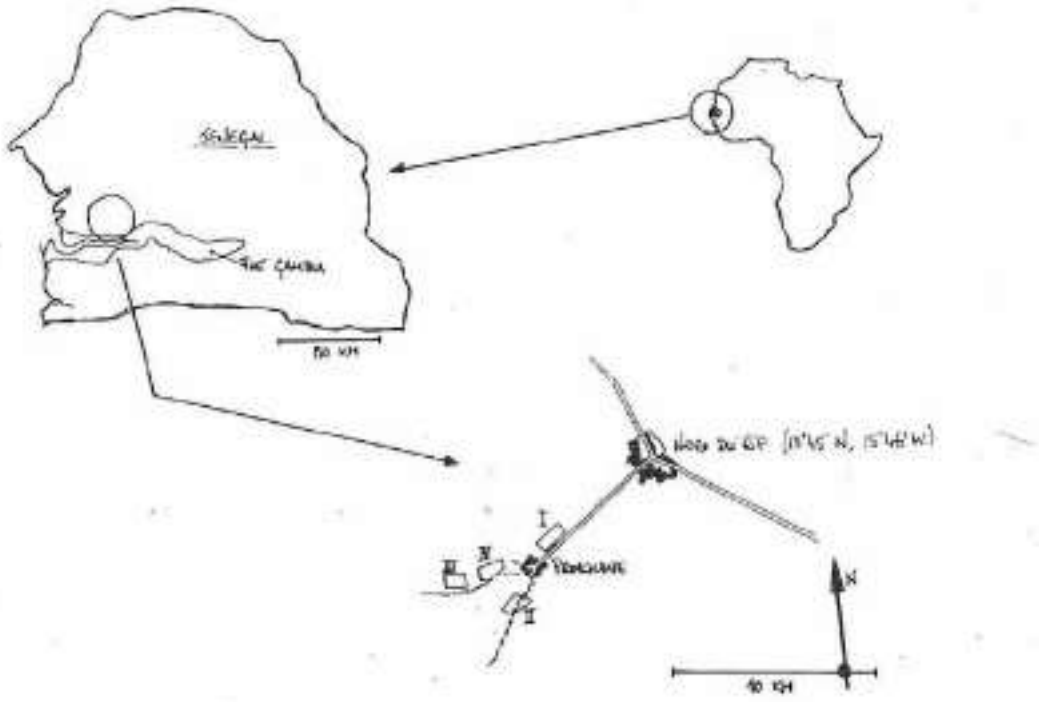


Fig. 3.2: Location of the study area near the village of Prokhane in central Senegal. Roman numbers refer to the four blocks of 3 fields each.

Insecticide treatments

Insecticides were applied on the different plots in the period from 11 to 14 September. Four plots were treated with the organophosphate fenitrothion (Sumithion® 500 g a.i./l ULV formulation); four others were sprayed with diflubenzuron (Dimilin® 450 g a.i./l ODC formulation). Fenitrothion was applied undiluted; diflubenzuron was diluted down to a 60 g a.i./l formulation with diesel oil before

application. Four control plots were left untreated.

All treatments were made using a Micro-ULVA® battery-powered spinning disk sprayer. The sprayer was equipped with four 1.5V dry cell batteries which gave a disk speed ranging from 6000 to 6400 rpm (measured on a pesticide loaded disk with a Vibratak® tachometer). According to manufacturers documentation this disk speed results in a droplet VMD varying between 80 and 100 µm. Spray passes were always approximately perpendicular to the direction of the wind. The sprayer head was held at the height of the millet ears (1.50 - 2 m). Track spacing was kept standard at 10 meters. Spray passes were marked by flagmen, one at each side of the plot.

Flow rate and operator walking speed were calibrated before application. 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 measured with the help of a calibrated distance wheel and a compass. Area dosage was subsequently calculated based on area treated and volume of insecticide used.

Windspeed was measured at the start and the end of each treatment using a cup-anemometer held at approximately 1.75 m. above the gound. Dry and wet bulb temperatures were determined with an aspired psychrometer which was whirled in the shade.

Insecticide deposition estimation

On fenitrothion plots a row of 10 oil-sensitive spray cards were put out in a line perpendicular to the spray tracks. Droplet deposition cards measured approximately 1 x 6 cm and were mounted, one vertically and one horizontally, on a washpeg. The pegs with the cards were then clipped on millet stalks at 1.4-1.75 m height (approximately 15 cm below the millet earhead), the oil-sensitive surface of the vertical card facing the wind. Droplets deposited on the cards were counted the evening following the treatment using a stereo-microscope. Since diflubenzuron does not stain well on the cards, droplet counts were not carried out for these treatments.

Insecticide residue analysis was performed on millet leaves and earheads which were sampled 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 gas chromatography (fenitrothion) or high performance liquid chromatography (diflubenzuron). A full description of the sampling procedures, extraction and analysis is given by Gadji (1993).

Arthropod assessments

Malaise traps

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

One malaise trap was erected in the centre of each plot. It had two opposed interception areas of 1 x 1.50 m. The tops of the traps were made out of fine, white mosquito netting, the lower part was dark red mosquito netting. The traps were always placed in such a way that the interception sides were facing east and west. The collection bottle at the top of the trap was filled with 4% formol in water.

Malaise traps were installed first on 29 and 30 July. Thereafter, collection bottles were changed on a weekly basis until harvest of the millet. Insect catches were taken back to the laboratory, washed with water and stored in 70% ethanol while awaiting further sorting.

Direct insect counts

Once a week, every field was visually inspected for the presence of *Heliocheilus albipunctella* (eggs or larvae) as well as beneficial arthropods such as *Alesia striata* (Coccinellidae) . Thirty to sixty millet hills, the number depending on the growth stage of the crop, were thoroughly inspected. Hills were chosen in a systematic way (e.g. every 5th, 6th or 7th hill in 3 or 4 rows) in the centre of the plot. The number of millet stems per observed hill was noted. Incidence was subsequently expressed as number of insects per stem.

Insect counts started on 20 August and continued until just before harvest of the millet.

Parasitism of Heliocheilus eggs

Once before and twice after treatment, eggs of *Heliocheilus albipunctella* were sampled to assess the rate of parasitism by *Trichogrammatoidea spp.* On each occasion 10 millet earheads which had emerged from the flagleaf but on which male flowers had not yet or only just started flowering were cut in each field. *Heliocheilus albipunctella* oviposits on these just emerged ears before male flowering. Ears were individually put in small paper bags and transported to the laboratory. There, the contents of the bags were inspected thoroughly and any eggs found were counted and carefully put in a petri dish. Subsequently, eggs were individually incubated in gelatine capsules at the ambient temperature of the laboratory (26-31 C). After a minimum of 15 days, emergence of *Heliocheilus* larvae or *Trichogrammatoidea* adults was scored under a stereo-microscope.

Parasitism of Heliocheilus larvae

Depending on the stage of maturation of the millet ears two or three times after treatment larvae of *Heliocheilus albipunctella* were sampled in each field. These larvae were put in glass pots at ambient laboratory conditions. Every pot contained a layer of slightly moist soil and a number of fresh parts of millet ears which were slit open lengthwise to facilite access for the larvae. Larvae were transfered to fresh millet ears every week. These pots were observed regularly for emergence of potential parasitoids until all larvae had either died or pupated. Living pupae were subsequently taken from the soil and transfered to glass tubes, closed off with a piece of cotton, until adult emergence the following year.

Incidence of stemborers

In the week of 15-22 October, just after the harvest of the millet by the farmers, 100 millet stems were collected at random in the centre of each field and taken to the laboratory. All stems were subsequently dissected (in the period of 24 October to 1 November) for presence of stemborers, in particular *Coniesta ignefusalis.*

Residual populations in the soil

To assess if the treatments had had any effects on residual populations of *Heliocheilus* pupae in the soil as well as certain natural enemies, soil samples were taken in all fields during the second two weeks of November. Ten holes, situated on the two medians, were dug in each field. Each sample 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*, as well as cocoons of *Cardiochiles sp.* and *H. albipunctella* larvae mummified by *Copidosoma sp.* were collected. Chrysalids, mummies and cocoons were put in glass tubes closed off with cotton and incubated at ambient laboratory temperatures untill emergence the year after

DATA ANALYSIS

Time series

The catches from the malaise traps result in short time series of ten observations (over 10 weeks) with an insecticide treatment during week 6. Such series are too short to perform standard time-series analysis. Therefore analysis of variance was chosen as method of statistical comparison based on the BACI (Before-After-Contol-Impact) principle (Stewart-Oaten et al. 1986, Underwood 1991).

All insect counts were transformed as ln(count+1) to improve normality of data and homogeneity of variances. For each block, the value of the untreated control was then subtracted from each of the treatment values. This basically results in a weekly transformed insect count for the treatment plot corrected for control fluctuations (called here "effect parameter" or E^p for convenience). A two-way ANOVA is subsequently carried out with as factors "block" and "period" (= before and after treatment), and using the E^p's per week as replicates. One such ANOVA is carried out independently for every post-treatment week, comparing the latter with the whole pre-treatment period. In other words, what is being tested is if the effect-parameter in any week after treatment differs significantly from the mean effect-parameter before. Since "block" is a second factor in the ANOVA, any variability caused by block (=location) differences can be separated from the impact of the treatment. The analyis is summarized below:

1. Effect parameter E^p = ln(treatment count+1) - ln(control count+1)

Table 3.2. Data to be analysed (* = E^p for that week):

	BEFORE TREATMENT WEEK					AFTER TREATMENT WEEK
	1	2	3	4	5	"n"
BLOCK						
I	*	*	*	*	*	*
II	*	*	*	*	*	*
III	*	*	*	*	*	*
IV	*	*	*	*	*	*

Table 3.3. ANOVA

SOURCE OF VARIATION	degrees of freedom
Subgroups	7
Block	3
Period (before - after)	1
Interaction	3
Within (error)	16

In case of a significant period effect (=treatment) as well as a significant interaction, i.e. the effect of the treatment depends in some systematic way on location (=block), the treatment effect is apparently not "generalized" over the whole study area. Such cases will as a rule, conservatively, be considered as showing no effect of the treatment. When exception is taken to this rule, this is explained in the text.

In the case of the malaise trap data, a small number of "missing values" had to be accounted for (i.e. 4 out of a total of 112 data points per analysis). These were non-valid catches, mostly collection bottles which had fallen. The missing values were estimated using the method described by van der Valk (1990), which calculates the means of the trends in catches in the blocks not having missing values. For the statistical analysis, however, missing value estimates were considered as "real"; i.e. the standard ANOVA procedures were executed, not correcting for the number of degrees of freedom (Sokal and Rohlf, 1981).

Other data sets

Whenever comparisons are made between data gathered at one specific point in time (e.g. density of eggs, percentage parasitism), Analysis of Variance (ANOVA), mostly following Randomised Complete Block Design (RCB), is used. Calculations are carried out by hand or using the MSTATC (Michigan State University) statistical package for personal computers. All analyses follow Sokal and Rohlf (1981). Absolute numbers (#) are as a rule transformed to LN(#+1); all percentages (%) to arcsin[square root (%/100)]. In ANOVA's without replication, Tukey's test for non-additivity was applied to verify the assumption of additivity of the main effects (absence of interaction) (Sokal and Rohlf, 1981)

After significant differences are found in the ANOVA, separation of means is done with Student-Neuman-Keul's Multiple Range Test. This test is a relatively conservative one among the multiple comparison tests: i.e. the actual chance of wrongly concluding that a difference between treatment and control fields exists is close to the intended error rate (in our case the experiment-wise error set at 5%) (Jones 1984). Otherwise said, if we conclude that a treatment causes an effect, there is less chance we are wrong.

Whenever data in the form of frequencies are pooled per treatment (i.e. data from the four blocks within a treatment are lumped), the G test of independence (Sokal and Rohlf 1981) has been used to test for differences between treatments.

Type I error levels (α) were set at $p < 0.05$ (i.e. up to 5% chance of concluding (wrongly) from the given statistical test that the treatments do have an effect while in reality they do not)
Type I error levels of $0.05 < p < 0.10$ will be considered as a "strong indicator" of a statistically significant effect.

Meteorological data

Rainfall and minimum and maximum temperatures were collected at the Nioro du Rip laboratory (Annex 3.2). Since rainfall tends to be localized, it may have differed somewhat on the experimental plots, though showing a similar pattern.

Annex 33. shows schematically the development of the millet in the fields during the study period.

RESULTS

Insecticide treatments

Details on spray parameters and results of the applications are given in table 3.2. All treatments, but the one of field 5, were carried out in steady winds varying from 1.1 to 2.5 m/s. In field 5 the tracks had to be re-aligned during spraying after an important change of wind direction had taken place. It does not appear that this has much influenced pesticide deposition, however (table 3.3). Windspeeds were sometimes at the lower end of what would be considered ideal for ULV drift spraying, but never dropped below 1 m/s. The measured area dosages differed always less than 10% from the recommended (nominal) dose rate for fenitrothion, and 0 - 16% from the nominal dose for diflubenzuron. Calibration of the applications was therefore good.

Pesticide deposition on vegetation and oil-sensitive cards is shown in table 3.4. Droplet deposition on the oil-sensitive cards is very variable, the Coefficient of Variation ranging from 40 to 120%. There is no clear correlation between mean droplet density and initial residue levels. Initial residue levels for fenitrothion are comparable to those reported by Ciss and Niane (1990) after experimental aerial locust control treatments in northern Senegal, but about 2-3 times higher than those measured by Dynamac (1988) in Sudan. The latter difference may have been caused by differences in application efficacy. Initial residue levels of diflubenzuron in all plots but field 3 are high relative to their application rate. They are comparable, however, to the residue levels found by Ciss and Niane (1990) after application on grazing land. Residues found on leaves were considerably higher than those on millet ears.

Table 3.4: Initial deposition of the insecticides on vegetation in the treated plots. All samples for residue analysis were taken 1 hour after treatment and were composites of a large number of subsamples from different locations in the field (Gadji 1993). Oil sensitive spray cards measured 6 x 1 cm; values shown are means of 10 cards.

Field	applied dose (g a.i./ha)	initial residues (mg/kg fresh vegetation)		spray card deposition (mean # drops/cm² [S.D.])	
		millet leaves	millet ears	vertical	horizontal
fenitrothion					
1	485	67.5	--	115 [101]	91 [111]
4	455	58.9	2.3	34 [37]	106 [131]
9	410	71.7	6.4	84 [54]	45 [36]
11	430	55.1	2.6	104 [41]	33 [15]
diflubenzuron					
3	60	9.2	--		
6	55	26.7	--		
8	50	38.6	2.3		
10	55	33.3	--		

Table 3.5: Treatment parameters for the fields sprayed with fenitrothion and diflubenzuron at nominal application rates of 450 g a.i./ha and 60 g a.i./ha respectively. All applications were carried out with a hand-held Micro-ULVA® spinning disk sprayer; track spacing was 10 m.

Plot	date of treatment	hour begin-end[3]	restrictor nozzle	flow rate (ml/min)	number of passess	area treated (ha)	volume applied (ml)	area dose[1] (g a.i./ha)	temperature (C) begin-end	relative humidity (%) begin-end	windspeed (m/s) begin-end
FENITROTHION											
1	12 Sep 91	16^{51}-17^{33}	orange	50	8	0.99	964	485	34-33	71-76	1.9-1.7
4	12 Sep 91	11^{11}-12^{55}	orange	50	8	1.3	1180	455	32-32	65-71	1.4-1.1
9	11 Sep 91	15^{51}-16^{20}	orange	50	17	1.56	1273	410	25-27	88-82	1.1-0.6
11	12 Sep 91	09^{51}-10^{15}	orange	50	16	1.89	1620	430	27-31	85-76	1.7-1.8
DIFLUBENZURON											
3	14 Sep 91	15^{31}-16^{45}	red	55	8	0.93	945	60	33-32	69-71	1.4-1.1
5	14 Sep 91	17^{46}-18^{45}	red	55	17^{2}	1.56	1470	55	33-27	74-85	1.7-2.5
8	13 Sep 91	09^{16}-10^{10}	red	55	10	2.25	1907	50	28-30	93-83	1.4-1.7
10	13 Sep 91	11^{26}-12^{55}	red	55	15	2.24	2010	55	31-34	80-71	1.4-1.1

[1]: area dose rounded off to nearest 5 g/ha
[2]: due to a change in wind direction, spray tracks were re-aligned halfway the treatment
[3]: data for begin and end of treatment

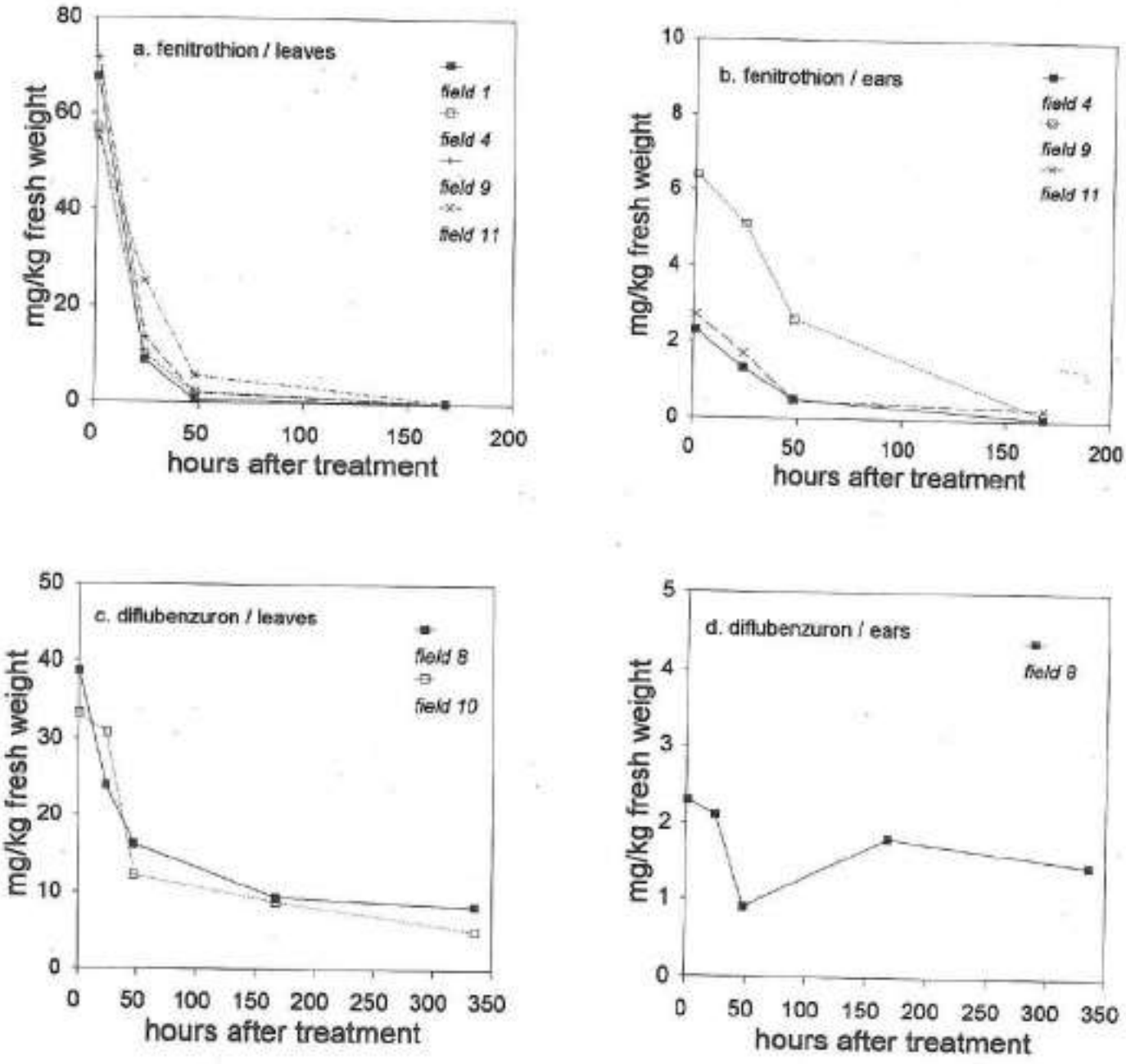


Fig. 3.3: Persistence of residues of fenitrothion and diflubenzuron after ULV application in millet fields. a:fenitrothion on millet leaves, b:fenitrothion on millet ears, c:diflubenzuron on millet leaves, d:diflubenzuron on millet ears. All data from Gadji (1993).

Insecticide persistence

For a number of fields insecticide persistence was followed over time (fig.3.3). Fenitrothion residues on millet leaves disappeared rapidly. Gadji (1993) estimated a half-life of 12 to 18 hours. Fenitrothion residues on millet ears, though much lower initially than on leaves, disappear more slowly, with an estimated half-life of 31-40 hours. Diflubenzuron persisted much longer, as was expected from

earlier studies. Half-life on millet leaves ranged from 3 to 6 days. Only one estimate for difubenzuron on ears was available, its half life calculated as 22 days. This latter value needs further confirmation (see Gadji 1993).
It should be noted that these are total residues following extraction of the whole vegetation sample. They do not necessarily reflect biological availability of these residues to non-target arthropods.

Arthropod assessments

Parasitism of Heliocheilus eggs

To evaluate if the treatments had an effect on the degree of parasitism of *H. albipuntella* eggs by *Trichogrammatoidea sp.* only the data of the first sampling round after treatment are used (20-21 September). The second sampling round, at the end of September, yielded very few eggs, since most millet ears were in the maturation stage. Therefore, in all treated plots sampling was carried out 7 to 9 days after the pesticide application. Since this variety of millet takes approximately 7 days from flag leaf emergence to first flowering, most if not all *Heliocheilus* eggs were laid after treatment. If egg densities (hosts) in the fields are approximately the same, any large differences found in degree of parasitism are probably caused by unequal parasitoid densities.

Densities of *H. albipunctella* eggs as well as the degree of parasitism by *Trichogrammatoidea* are given in table 4.

Table3.5: Egg density of millet earhead miner *Heliocheilus albipunctella* on millet ears 7-9 days after spraying with locust control pesticides, and incidence of parasitism by *Trichogrammatoidea sp.* after incubation of earhead miner eggs in the laboratory.

Treatment	Number of H.albipunctella eggs per 10 millet ears					Degree of parasitism by Trichogrammatoidea (%)				
	Block					Block				
	1	2	3	4	mean'	1	2	3	4	mean
FENITROTHION	65	30	28	93	62	17	4		17	13
DIFLUBENZURON	41	53	0	22	39	5	17		9	10
UNTREATED	22	44	0	114	60	14	7		16	12
mean	43	42	9	76						

; mean of blocks 1,2 and 4 only
Statistics for differences between treatments:
- egg density: ANOVA (RCB; blocks 1,2,and 4 only) p=0.76
- percentage parasitism: ANOVA (RCB; blocks 1,2, and 4 only) p=0.92

No significant difference was found in egg densities between treatments. However, a significant difference between blocks exists as well as a significant interaction between treatments and blocks (main effects are non-additive). The latter means that any effects of the treatments depends in some systematic way on the location, and is thus not generally valid. Since Block 3 obviously has a much lower egg density, it was omitted from further analysis. This lower egg density is probably caused by the fact that in 2 out of 3 fields the millet was already largely in maturation stage. Repeating the ANOVA on blocks 1, 2, and 4 yields no significant difference between treatments nor blocks, and fulfilled the statistical assumption of additivity of main effects.

Mean percentage parasitism by *Trichogrammatoidea* varied between 10 and 13 %. There was no significant difference between treatments.

Incidence of Heliocheilus larvae in the field

Larval incidence of millet earhead miner are compared between treatments. For every field the maximum density of *Heliocheilus* larvae observed during the weekly observations is given in table 3.6.

Maximum counts were as a rule observed near the end of the maturation period of the millet ears (24 Sept. - 5 Oct.). Recruitment into the larval stage from the egg stage had almost ceased during this period, and was definitely very small compared to the existing larval population. Larval development is quoted to be 23-39 days (Gahukar et al 1986) and 30-35 days (Ndoye 1991). Since egg density on 4 September was still very low, few larvae would already have left the millet ears to pupate in the soil. The maximum density measured during the period mentioned above can therefore be considered to be close to the real maximum density of *Heliocheilus* larvae.

Table 3.6: Maximum density of larvae of *Heliocheilus albipunctella* in treated and untreated millet fields. Incidence is expressed as number of larvae counted per 30 millet hills.

Treatment	# larvae / 30 hills					
	Block				mean[1]	
	1	2	3	4		
FENITROTHION	199	261	63	167	172	a
sampling date	04 Oct	30 Sep	5 Oct	5 Oct		
DIFLUBENZURON	58	67	27	47	49	b
sampling date	26 Sep	30 Sep	26 Sep	5 Oct		
UNTREATED	161	94	53	83	98	c
sampling date	24 Sep	30 Sep	26 Sep	26 Sep		

Statistics for differences between treatments:
• max. # larvae per 30 hills: ANOVA (RCB) p=0.001

[1]: means followed by the same letter are not significantly different (Student-Neuman-Keul's Multiple Range Test, α= 0.05)

Maximum density of *Heliocheilus* larvae in the fenitrothion treated fields is almost double that in the control fields, while the diflubenzuron treated fields contain about half of the control larval density. These differences are highly significant. It is unlikely that this effect is caused by differences in egg density or egg parasitism since these were found not to differ significantly between treatments.

Survival and parasitism of Heliocheilus larvae

Mortality of larvae which were incubated in the laboratory, due to other factors than parasitism, was very high, on average 71 % (but varying from 13 to 100%). This is quite probably due to the fact that the pieces of millet ears offered to the larvae were drying out too rapidly and consequently larvae had to be provided with fresh ones. This resulted in too much handling and probably an "adaptation shock" to new ears. No significant difference in larval mortality was observed between treatments, but given the above, this conclusion is probably not very meaningful.

Of those larvae surviving until the last larval stage, 0 to 26% were parasitized by *Copidosoma sp. nr. truncatellum* (Table 3.7). Between 67 and 71% of these mummified larvae yielded *Copidosoma* parasitoids during the rainy season of 1992. The number of *Copidosoma* adults emerging was very variable, ranging from 18 to 390 per mummy. There was no significant difference between treatments for any of the above parameters.

Of the viable *Heliocheilus* chrysalids incubated at the end of the rainy season, adults emerged in 50 - 100% of the cases (table 3.7). No significant difference was observed between treatments.

Table 3.7: Fraction of surviving incubated larvae of *Heliocheilus albipuntella* parasitised by *Copidosoma sp.nr. truncatellum*, and results of incubation of chrysalids of *H. albipunctella* and its mummified larvae.

Treatment		% parasitization by *Copidosoma sp.*[1]					Emerged *Heliocheilus* adults as % of total live chrysalids incubated					Hatched *Copidosoma* mummies as % of total mummies incubated
		Block					Block					All Blocks combined
		1	2	3	4	mean	1	2	3	4	mean	
FENITROTHION		12	0	26	11	12	94	67	73	91	81	67
	n	*26*	*5*	*19*	*39*		*17*	*3*	*11*	*32*		*12*
DIFLUBENZURON		14	22	7	3	12	80	50	60	100	73	67
	n	*14*	*9*	*14*	*29*		*10*	*4*	*10*	*24*		*6*
UNTREATED		7	12	15	23	14	83	100	67	82	83	71
	n	*15*	*17*	*27*	*30*		*6*	*15*	*21*	*22*		*14*

[1]: percentage of total *Heliocheilus* larvae surviving until last instar.

Statistics for differences between treatments:
- percentage parasitism by Copidosoma: ANOVA (RCB) p=0.86
- percentage of viable chrysalids yielding adults: ANOVA (RCB) p=0.84

Incidence of stemborers

The stemborers found in millet stems after the harvest were separated in two groups: *Coniesta (=Acigona) ignefusalis* and "others" (not further identified). In addition to the number of stemborers observed, the number of holes in the side of the stems was also noted as an index of stemborer attack. Table 3.8 gives data for all fields.

Differences between treatments in the above parameters for stemborer attacks were not statistically significant at the 5% level. However, there is a strong indication that the number of *Coniesta* is higher in the diflubenzuron fields compared to the untreated control.

Table 3.8: Incidence of stemborers in millet stalks just after harvest for fields treated with fenitrothion, diflubenzuron and untreated.

Treatment	# holes / 100 stems					# Coniesta / 100 stems					# other stemborers / 100 stems				
	Block					Block					Block				
	1	2	3	4	mean	1	2	3	4	mean	1	2	3	4	mean
FENITROTHION	8	18	44	28	25	7	12	16	14	12	0	3	5	1	2.3
DIFLUBENZURON	36	54	37	19	36	9	36	20	9	17	8	6	2	3	5
UNTREATED	28	24	19	39	27	8	8	8	7	8	4	11	1	9	6.2

Statistics for differences between treatments:
- number of holes: ANOVA (RCB) p=0.76
- number of Coniesta: ANOVA (RCB) p=0.10
- number of other stemborers: ANOVA (RCB) p=0.35

Residual populations in the soil

Soil sampling in the treated and untreated control fields yielded chrysalids of *Heliocheilus albipuntella* as well as its larvae mummified by *Copidosoma sp.nr. truncatellum*. Very few cocoons of diapausing *Cardiochiles spp.* were found in the samples, and they are not further included in the analysis. In the diflubenzuron fields, the total larval population which attained the soil (chrysalids + mummies) was significantly reduced (80%) compared to the control fields (table 3.9).

Table 3.9: Number of chrysalids of *Helicoverpa* abipunctella and its larvae mummified by *Copidosoma sp. nr. truncatellum* in 10 samples of 0.25 m^2 per field.

Treatment	# chrysalids / 2.5 m^2					# mummies / 2.5 m^2					total / 2.5 m2
	Block					Block					mean[1]
	1	2	3	4	mean[1]	1	2	3	4	mean	
FENITROTHION	11	14	18	17	15 a	2	3	4	4	3.3	18.3 a
DIFLUBENZURON	8	16	1	0	6.3 b	0	2	3	3	2	8.8 b
UNTREATED	26	44	14	15	25 a	14	13	0	1	7	31.8 a

Statistics for differences between treatments:
- number of chrysalids / 2.5 m^2: ANOVA (RCB): p=0.039
- number of mummies / 2.5 m^2: ANOVA (RCB): p=0.74
- number of total / 2.5 m^2: ANOVA (RCB): p=0.026

[1]: means in the same column followed by the same letter are not significantly different (Student-Neuman-Keul's multiple range test; α=0.05)

To determine impact of treatments on mummification frequency, the different counts were pooled per treatment. This was done given the often low number of counts per sample which would distort mummification percentages if calculated and analysed on a per field basis. Two-way tests of independence do not show significant dependence between treatment and mummification frequency for neither treatment (table 3.10).

Table 3.10: Frequency of mummification of *Helicoverpa abipunctella* larvae by *Copidosoma sp. nr. truncatellum*. Counts per treatment are pooled over 4 fields.

	mummies	chrysalids	total	% mummification[1]	G-statistic [significance]
FENITROTHION	13	60	73	18	G=0.5 [p=0.48]
UNTREATED	28	99	127	22	
DIFLUBENZURON	8	25	33	24	G=1.64 [p=0.20]
UNTREATED	28	99	127	22	

[1]: expressed as percentage of total chrysalids + mummies in the soil sample

A number of the collected chrysalids and mummified larvae were incubated in the laboratory. Table 3.11 lists the adult emergence rates at the end of October 1992, i.e. one year after sampling. Since the number of incubations per field was low, they are pooled by treatment and emergence frequency statistically analysed using a two way test of independence. It is assumed that those chrysalids and mummies not emerged after one year will not do so later.

Table 3.11: Emergence frequencies of *Hellochelus albipunctella* chrysalids and its larvae mummified by *Copidosoma sp.nr.truncatellum* which were sampled from soil under millet fields. Numbers per treatment are pooled over 4 fields.

Chrysalids	emerged	not emerged	total	% emergence	G-statistic[significance]
FENITROTHION	8	26	34	24	G=0.40 [p=0.53]
UNTREATED	14	33	47	30	
DIFLUBENZURON	7	4	11	64	G=4.28 [p=0.039]
UNTREATED	14	33	47	30	
mummified larvae	**emerged**	**not emerged**	**total**	**% emergence**	
FENITROTHION	7	5	12	58	G=0.12 [p=0.73]
UNTREATED	16	9	25	64	
DIFLUBENZURON	2	5	7	29	G=2.80 [p=0.094]
UTREATED	16	9	25	64	

Emergence of *Hellochelus* chrysalids in the diflubenzuron treated fields is double that of the control fields. Although this increase is statistically significant, it is based on only a few chrysalids. There is an indication that emergence of *Copidosoma* parasitoids in the diflubenzuron fields is reduced by approximately 50%, but again this is based on only a few individuals. Emergence in fenitrothion and untreated plots did not differ.
A summary of the fluctuations in population dynamics parameters of millet earhead miner and some of its natural enemies which are described above, is given in table 3.12.

Table 3.12: Summary of results from *Hellochelus albipunctella* dynamics and comments on validity of these results.

parameter	fenitrothion		diflubenzuron		comments
	difference from control	statistical significance	difference from control	statistical significance	
egg incidence	+3 %	ns	-35 %	ns	peak density
egg parasitism	+8 %	ns	-17 %	ns	eggs incubated
larval incidance	+76 %	***	-50 %	***	peak density
adult emergence (from live chrysalids)	-2 %	ns	-12 %	ns	larvae incubated
parasitism by copidos.	-14 %	ns	-14 %	ns	larvae incubated
copidos. emergence	-6 %	ns	-6 %	ns	larvae incubated
chrysalid density	-40 %	*	-75 %	*	real density
copidos. mummy density	-53 %	ns	-71 %	ns	real density
total larvae which attained the sub-soil	-42 %	*	-82 %	*	real density
parasitism by copidos.	-18 %	ns	+9 %	ns	direct calculation, no incubation
adult emergence (from live chrysalids)	-20 %	ns	+113 %	*	chrysalids incubated
copidos. emergence	-9 %	ns	-55 %	ns	mummies incubated

Malaise trap catches

Weekly catches from malaise traps in all fields were available for a period of 5 weeks before and 4-5 weeks after treatment (Annex 3.1). They were sorted and identified for a limited number of taxa which have known or potential functions as predators or parasitoids of millet pests or pests of groundnuts. The latter are often grown in rotation with millet in this part of Senegal. Table 3.13 lists the identified taxa, their hosts, as well as the total number of specimens sorted. All raw data are listed in Annex 3.4.

Table 3.13: Taxa identified in this study and their hosts in millet or groundnuts in Senegal; and the total number identified per taxon over the duration of the study.

Taxon	class	importance	total number sorted (rounded off)
Diptera			
Syrphidae: *Ischiodon aegyptcus* (Wiedemann)	predator	predators of aphids: observed on *Aphis craccivora*, *Rhopalosiphum maidis* in Senegal [1];	320
Asilidae: total	predator	polyphagous predators of e.g. grasshoppers, lepidoptera [1,2,3]	360
Bombylidae: *Exoprosopa sp.* (probably *E. tricolor* Macquart)	predator	unknown for Senegal/Sahel. *Exoprosopa sp.* known to attack grasshopper eggpods [2].	330
Tachinidae: Spec. 1 (possibly *Palexorista sp.*, is being verified)	parasitoid	Spec.1 bred out of *H. albipunctella* larvae [4]; importance not clear	3000
Tachinidae: total except for Spec. 1	parasitoid	attack several lepidopteran pests in Senegal/Sahel [1].	820
Hymenoptera			
Hymenoptera: total	parasitoid predator polinator	attack many pests in Senegal [1,2,5]; locally important pollinators [9,10]	8000
Braconidae: Cardiochiles spp.	parasitoid	Several species of this genus attack *H. albipunctella* and *Helicoverpa armigera* in Senegal [1,6]	600
Braconidae: total except *Cardiochiles spp.*	parasitoid	attack many lepidopteran pests in Senegal [1]	650
Ichneumonidae: total	parasitoid	attack several lepidopteran pests in Senegal [1]	700
Sphecidae: *Tachytes spp.*	predator	specialist predators on grasshopper nymphs [3,7]. Status in Sahel unknown.	750
Tiphiidae: Mesa sp.	parasitoid	attacks larvae of Scarabaeidae, Tenebrionidae and Cicindelidae [3,8] status in Sahel unclear.	740

References: [1]:Bhatnagar 1987 [2]:Greathead 1963 [3]:Borror et al. 1989 [4]:this study [5]: Risbec 1950 [6]:Huddleston and Walker 1988 [7]:Bohart and Menke 1976 [8]:Kimsey 1991 [9]:Crane and Walker 1983 [10]:Roubik 1989

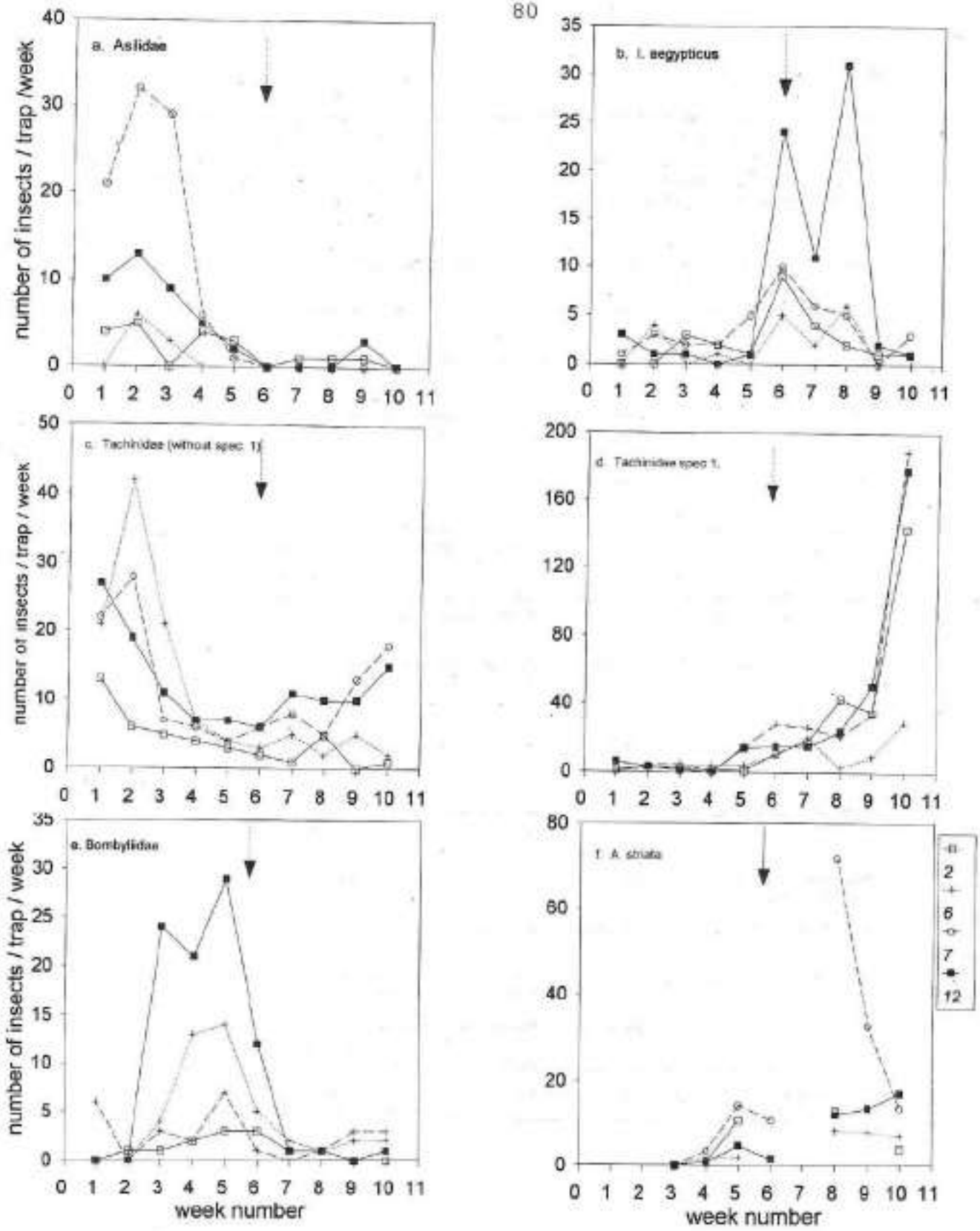


Fig. 3.4: Numbers of different taxa of Diptera caught in malaise traps in the untreated control plots: a. total Asilidae, b.*Ischiodon aegyptiicus* (Syrphidae), c.total Tachinidae but species 1. excluded, d. Species 1. (Tachinidae), e.*Exoprosopa sp.* (Bombyliidae), f.Number of *Alesia striata* (Coccinellidae) counted per 100 millet stems. Arrow indicates week of insecticide treatment. All legends according to fig. 3.4f.

Trapping fluctuations in control plots

All trapping data are expressed as number of insects caught per trap per seven days. In the few cases that traps were in the field for longer or shorter periods, catches were proportionally corrected to a count per week. Abundance of insects in the traps depends on various factors, including the insect's actual density, its activity, meteorological conditions, and trap placement (Muirhead-Thomson, 1991). Counts in traps such as the malaise trap will therefore further be referred to as the insect's "activity density".

To assess the presence of the taxa in table 3.13 potentially susceptible to the insecticide treatments, catches on the different untreated control fields are shown in figs.3. 4 and 3.5. Obviously, absence of susceptible life stages of a given taxon at the moment of treatment, results in absence of exposure and any insecticide impact is unlikely.

Diptera

Asilidae peak in the traps during the first three weeks of the study and may have been abundant even previously (fig. 3.4a). At the moment of treatment these flies have almost completely disappeared from the traps. This can be partly explained by the fact that asilid adults tend to emerge shortly after the first rains. It is not clear, however, if the flies start avoiding millet fields because vegetation becomes too dense for hunting, or because they have laid their eggs and die off. Bhatnagar (1987) observed peaks in malaise trap captures of *Promachus spp.*, a predominant asilid genus, late June or early July in millet in central Senegal, while van der Valk (1990) found catches of Asilidae diminishing considerably after mid-August in semi-arid pastures in Northern Senegal. Given the fact that Asilidae larvae burrow in the soil, it is unlikely that any life stage of this group of predators could have been exposed to the treatments in this study.

Exoprosopa sp., the principal Bombyliid fly caught in the traps, peaked from mid-August to early-September (fig. 3.4e). At the time of treatment counts were already going down sharply. As with the Asilidae, bombyliid larvae burrow in the soil and are not likely to be exposed to the treatments. Larvae often need repeated wetting from rain before pupation starts (Greathead, 1963) which may explain the later peak activity density observed in the study when compared to the Asilidae. *Exoprosopa sp.* was observed to be very active in millet fields at the tillering stage, flying low over the soil in between the plants and making characteristic "flicking movements" with its abdomen. The latter has been described as typical egg laying behaviour (Greathead 1963). Again, it is not clear if *Exoprosopa* adults merely displace their activity from millet to other biotopes, or if the drop observed in activity density represents a real drop in overall density. *Exoprosopa* larvae were reported being a predator of grasshoppers in southern Russia, Madagascar and Zambia (Greathead 1963), but no data on hosts are known for the Sahel.

Tachinidae *"species 1"* were not very abundant until late September, when millet was maturing (fig. 4d). A small number of adults of this species was obtained from laboratory incubated larvae of *Heliocheilus albipunctella* in 1992. *H. albipunctella* was by far the most abundant lepidopteran host available in the millet fields at the time of peak *spec.1* adult catches in the traps. Bhatnagar (1987) regularly obtained the tachinid fly *Palexorista quadrizonula* Thomson from incubating late instar larvae of *H. albipunctella*. He found this species attacking several lepidopteran pests in Senegal, including *Amsacta moloneyi* Drc. and *Helicoverpa armigera* (Hb.). It is not yet clear if *spec.1* is the same species as described by Bhatnagar. Insecticide treatments in our study could affect *spec.1* both directly as well as through mortality in its host.

Other species of Tachinidae were present during the whole study period at varying activity densities (fig. 3.4c).

Ischiodon aegypticus (Wiedemann) was by far the most abundant syrphid fly caught in the traps. Catches in the millet fields peaked during the month of September (fig. 3.4b). Syrphid larvae are predators of aphids and *I. aegypticus* has been reported attacking *Aphis craccivora* KOCH and *Rhopalosiphum maidis* FITCH, localised pests of groundnut and niébé in Senegal (Bhatnagar 1987) *Ischiodon scutellaris* (Fabricius) has been reported attacking at least 12 different species of aphids and one species of Agromyzidae on a variety of crops in India (Fry 1989). However, no species of

aphid nor Agromyzidae is abundant on millet in the study area. It seems most likely that the adult *I. aegypticus* found in the malaise traps were foraging for pollen (Greathead et al. 1990). Their peak activity density coincides with the flowering period of the millet (fig. 3.4b). Egg laying and larval stage of this species is probably linked with groundnuts, a crop widely grown in the study area and rotated with millet. In this respect it is worth noting that in the control fields #7 and #12, which have the highest overall activity density of adult *I. aegypticus*, more than 60% of the fields bordering the trial field were groundnuts, while for fields #2 and #6 this was less than 25%.
In this study, adult Syrphidae may be exposed to the insecticides both through direct contact as well as through ingestion of contaminated pollen.

Hymenoptera

Hymenoptera were present in millet during the whole study period in large numbers (fig. 3.5a). Certain taxa within this order were followed in more detail.

The parasitoid family of Braconidae accounted for approximately 15% of the total Hymenoptera sample. Almost 50% of the Braconidae consisted of *Cardiochiles spp.* (fig. 3.5c) Several *Cardiochiles* species are known to parasitize Sahelian lepidopteran pests: *Cardiochiles sahelensis* on *H. albipunctella* and *Cardiochiles variegatus* on *Helicoverpa armigera* (Bhatnagar 1987, Huddleston and Walker 1988). In this study some *Cardiochiles sahelensis* were caught in the traps, but the most predominant species was *C. punctatus* and/or *C. testaceus* (definitive identification pending). For these species no hosts are known (Huddleston and Walker 1988) even though they are very regularly encountered not only in millet but also in semi-arid grassland in Senegal (van der Valk 1990). *Cardiochiles spp.* were abundant in the traps as of the start of September until the end of the season.

Other Braconidae are lumped together, since no single species was caught in large enough quantities to allow insecticide impact assessment (fig. 3.5d)

Ichneumonidae, another group of parasitoids, were caught to a lesser extent than Braconidae (fig. 3.5b). They are considered here grouped on family level, since no single species was abundant enough to allow pesticide impact assessment. *Syzeuctus spp.*, described as a principal parasitoid of the millet stemborer *Coniesta ignefusalis* (Bhatnagar, 1987), was only encountered rarely. Stemborer incidence in this area was low, however (see above).

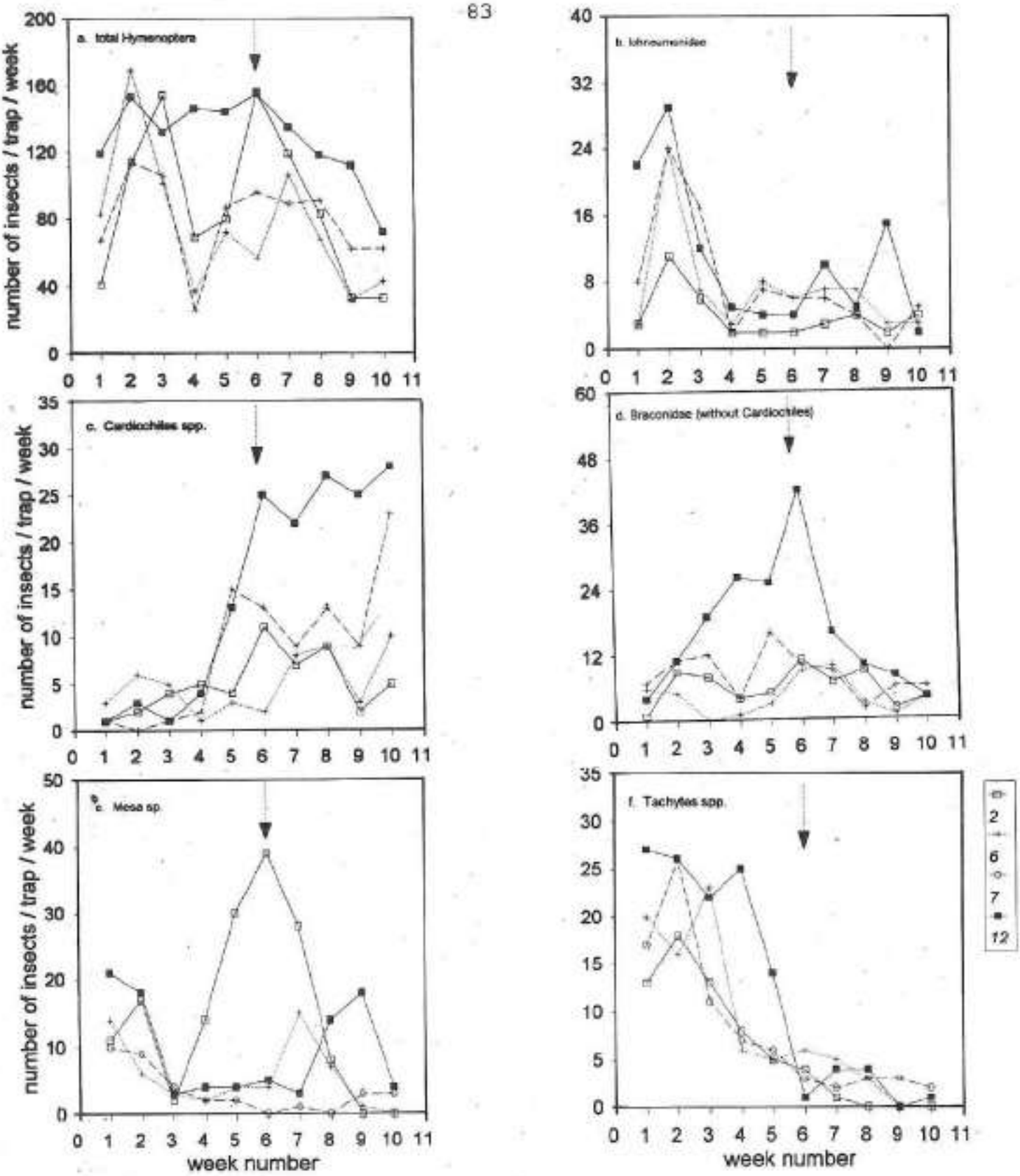


Fig. 3.6: Numbers of different taxa of Hymenoptera caught in malaise traps in the untreated control plots: a.total Hymenoptera, b.total Ichneumonidae, c.*Cardiochiles* spp. (Braconidae) d.total Braconidae excluding *Cardiochiles spp.*, e.*Mesa sp.* (Tiphiidae), f.*Tachytes spp.* (Sphecidae). Arrow indicates week of insecticide treatment. All legends according to fig. 3.5f.

Mesa sp. (Tiphiidae) was encountered regularly in the traps (fig. 5e). This species (or species complex) was found to be abundant in millet by Bhatnagar (1987) as well as in semi-arid grassland by van der Valk (1990). A computerized literature search did not reveal any data on host relationships for this genus. *Mesa sp.* belongs to the subfamily of Myzininae, which are parasitoids of larvae of Scarabaeidae, Tenebrionidae and Cicindelidae (Borror et al. 1989, Kimsey 1991). The first two families of hosts are abundant as larvae in the soil in central Senegal.

Tachytes spp. belong to the Sphecidae (subfamily Larrinae) and are specialized hunters on grasshoppers (Bohart and Menke 1976). They are mostly caught in the traps early in the study period (August) (fig. 5f). This coincides with the period in which grasshopper nymphs are most active in millet. *Tachytes* adults build nesting chambers in the soil. Eggs are laid on the paralyzed prey which the wasp brings into the nesting chambers (Bohart and Menke 1976). The importance of these wasps in regulating grasshopper populations in Africa is completely unknown (Greathead, 1963). Several different species of Tachytes were abundant in the malaise traps, but given their similar behaviour and prey, they are considered as one group for insecticide impact analysis.

Coleoptera

The coccinellid beetle *Alesia striata* F. was counted weekly on millet during the millet earhead miner assessments (fig. 4f). Adults started appearing in the fields with the emergence of the millet earhead and remained present almost until harvest. Larvae were first observed approximately two weeks later. Adults would often be found on the earhead and seemed to be searching between the flowers. Risbec (1950) reports that this species was very abundant on millet in Senegal and attacked aphids. We observed adult *Alesia striata* attacking young (1st or 2nd instar) larvae of *Heliocheilus albipunctella*, but we don't know if this is a preferred prey. Aphids were virtually absent on millet earheads. Larvae were mostly observed on the leaves, and were very active during the day.

Of all the above mentioned taxa, the Asilidae and the Bombyliidae cannot further be analysed because they had practically disappeared by the time the treatment was carried out. For the other taxa, potential insecticide impact will be analysed below. However, especially in the case of *Tachytes spp.* (Sphecidae) and the Ichneumonidae, insecticide applications in this study were carried out during a period that these taxa appear not to be very active in millet.

Impact of the treatments on malaise trap catches

The average numbers of insects caught in the malaise traps for the two treatments and the untreated controls are shown in figs. 6 and 7. Statistically significant effects are marked by asterisks in the graphs. All differences in catches due to the treatment are in comparison to mean before-treatment levels and take into account fluctuations in the untreated fields (see "methods" for more details). Results are summarized in table 13. When, in this section, we speak of presence or absence of effects, this always implicitly means "as measured by malaise trap catches".

Effects of fenitrothion and diflubenzuron on the dipteran taxa evaluated appears to be very small. Of the three taxa for which susceptible life stages were expected to be present during treatment, only a temporary increase in Tachinidae is observed. There was no effect on the syrphid species, *Ischiodon aegypticus*. This may have been because large numbers of Syrphidae were available in neighbouring fields, and this species rapidly re-invaded the treated fields.

No impact of treatments was discernible on Tachinidae *Spec.1*. If indeed this species attacks the millet earhead miner *Heliocheilus albipunctella*, it may have been well protected as larvae inside its host at the time of treatments. However, millet earhead miner incidence at the end of the season in diflubenzuron treated fields was significantly reduced (table 5). One would expect parasitoid activity density to be less with increasing host mortality. More data on the taxonomy and ecology of *Spec.1* are needed.

Total Hymenoptera catches are reduced considerably for the whole after-treatment period in the fenitrothion fields, but were not affected by diflubenzuron. Ichneumonidae were not affected, except in the fenitrothion fields during the 4th week after spraying. It is not clear if this effect would have persisted had the spraying been done earlier in the season.

The wasps belonging to the braconid genus *Cardiochiles* were affected by fenitrothion applications. Counts in the traps were reduced 80% during the first two weeks after treatment and still 55% in the 4th week. The temporary increase in the malaise trap catches in week 9 was caused by a single influx of wasps in the trap of field 4 only. The ANOVA for this week also gives a significant interaction between the effects of insecticide and block. Because the other fields did not show such an increase, the reduction of *Cardiochiles spp.* after fenitrothion treatments will be considered to last at least 4 weeks. This group of parasitoids was significantly reduced in the diflubenzuron fields only the 4th week after treatment. Other Braconidae, pooled at family level, did not seem to be affected by either treatment, but pooling may have masked impact on individual species.

The tiphiid wasp *Mesa sp.* was greatly affected by fenitrothion treatments, showing a reduction of 90% over the first 2 weeks after application. That later differences between treatment and control were not significant anymore was due to a decrease of the population in the control fields rather than an recovery in the treated ones. Diflubenzuron did not appear to affect this wasp.

Not much can be said about impact on the specid wasps belonging to the genus *Tachytes*. No effects were observed after either treatment, but activity densities had already decreased considerably in the control plots at the time of insecticide application.

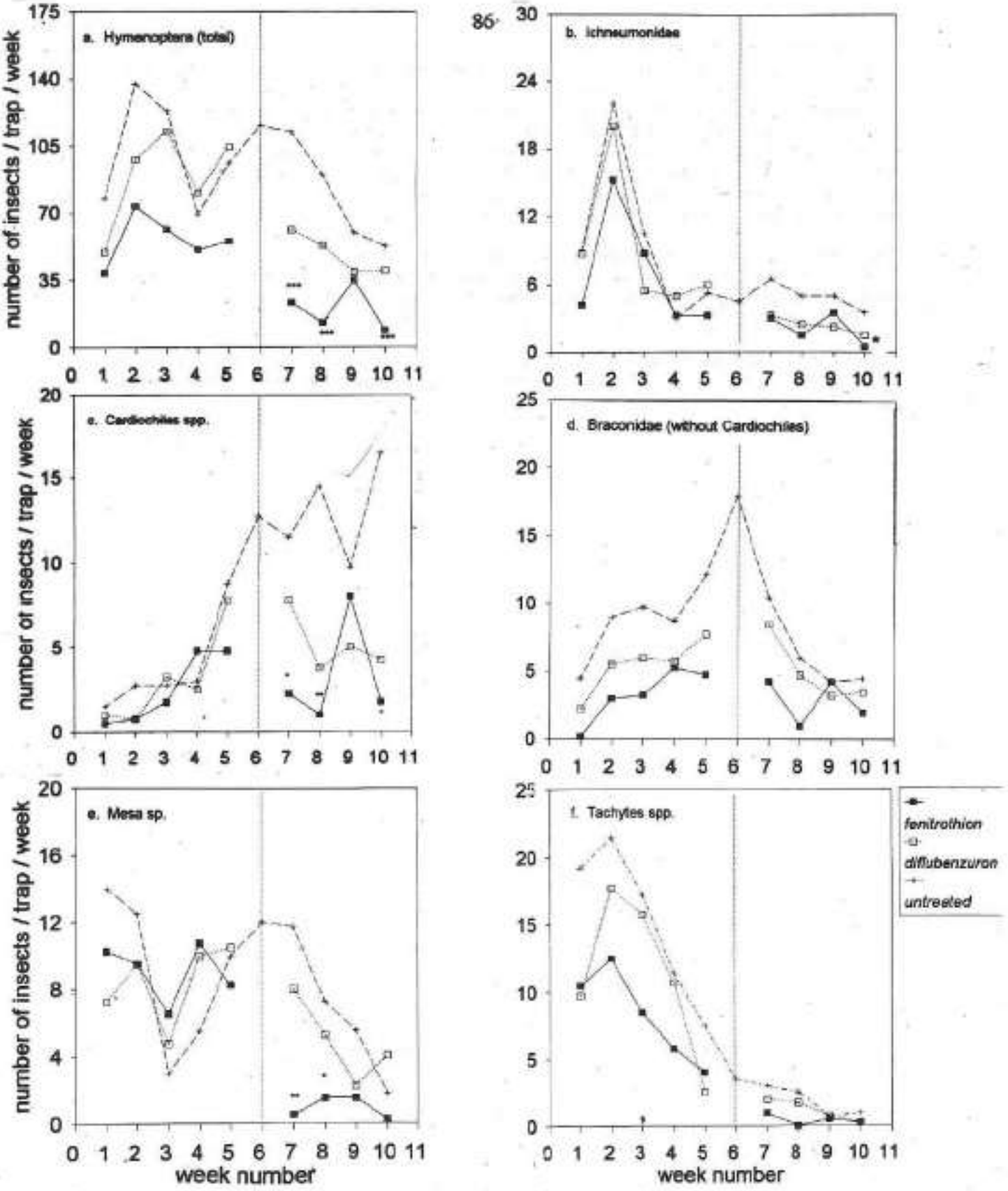


Fig. 3.6: Mean number of insects caught per malaise trap per week in fields treated with fenitrothion, diflubenzuron and in untreated control fields (means are of 4 fields). a: *Ischiodon aegyptious* (Syrphidae), b: Tachinidae *Spec.1*, c: Tachinidae (all species except *Spec.1*), d: *Alesia striata* (Coccinellidae). Treatments carried out in week 6 (vertical dotted line). Those counts which are significantly different from mean before treatment levels, when corrected for control fluctuations, are marked with asterisks: *: $p<0.05$, **: $0.05<p<0.01$, ***: $0.01<p<0.001$. (Details on the statistical analysis in text).

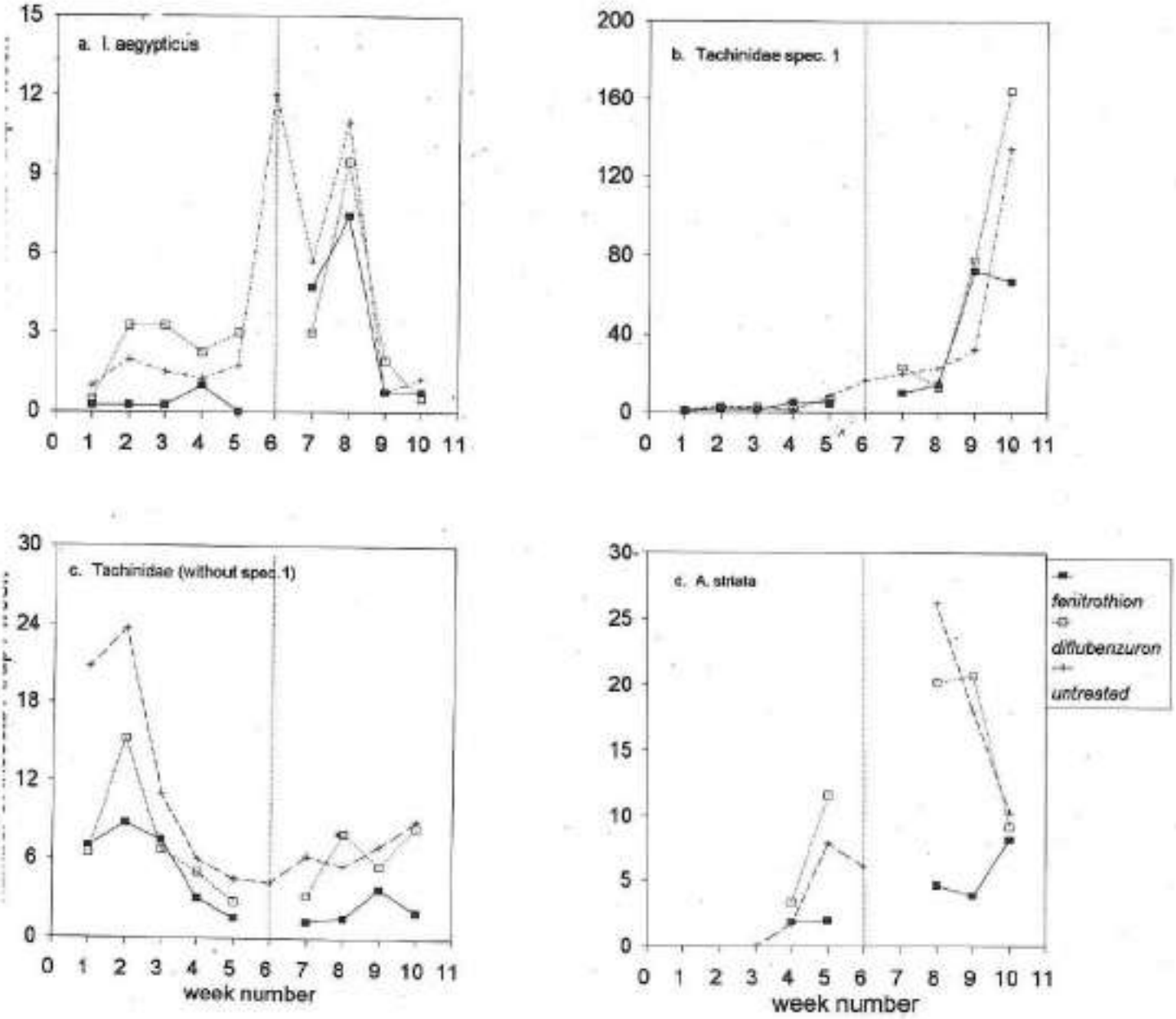


Fig. 3.7: Mean number of insects caught per malaise trap per week in fields treated with fenitrothion, diflubenzuron and in untreated control fields (means are of 4 fields). a: Hymenoptera (total), b: Ichneumonidae (total), c: *Cardiochiles spp.* (Braconidae), d: Braconidae (all species except *Cardiochiles spp.*), e: *Mesa sp.* (Tiphiidae), f: *Tachytes spp.* (Sphecidae). Treatments carried out in week 6 (vertical dotted line). Those counts which are significantly different from mean before treatment levels, when corrected for control fluctuations, are marked with asterisks: *: $p<0.05$, **: $0.05<p<0.01$, ***: $0.01<p<0.001$. (Details on the statistical analysis in text)

Table 3.14: Summary of results of the malaise trap catches and counts of beneficial insects.

Taxon	Susceptible stage present[1]	fenitrothion			diflubenzuron			remarks
		impact[2]	mean % reduction[3]	duration of effect[4]	impact	mean % reduction	duration of effect	
DIPTERA								
Asilidae	no							peaks early in season, virtually absent during treatment
Exoprosopa sp. (Bombyliidae)	no							peaks early in season, very low catches during treatment
Ischiodon aegyptius (Syrphydae)	yes	no			no			probably high immigration from neighbouring peanut fields
Spec.1 (Tachinidae)	yes	no			no			possibly recruitment from protected hosts in field
Tachinidae (all except *Spec.1*)	yes	no			yes	140 (increase)	2nd week	one week of sign. increase observed; no obvious explanation.
HYMENOPTERA								
Hymenoptera (total)	yes	yes	50	4 weeks	no			70% decrease during first two weeks after application of fenitrothion.
Ichneumonidae	yes	yes	60	4th week	no			peak catches early in season; impact probably underestimated.
Cardiochiles spp. (Braconidae)	yes	yes	55	4 weeks	yes	65	4th week	80% decrease during first two weeks after application of fenitrothion
Braconidae (all except *Cardiochiles spp.*)	yes	no			no			
Mesa sp. (Tiphiidae)	yes	yes	90	2 weeks	no			steep natural population decline after treatment; probably underestimation of impact.
Tachytes spp. (Sphecidae)	yes	no			no			peaks before treatment; low numbers after treatment; probably underestimation of impact.
COLEOPTERA								
Alesia striata (Coccinellidae)	yes	no			no			high immigration from neighbouring (peanut-)fields?

[1]: if a susceptible life stage of the taxon is expected to have been present during treatment. [2]: statistically significant reduction over period of effect compared to average before treatment levels.
[3]: corrected for control fluctuations. [4]: four weeks after treatment harvesting started in the study fields,and trapping was ended.

DISCUSSION

Pest-pesticide-natural enemy interactions

In the introduction we mentioned that the main objective of this study was to assess to what extent insecticide applications against locusts in millet could increase the likelihood of pest resurgence. For a proper understanding of the value as well as the inadequacy of the study methods used to attain this objective, pest-pesticide-natural enemy interactions will be briefly discussed.

An insecticide as used in locust control may affect a pest - natural enemy system in various ways. It could kill part of the natural enemy population. This may or may not reduce mortality of the pest, depending on the importance of the natural enemy in question as regulating factor, as well as on the presence of other parasitoids or predators. For instance, the place in the system which is freed by killing parasitoid A may be filled up by parasitoid B, which never had a chance before to do so effectively, and natural mortality of the pest is not affected. The insecticide could also, and will often, reduce pest densities or change their spatial distribution. This can affect parasitization or predation rates both positively as well as negatively, depending on natural enemy biology. Apart from lethal effects, the insecticide may have sublethal effects on longevity and reproduction of both pest and natural enemy, effects which again can reduce as well as increase natural mortality in the pest and densities of the pest. Croft (1990) and Elzen (1989) review sublethal and indirect effects of pesticides on natural enemies.

It is easy to see that assessing the potential impact of an insecticide on the pest - natural enemy system may be very complicated. Just looking for changes in trapping data, in parasitization rates or in pest densities may not reveal real impact. Waage (1989) and Van Driesche (1983) discuss this topic in more detail.

From the above it follows that the value of our malaise trap data (or from any natural enemy trapping data) is limited with respect to being able to predict resurgence of pest populations. This is even more so the case since the actual importance of several of the insects trapped in the millet ecosystem is not well known. However, we can say for a given crop that resurgence or secondary pest development is more likely in areas with consistently lower natural enemy populations (although we do not know the probability nor the size of the resurgence). Our data therefore have comparative rather than predictive value: all other things being equal the chance of resurgence or secondary pest development is bigger for insecticide A than for B.

Assessing the degree of natural mortality rather than natural enemy (activity) densities comes already a step closer to our goal of assessing risk of resurgence. At least we measure an actual effect of the presence of the natural enemy. The problem here is that in measuring such rates, for instance percent parasitism, we don't necessarily measure the real contributon of the natural enemy in regulating its host. The pitfalls of using such parasitization rates in the assessment of the importance of insect parasitoids are discussed in a classical publication by Van Driesche (1983). The way in which our study has been set up is inadequate for the real parasitization or predation rates to be calculated. Many more samples should have been taken, and more often. However, for insecticide assessments sufficient treatment replicates are a principal requirement. The two questions, actual importance of a parasitoid and the size of pesticide side-effects, cannot be answered in the same study. The study would become too big to handle with the limited number of personnel generally available.

However, if we only want to compare treatments rather than quantify natural mortality for each treatment, errors made in actual parasitization or predation rates are probably less important. This is because, if sampling methods and times are standardized, phenological biases causing such errors can often be expected to be equal across treatments (van Driessche 1983).

One of the few tools available to assess the actual size of the effect of insecticides on pest regulation, are detailed life tables. Life tables describe the population dynamics of a pest, and include a quantification of mortality factors such as natural enemies. The detailed biological and ecological

data required do not yet exist for any millet pest, however, and it would take several scientists a number of years of work to compile them. Waage and Mills (1990) provide an introduction to life table analysis for evaluation of pest-natural enemy interactions. Manly (1989) and Bellows et al (1989, 1992) recently reviewed this topic in detail and the reader is referred to these publications for more information on the approach.
Alternative to life table analysis, computer simulation studies could be attempted (e.g. Rabbinge et al. 1989), but again the basic biological data needed for such an analysis are not yet available.

So for the time being the evaluation of side-effects of insecticides on the natural enemy -millet pest complex will necessarily be of a comparative nature. A major effort will be necessary in basic research on biology and population dynamics of millet pests and their natural enemies if ever this work can become really predictive (Incidentally, the same would be true for the introduction of viable Integrated Pest Management systems in millet in Africa).

Results of the study

Heliocheilus population dynamics parameters

Taking into account the above considerations, what can be concluded from this study? While discussing this we will follow the life history of the millet earhead miner, our principal candidate for resurgence.

Parasitism of *Heliocheilus* eggs by *Trichogrammatoidea spp.* of was relative low. However, the percentages observed with the techniques used in this study do not necessarily represent the real levels (see above). No significant differences between the treatments were found on egg parasitism. The rate of parasitism is, apart from the density of the natural enemy, also influenced by the host (=egg) density. Because no difference was found in egg incidence between fields, it is not likely that this has played a major confounding role, however.

From laboratory studies *Trichogramma spp.* are known to be very susceptible to fenitrothion but diflubenzuron does not cause much mortality at doses equal or higher than those used in locust control (Hassan et al. 1987). The fact that no effect on parasitism was found in the fenitrothion fields may mean that the wasps successfully re-invaded the treated fields in the period between spraying and egg sampling (approx. 7 days). If this was the case, it also means that fenitrothion is only toxic on millet earheads for a relatively short period. *Trichogrammatoidea* wasps necessarily come into contact with sprayed vegetation while searching for host eggs. The toxic activity of fenitrothion on foliage under the conditions of this study has indeed been shown to be very short (< 48 hours) using bio-assays with *Bracon hebetor*, a braconid parasitoid (van der Stoep 1993)

The peak density of larvae of millet earhead miner was halved in diflubenzuron fields compared to the untreated controls. This is not completely unexpected, given the mode of action of this insecticide, which disturbs moulting, and its persistence on vegetation. The effect was not only very significant but also consistent over all blocks.
In the fields treated with fenitrothion peak densities were on average 75% higher than in the controls. It is not clear what exactly has caused this increase. It is not unlikely that it occurred due to a reduction in the natural enemy population. According to our present knowledge the main natural enemies of the larval stages of millet earhead miner are *Bracon hebetor*, *Cardiochiles sahelensis* (both braconid hymenopterans) and a tachinid fly, *Palexorista quadrizonula*. Furthermore several hymenopteran predators have regularly been observed attacking the larvae (table 1). Hymenoptera as a whole were found to be markedly reduced in the malaise traps for a prolonged period after the fenitrothion treatments, and this was more specifically the case for *Cardiochiles spp.* However, few *C. sahelensis* were captured in the traps and very little diapausing cocoons of *Cardiochiles* were found in the soil after harvest. This places some doubt about their importance as parasitoid of *Heliocheilus* during this study. *Bracon hebetor* was also found rarely in the malaise traps, but this is quite certainly so because the trap is not appropriate for monitoring this species. No large reductions in catches of Tachinidae were observed either. This leaves us with either *Bracon hebetor* or

hymenopteran predators, rather than parasitoids, which may have been mostly affected. We have some indications, both from laboratory experiments and from monitoring of normal field applications by the Senegalese Crop Protection Service, that *Bracon hebetor* may actually be affected by fenitrothion treatments (van der Stoep 1993, Bèye 1993). This has not yet been shown in well controlled field experiments, however.
Whatever the explanation, the increase in millet earhead miner peak density in fenitrothion fields is both very significant and occurs consistently in all blocks.

Assuming that no mortality has taken place in the soil before sampling, the total number of last instar larvae which has reached the soil for pupation is represented by the number of chrysalids, larvae mummified by *Copidosoma*, and coccoons of *Cardiochiles*. The latter were virtually absent during this study. Combined chrysalid and mummy density was reduced both in fenitrothion and in diflubenzuron plots. For the latter insecticide this seems to correspond with the reduced larval incidence. However, in fenitrothion fields larval incidence was considerably higher than in untreated ones. We cannot explain this apparent contradiction. Sampling error may have played a role although it should have been similar over all plots. Although sampling error will influence the validity of a given density, it would have much less influence on ratios between treated and control plots which we have used.

No effect was observed in degree of mummification when expressed as percentage of total chrysalids plus mummies in the soil samples. This shows that the insecticides apparently did not have an effect on the development of *Copidosoma sp. nr. truncatellum* while inside its host, at least until mummification. Note that the percentage mummification used here is not the same as the actual degree of parasitism which should have been expressed as percentage of the egg density since *Copidosoma* oviposits in the eggs of *Heliocheilus*.

Emergence of *Heliocheilus* chrysalids collected from the soil while in diapause was not affected in fenitrothion fields. However, in diflubenzuron fields moth emergence rates were doubled. This may be explained by a sort of selection pressure by the insecticide on *Heliocheilus*: those larvae which succeed in surviving until the chrysalid stage in spite of the pressure by the IGR are "fitter" than the average larvae which have not been exposed. Therefore, relatively more also survive the chrysalid stage until emergence. That the same does not seem to occur in the fenitrothion fields may be because larval mortality, and thus selection pressure, was much less pronounced. Although this explanation may be plausible, it is by no means necessarily the right one. For one thing, numbers of chrysalids and mummies collected from the diflubenzuron fields was very low. This point needs further clarification.

Emergence of *Copidosoma* adults from incubated mummies was not influenced by fenitrothion. There is an indication in diflubenzuron fields, however, of a 50% reduction in adult emergence. Again, numbers collected were very low. If this is a true effect, however, it suggests that diflubenzuron exerts an effect on the development of parasitoid larvae inside its host. Such an indirect effect by diflubenzuron has been observed for other parasitoids before (Croft 1990).

We can now combine residual densities in the soil with the data on emergence to estimate initial densities of adult moths and parasitoids the year after treatment (fig. 8). These estimates suggest that for both treatments approximately half the number of moths would emerge compared to the untreated fields. The reduction in abundance of *Copidosoma* parasitoids is much higher, however.

From a biological control point of view it is not only the number of emerged host or parasitoids per se which is important but their ratio: Does the insecticide treatment influence how many hosts will be available per parasitoid in a next generation? If we compare the number of mummified larvae (*Copidosoma* parasitoids) per chrysalid (hosts) collected from the soil, no large differences are found between treatments (fig. 9a). However, if we include emergence rates of both species, the picture changes dramatically (fig. 9b). For every *Heliocheilus* adult expected to emerge the year after treatment with diflubenzuron we find a 75% reduction in number of available *Copidosoma* parasitoids. This suggests that parasitism rates by *Copidosoma* will be lower in the year following treatment.
It is unlikely, however, that the reduction in *Copidosoma* will increase Heliocheilus larval densities the year after treatment because this parasitoid only kills last instar larvae, when the damage would already be done. Any effect from diflubenzuron as observed in this study would only manifest itself

two years after treatment. In the treatment year survival rates of *Copidosoma* would be affected. In the year following treatment there would be reduced parasitism by *Copidosoma*, but no effect yet on *Heliocheilus* larval density and hence no increased crop damage would be found. Finally, in the second year after treatment an increased host density would be observed, followed by increased crop damage.

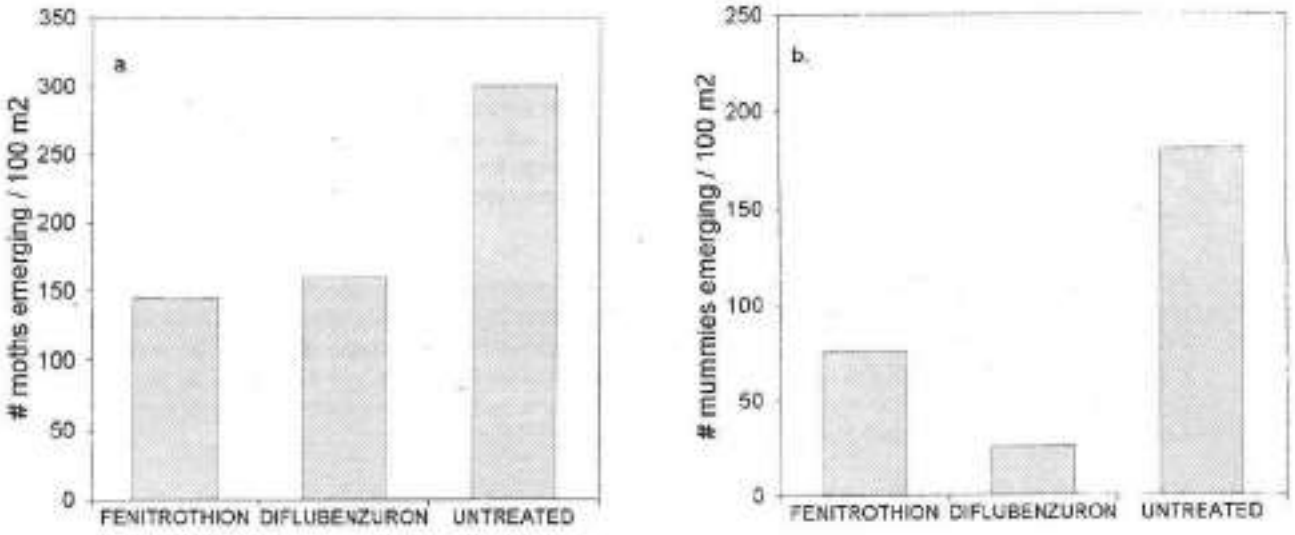


Fig. 3.8: Estimated number of emerging *H. albipunctella* and *Copidosoma* mummies per 100 m² the year after treatment with fenitrothion or diflubenzuron and in untreated control fields.

Although this scenario is biologically quite possible, we are obviously entering the domaine of heavy speculation. Given all other mortality factors and the effects of immigration of parasitoids from non-treated areas, it is not possible to predict such effects with any degree of certainty. Multi-year field trials or, when more biological data become available, computer simulation studies may clarify this.

The example above does show, however, the complexity of studying insecticide impact on the effect of natural enemies of crop pests.

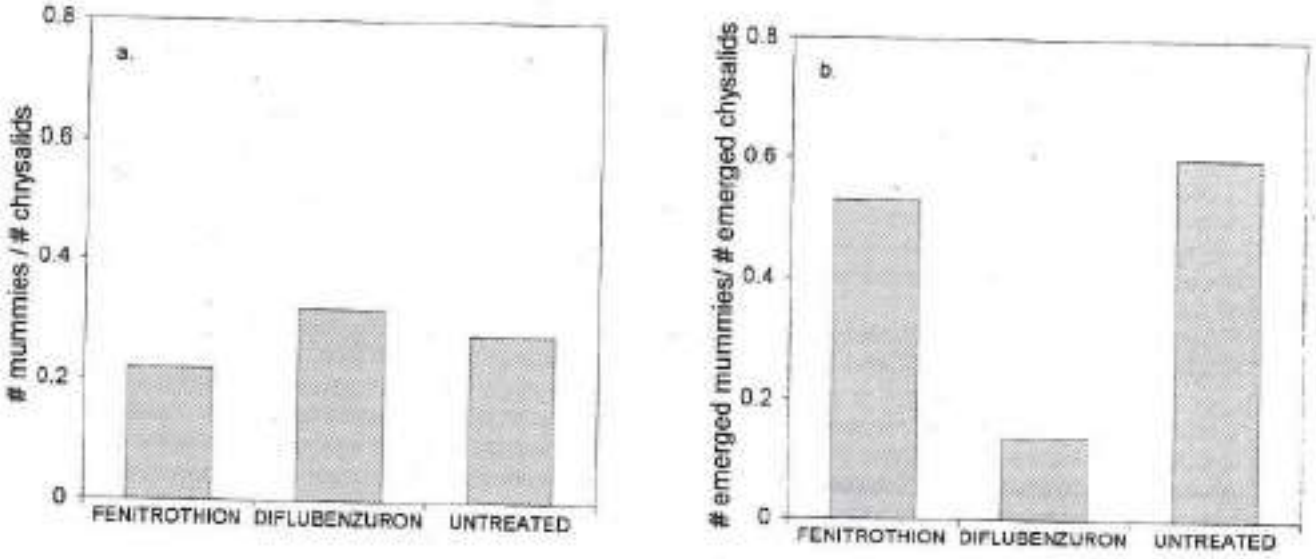


Fig. 3.9: a. ratio of mean numbers of mummies of *Capidosoma* to numbers of chrysalids of *Heliocheilus* found in soil samples after the millet harvest in fields treated with fenitrothion, diflubenzuron and untreated fields. b. ratio of mummies yielding parasitoids to emerged Heliocheilus adults in October 1992, a year after treatment.

Trapping data

The malaise trap data show a clear difference in impact caused by the two insecticides. Catches in several groups of Hymenoptera were reduced considerably after fenitrothion applications. The major braconid genus, *Cardiochiles spp.* and the tiphhid *Mesa sp.* were especially affected. Dilfubenzuron did not show reductions in catches of natural enemies, except for *Cardiochiles spp.* at the very end of the season. As discussed above, the impact on captures of certain Hymenoptera seems to correspond fairly well with the effects observed in *Heliocheilus* population dynamics. Van der Valk (1990) observed large reductions in Braconidae after fentrothion and diflubenzuron applications in semi-arid savanna in Northern Senegal.

The slight impact observed on Diptera is remarkable. In some cases this was probably because the taxa were no longer present at the time of treatment. This is the case for Bombyliidae and Asilidae, which were greatly affected by fenitrothion in the semi-arid savanna in northern Senegal (Van der Valk 1990) In others, the absence of effects may be explained by either an inherently lower susceptibility or a rapid recolonization of the treated fields. *Metasyrphus corollae*, a syrphid fly which is standardly tested for pesticide side-effects, was considered as one of the most susceptible "standard test species" (Croft 1990), although often the larva rather than the adult is exposed to the pesticide. Similarly, Hassan et al. (1987) report 50-75% mortality in another syrphid fly after treatments at 330 g a.i./ha of fenitrothion in Europe. Therefore, larger scale locust control treatments using fenitrothion, which would in Senegal almost always cover both millet and peanut fields, may cause much more impact on syrphids than this study suggests.

Data which Croft (1990) presents on several species of Coccinellidae suggest that the slight impact observed by fenitrothion in this study does not correspond with the literature. Hassan et al. (1987) found fenitrothion to be harmful to coccinellids in the field, but not diflubenzuron. It is not clear if *Alesia striata* is a relatively non-susceptible species or if recovery after treatment was very fast. In a future study, adult and larval counts need to be reported separately.

Future research

Even though the present study has been relatively detailed, a large number of questions remain to be answered.

It needs to be confirmed that those effects observed, as well as the absence of effects in other cases, occur consistently under similar situations. This would require similar studies to be repeated. It has also become clear that specific natural enemies are present only during fairly limited periods in the rainy season. This study simulated spraying during the maturation period of the millet grain, as it occurs widely against grasshoppers and locusts. Other periods in millet development which are often treated because of susceptibility to grasshoppers are the emergence and tillering stages. The trapping data clearly show that early season applications will expose very different groups of beneficials to the insecticide. It is not clear to what extent this may affect the likelihood of resurgence.

Insecticide impact can be divided into two stages: the initial effect of the insecticide (e.g. percentage reduction in density directly after treatment) and the duration of the effect (e.g. the time needed for populations to have recovered to pre-treatment levels). This study has assessed initial effects as well as recovery. The latter was measured over the remaining part of the millet growing season. Recovery is affected by life-history traits of the species (e.g. dispersal capacity) as well as by the scale over which it has to take place. The larger the area which has been depleted from a certain species, the longer it generally will take for recovery to take place (Everts 1983, Jepson 1989, Jepson and Thacker 1990, Duffield and Moffat 1991). In our study, sprayed fields were smaller than what one would normally encounter in locust or grasshopper control. For certain species recovery after actual locust control treatments will take more time than observed here. It needs to be assessed in what way these spatial aspects can be included in the study of the risk of resurgence. Quite certainly more data regarding dispersal capacity of the major natural enemies in millet will be required.

With respect to the methodology used we suggest a number of changes. Even though this means a considerable increase in the workload, a more frequent sampling pattern for the different *Heliocheilus* stages will facilitate later interpretation of the results. Similarly, doubling the number of malaise traps per field will probably reduce interplot variability and allow for better separation of treatment effects. The impact of the treatments on *Bracon hebetor* needs to be assessed. Activity densities can be measured using yellow sticky traps (Bhatnagar 1987) Actual density of *B. hebetor* cocoons can be fairly easily determined after harvest of the millet (Beye 1993) which would give an exact measure of generational mortality of *Heliocheilus* by this parasitoid. There is an urgent need to develop a good laboratory diet for *H. albipunctella*. Without such a diet a proper evaluation, through incubation, of mortality of larvae caused by parasitoids is all but impossible. Simple artificial diets need to be tested on millet head miner.

CONCLUSIONS

Fenitrothion applied at approximately 450 g a.i./ha on maturing millet considerably reduced populations of Hymenoptera, in particular the parasitoids *Cardiochiles spp.* (Braconidae) and *Mesa sp.* (Tiphiidae). Recovery was not complete at harvest, the end of the study. Coccinellidae and dipteran natural enemies were not affected. No reduction was found in natural mortality of *H. albipunctalla* eggs. Probably the most important observation was a temporary resurgence of *H. albipunctella* larvae, amounting to a 75% increase in larval peak density, in all fenitrothion treated plots. No effect was found on emergence of diapausing millet head miner chrysalids as well as of *Copidosoma* parasitoids the year after treatment. Initial densities of emerging *Heliocheilus* moths the year after treatment were estimated to be lower than in untreated control plots.

Diflubenzuron applied at approximately 60 g a.i./ha did not cause any reductions in natural enemy taxa monitored by malaise traps, except the braconid parasitoid *Cardiochiles spp.* The latter was affected at the very end of the growing season. This insecticide did not appear to cause a reduction in natural mortality of *H. albipunctella* eggs. A 50% reduction in peak density of *Heliocheilus* larvae was observed in diflubenzuron treated fields. A 110% increase in *Heliocheilus* chrysalid emergence as well as a 50% reduction in *Copidosoma* parasitoid emergence was found the year after treatment. These two effects combined would result in a 75% reduction in the number of *Copidosoma* parasitoids available per emerged millet head miner the year after treatment, when compared to the untreated fields. Initial densities of emerging *Heliocheilus* moths the year after treatment were estimated to be lower than in untreated control plots.

A number of taxa of beneficial arthropods has been identified which may be affected by treatments carried out earlier in the season.

REFERENCES

Bellows TS, van Driesche RG and Elkinton JS (1989) Life tables and parasitism: estimating parameters in joint host-parasitoid systems. pp. 70-80 in: McDonald, L, Manly, B., Lockwood, J. and Logan, J. (eds.) Estimation and analysis of insect populations. Lecture notes in statistics 55. Springer-Verlag. Berlin etc.

Bellows TS, van Driesche RG and Elkinton JS (1992) Life-table construction and analysis in the evaluation of natural enemies. Annual Review of Entomology 37:587-614

Bèye, A (1993) Impact des applications de fénitrothion sur le complexe Heliocheilus et ses principaux antagonistes. LOCUSTOX rapport d'avancement. FAO projet ECLO/SEN/003/NET. Dakar.

Bhatnagar VS (1987) Recherche et développement de la lutte intégrée contre les ennemis des principales cultures vivrières dans les pays du Sahel. Sénégal. Programme de lutte biologique. Rapport Technique Final GCP/RAF/128/CLS. FAO. Rome. pp.168

Bohart RM and Menke AS (1976) Sphecid wasps of the world. A generic revision. University of California Press. Berkeley and Los Angeles.

Borror DJ, Triplehorn CA and Johnson NF (1989) An introduction to the study of insects. Sixth edition. Saunders College Publishing. Philadelphia etc. pp.875

Ciss M and Niane B (1990) Evaluation chimique; l'analyse des résidues de pesticides et détermination de l'activité cholinestérasique. in: Everts, JW (ed.) Environmental Effects of chemical locust and grasshopper control. A pilot study. Project Report ECLO/SEN/003/NET. FAO. Rome.

Crane E and Walker P (1983) The impact of pest management on bees and pollination. Tropical Development Research Institute. London.

Croft BA (1990) Arthropod biological control agents and pesticides. John Wiley and Sons. New York etc. pp.723

Duffield SJ and Moffat J (1991) Does scale influence the recovery of dimethoate-depleted invertebrate populations in winter wheat? pp.305-311 in: Polgár et al. (eds.) Behaviour and Impact of Aphidophaga. SPB Academic Publishing. The Hague.

Dynamac (1988) Results of the locust pesticide testing trials in Sudan. Technical Report. Dynamac Corporation and Consortium for International Crop Protection. Rockville MD.

Elzen GW (1989) Sublethal effects of pesticides on beneficial parasitoids. pp. 129-150 in: Jepson P (ed.) Pesticides and non-target invertebrates. Intercept. Wimborne.

Everts JW (1983) Animal indicators for side-effects of chemical vector control. Environmental Monitoring and Assessment 3:229-236

Fry JM (1989) Natural enemy databank 1987. CAB International. Wallingford. pp.185.

Gadji B (1993) La dégradation du fénitrothion et du diflubenzuron en milieu Sahélien: campagne 91. rapport d'avancement LOCUSTOX. Projet FAO ECLO/SEN/003/NET. Dakar

Gahukar RT, Guevremont H, Bhatnagar VS, Doumbia YO, Ndoye M and Pierrard G (1986) A review of the pest status of the millet spike worm, Rhaguva albipunctella De Joannis (Noctuidae:Lepidoptera) and its management in the Sahel. Insect Science and its Application 7(4):457-463

Geddes AMW (1990) The relative importance of crop pests in sub-Saharan Africa. Natural Resources Institute Bulletin No. 36. Natural Resources Institute. Chatham Maritime. pp. vi.+69

Greathead DJ (1963) A review of the insect enemies of Acridoidae (Orthoptera). Transactions of the Royal Society of London 114(14):437-517

Greathead DJ, Waage JK, Evans HC and van den Berg H (1990) Understanding natural enemies. in: Girling DJ (ed.) UNDP/FAO Biological Control Training Manual. CAB International Institute of Biological Control. Ascot.

Hassan SA, Albert R, Bigler F et al. (1987) Results of the third joint pesticide testing programme by the IOBC/WPRS-Working Group "Pesticides and Beneficial Organisms". Journal of Applied Entomology 103:92-107

Huddleston T and Walker AK (1988) Cardiochiles (Hymenoptera: Braconidae), a parasitoid of lepidopterous larvae, in the Sahel of Africa, with a review of the biology and host relationships of the genus. Bulletin of Entomological Research 78:435-461

Jago ND (1992) IPM in the Sahelian zone, peasant-level farm environment of north-west Mali. pp.25-32 in: Gibson RW and Sweetmore A (eds.) Proc. Sem. Crop Protection for Resource-poor Farmers. CTA/NRI. Wageningen.

Jepson PC (1989) The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. pp.95-127 in: Jepson P (ed.) Pesticides and non-target invertebrates. Intercept. Wimborne.

Jepson PC and Thacker JRM (1990) Analysis of the spatial component of pesticide side-effects on non-target invertebrate populations and its relevance to hazard analysis. Functional Ecology 4:349-355

Jones D (1984) Use, misuse, and role of multiple-comparison procedures in ecological and agricultural entomology. Environmental Entomology 13(3):635-649

Kimsey LS (1991) Relationships among tiphiid wasp subfamilies (Hymenoptera). Systematic Entomology 16:427-438

Manly BFJ (1989) A review of methods for the analysis of stage-frequency data. pp. 3-69 in: McDonald, L, Manly, B., Lockwood, J. and Logan, J. (eds.) Estimation and analysis of insect populations. Lecture notes in statistics 55. Springer-Verlag. Berlin etc.

Muirhead-Thomson RC (1991) Trap responses of flying insects. The influence of trap design on capture efficiency. Academic Press. London etc. pp.287

Ndoye M (1991) Biologie et dynamique de populations de Heliocheilus albipunctella (De Joannis), ravageur de la chandelle de mil dans le Sahel. Sahel PV Info 39:11-20

Rabbinge R, Ward SA and van Laar HH (eds.) (1989) Simulation and systems management in crop protection. Simulation Monographs 32. PUDOC. Wageningen. pp.420

Risbec J (1950) La faune entomologique des cultures au Sénégal et au Soudan français. Station expérimentale de M'Bambey (Sénégal). Gouvernement général de l'Afrique occidentale française. pp.508

Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge University Press. Cambridge. pp.514

Sokal RR and Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research. Second edition. Freeman and Co. New York. pp.859

Stewart-Oaten A, Murdoch WW and Parker KR (1986) Environmental impact assessment: "pseudoreplication" in time? Ecology 67(4):929-940

Underwood AJ (1991) Beyond BACI: experimental designs for detecting human environmental impacts on temporal variations in natural populations. Australian Journal of Marine and Freshwater Research 42:569-587

Van der Stoep J (1993) The development of laboratory toxicity tests with Bracon hebetor (Say) for the study of the environmental effects of locust control. LOCUSTOX Progress Report. Projet FAO ECLO/SEN/003/NET. Dakar.

Van der Valk HCHG (1990) Beneficial arthropods. pp.171-224 in: Everts, JW (ed.) Environmental Effects of chemical locust and grasshopper control. A pilot study. Project Report ECLO/SEN/003/NET. FAO. Rome.

Van Driesche RG (1983) Meaning of "percent parasitism" in studies of insect parasitoids. Environmental Entomology 12(6):1611-1622

Waage J (1989) The population ecology of pest-insecticide-natural enemy interactions. pp. 81-93 in: Jepson P (ed.) Pesticides and non-target invertebrates. Intercept. Wimborne.

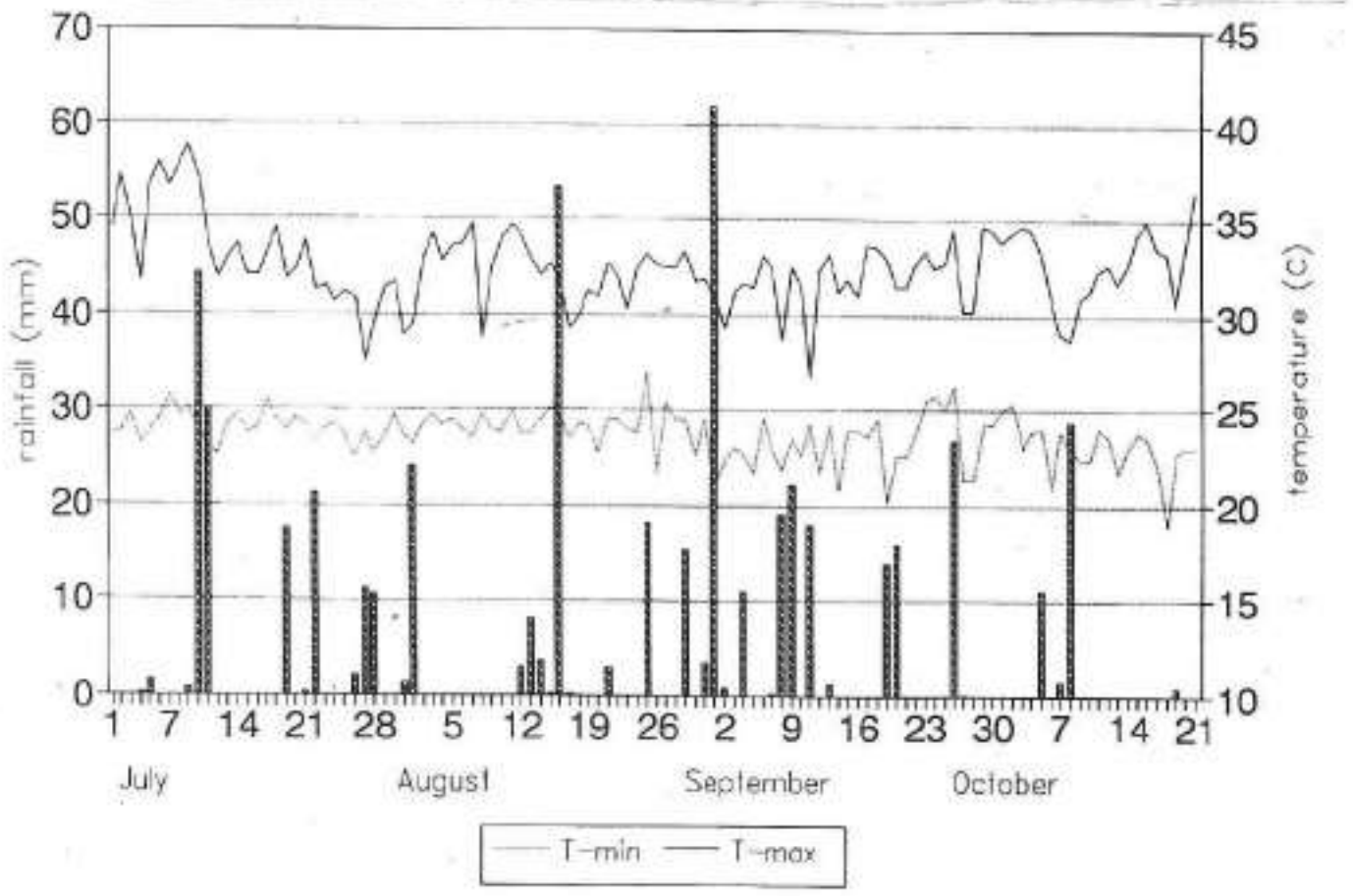
Waage J and Mills NJ (1990) Understanding and measuring the impact of natural enemies on pest populations. in: Girling DJ (ed.) UNDP/FAO Biological Control Training Manual. CAB International Institute of Biological Control. Ascot.

ANNEX 3.1: Trapping scheme and week codes for the malaise traps and direct insect counts.

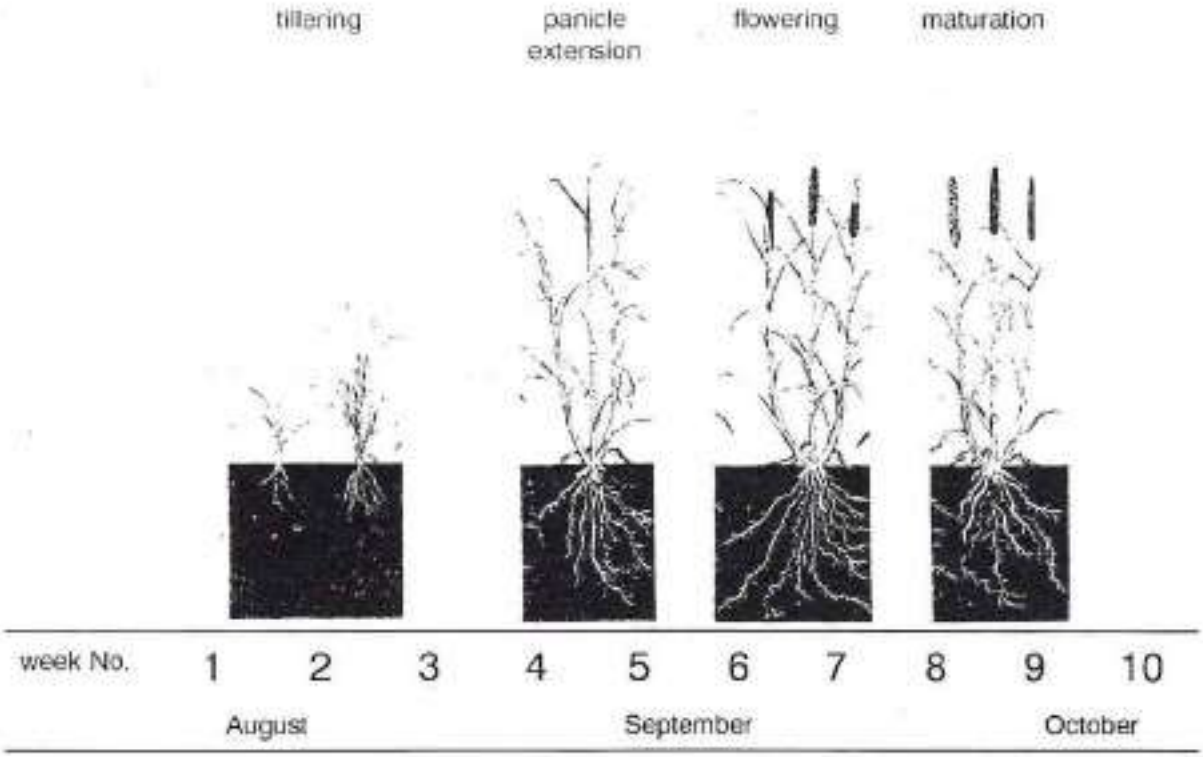
WEEK CODE	*PERIOD*
1	1-7 August 1991
2	8-14 August
3	15-21 August
4	22-28 August
5	29 August - 4 September
6 (treaments')	5-11 September
7	12-18 September
8	19-25 September
9	26 September - 2 October
10	3-9 October
11	10-16 October

': all treatments took place from 11-14 September; week 7 is after treatment for all fields.

ANNEX 3.2: Rainfall Nioro during the rainy season of 1991.



ANNEX 3.3: Schematic approximate development of the millet in the study fields in relation to the trapping weeks.



ANNEX 3.4: Numbers of insects caught in malaise traps in millet fields. Shaded numbers: trapping disturbed

Nioro du Rip 1991

HYMENOPTERA		TOTAL HYMENOPTERA											
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	15	43	39	44	52		19	24	41	10	7	294
4		46	156	122	90	55		10	9	81	17		586
9		60	35	65	35	63		15	9	12	5		299
11		34	60	20	34	51		49	8	5	3	21	285
	mean	38.8	73.5	61.5	50.8	55.3		23.3	12.5	34.8	8.8	14.0	366.0
3	DIFLUBENZURON	13	24	36	66	78		58	42	19	42		378
5		42	105	58	54	74		58	53	32	22	32	530
8		84	116	249	168	199		85	67	49	70		1089
10		60	145	109	35	67		43	51	56	25	12	603
	mean	49.8	98.0	113.0	80.8	104.5		61.0	53.3	39.0	39.8	22.0	650.0
2	UNTREATED	41	114	154	69	60	156	119	83	33	33		882
6		83	169	101	37	72	57	106	68	32	43	84	852
7		67	114	106	28	67	98	85	91	62	62		600
12		118	153	132	148	144	135	135	118	112	72		1266
	mean	77.5	137.5	123.3	69.5	95.8	116.0	112.3	90.0	59.8	52.5	84.0	955.0

ICHNEUMONIDAE		TOTAL											
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	1	9	0	3	1		2	1	6	0	2	34
4		8	38	12	5	5		1	2	8	1		80
9		7	3	11	2	6		5	2	0	1		37
11		1	11	3	3	1		4	1	0	0	2	26
	mean	4.3	15.3	6.6	3.3	3.3		3.0	1.5	3.5	0.5	2.0	44.3
3	DIFLUBENZURON	1	7	0	1	1		6	1	0	5		22
5		7	11	5	6	6		2	3	5	0	0	47
8		13	16	9	10	11		5	2	3	0		69
10		14	40	6	3	4		0	4	1	1	0	81
	mean	8.8	20.0	5.5	5.0	6.0		3.3	2.5	2.3	1.5	0.0	54.6
2	UNTREATED	3	11	6	2	2	2	3	4	2	6		39
6		3	24	7	3	6	8	7	7	3	3	12	83
7		8	24	17	2	7	5	6	4	0	5		79
12		22	39	12	5	4	4	10	5	15	2		108
	mean	9.0	22.0	10.5	3.0	5.3	4.5	6.5	5.0	5.0	3.5	12.0	77.3

ANNEX 3.4 (continued)

BRACONIDAE		CARDIOCHILES SPP.											
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	1	0	0	2	3		4	2	4	1	0	16
4		0	3	5	14	4		1	1	24	5		57
9		0	0	2	2	9		1	1	4	0		19
11		1	0	0	1	4		3	0	0	1	5	15
	mean	0.5	0.8	1.8	4.8	4.8		2.3	1.0	8.0	1.8	2.5	28.8
3	DIFLUBENZURON	0	1	1	1	3		2	4	0	5		17
5		0	0	1	5	3		6	3	4	1	2	21
8		3	2	11	7	13		15	1	4	7		63
10		1	0	0	1	12		8	7	12	4	5	50
	mean	1.0	0.8	3.3	2.5	7.8		7.8	3.8	5.0	4.3	3.5	37.8
2	UNTREATED	1	2	4	5	4	11	7	9	2	6		50
6		3	6	5	1	3	2	8	9	3	10	7	57
7		1	0	1	2	15	13	9	13	9	23		86
12		1	3	1	4	13	25	22	27	25	28		149
	mean	1.5	2.8	3.8	3.0	8.8	12.8	11.5	14.5	9.8	16.5	7.0	85.5

BRACONIDAE		ALL SPECIES LUMPED EXCLUDING CARDIOCHILES SPP.											
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	0	0	2	6	5		4	1	7	3	2	29
4		0	8	7	4	4		2	0	9	4		38
9		1	1	4	7	5		1	3	1	1		24
11		0	3	0	5	5		10	0	0	0	0	23
	mean	0.3	3.0	3.3	5.3	4.8		4.3	1.0	4.3	2.0	1.0	28.5
3	DIFLUBENZURON	0	2	2	1	2		12	5	1	12		27
5		4	7	3	3	6		1	6	2	3	5	40
8		1	9	15	17	17		16	7	6	7		95
10		4	4	4	2	6		5	1	4	2	3	35
	mean	2.3	5.5	6.0	5.8	7.9		8.5	4.8	3.3	3.5	4.0	49.3
2	UNTREATED	1	9	8	4	5	11	7	9	2	6		60
6		6	5	0	1	3	9	10	3	1	4	5	47
7		7	11	12	4	16	10	9	2	6	6		83
12		4	11	19	26	25	42	18	10	8	4		165
	mean	4.5	9.0	9.8	8.8	12.3	18.0	10.5	6.0	4.3	4.5	5.0	88.8

ANNEX 3.4 (continued)

Nkoro du Rip 1991

DIPTERA TACHINIDAE		SPECIES 1											
WEEK:		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	1	3	1	6	2		10	12	84	86	89	291
4		1	3	4	17	12		6	4	21	36		104
9		0	1	0	1	3		19	39	181	145		389
11		1	1	0	0	1		5	1	4	1	75	89
	mean	0.75	2.00	1.25	5.25	4.50		10.00	14.00	72.50	67.00	82.00	
3	DIFLUBENZURON	1	2	0	0	1		9	7	22	95		137
5		4	0	2	2	2		3	8	21	46	21	109
8		0	3	5	4	19		82	17	246	445		801
10		0	6	4	2	6		19	19	23	72	57	208
	mean	1.25	2.75	2.75	2.00	7.00		23.25	12.75	78.00	164.50	39.00	
2	UNTREATED	1	3	2	1	1	11	18	43	35	142		256
6		0	2	3	4	4	10	20	3	9	29	49	133
7		0	4	5	0	15	28	26	21	34	169		322
12		6	3	1	0	14	15	15	24	51	178		307
	mean	1.75	3.00	2.75	1.25	8.50	16.00	19.75	22.75	32.25	134.75	49.00	

DIPTERA TACHINIDAE		ALL BUT SPECIES 1											
WEEK:		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	3	2	8	3	1		1	2	2	1	12	35
4		15	19	14	6	5		1	0	8	1		69
9		7	7	5	0	0		0	3	4	3		29
11		3	7	3	3	0		3	1	1	3	21	45
	mean	7.00	8.75	7.50	3.00	1.50		1.25	1.50	3.75	2.00	16.50	
3	DIFLUBENZURON	1	13	9	5	4		5	9	6	14		66
5		6	9	9	8	1		1	7	8	8	4	57
8		13	20	8	7	4		2	12	5	14		85
10		5	18	1	2	2		5	4	2	0	3	44
	mean	6.50	15.25	6.75	5.00	2.75		3.25	8.00	5.50	8.50	3.50	
2	UNTREATED	13	6	5	4	3	2	1	5	0	1		40
6		21	42	21	7	4	3	5	2	5	2	12	124
7		22	28	7	6	6	6	8	5	13	18		117
12		27	19	11	7	7	6	11	10	10	15		123
	mean	20.75	23.75	11.00	6.00	4.50	4.25	6.25	5.50	7.00	9.00	12.00	

ANNEX 3.4 (continued)

ALISMA STRIATA	#/100 STEMS											
WEEK	1	2	3	4	5	6	7	8	9	10	11	total
						treatment						
FIELD	TREATMENT											
1	FENITROTHION				0.4	4.3		5.3		5.4		
4					4.9	0.8		4.7	3.9	3.9		
9					1.1	0.8		2.9		9.1		
11					1	2		5.8		19.4		
	mean				1.85	1.98		4.68	3.90	6.20		
3	DIFLUBENZURON				4.4	17.4		22.1	50.9	2.9		
5					0.4	1.5		20.6	4.5	11.5	0.7	
8					7.6	23.3		36.8	11.8	8.7		
10					0.8	4.1		1.5	16	15.8	0	
	mean				3.35	11.58		20.25	20.75	9.23	0.35	
2	UNTREATED			0	0.6	10.7		12.9		3.7		
6				0	1.9	1.9		8.2	7.6	6.8	4.1	
7				0	3.4	14.3	10.7	71.7	32.6	13.5	10.1	
12				0	0.7	4.6	1.5	12	13.5	17.1		
	mean			0	1.70	7.88	6.10	26.20	18.03	10.30	7.10	

DIPTERA: SYRPHIDAE	Ischiodon aegyptiacus											
WEEK	1	2	3	4	5	6	7	8	9	10	11	total
						treatment						
FIELD	TREATMENT											
1	FENITROTHION	0	0	0	1	0	2	4	2	0	0	9
4		0	1	1	1	0	0	1	0	2		6
9		0	0	0	2	0	15	15	0	0		32
11		3	0	0	0	0	2	10	1	1	0	15
	mean	0.25	0.25	0.25	1.00	0.00	4.75	7.50	0.75	0.75	0.00	
3	DIFLUBENZURO	1	7	6	2	0	0	3	1	0		20
5		1	2	1	1	2	3	16	0	0	5	31
8		0	3	5	5	10	3	13	6	0		45
10		0	1	1	1	0	6	6	1	2	0	18
	mean	0.50	3.25	3.25	2.25	3.00	3.00	9.50	2.00	0.50	2.50	
2	UNTREATED	0	0	3	2	1	9	4	2	1	1	23
6		0	4	0	1	0	5	2	6	0	0	18
7		1	3	2	2	5	10	6	5	0	3	37
12		3	1	1	0	1	24	11	31	2	1	75
	mean	1.00	2.00	1.50	1.25	1.75	12.00	5.75	11.00	0.75	1.25	0.00

ANNEX 3.4 (continued)

SPHECOME TACHYTES SP													
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	10	10	3	7	6		1	0	1	1	0	39
4		11	22	15	7	2		1	0	1	0		59
9		10	5	10	4	3		0	0	0	0		32
11		11	13	8	5	5		2	0	0	0	0	42
	mean	10.50	12.50	8.50	5.75	4.00		1.00	0.00	0.50	0.25	0.00	
3	DIFLUBENZURO	1	6	8	5	1		3	1	0	0		25
5		13	23	12	9	2		3	0	1	1	0	63
8		15	35	30	23	5		0	0	1	0		110
10		10	6	13	7	2		2	6	1	0	0	47
	mean	9.75	17.75	15.75	10.75	2.50		2.00	1.75	0.75	0.25	0.00	
2	UNTREATED	13	19	13	8	5	4	1	0	0	0		62
6		20	16	23	8	5	6	5	3	0	1	4	89
7		17	26	11	7	8	3	2	3	3	2		80
12		27	26	22	25	14	1	4	4	0	1		124
	mean	19.25	21.50	17.25	11.50	7.50	3.50	3.00	2.50	0.75	1.00	4.00	

MESA SPP													
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	0	1	5	10	5		0	6	3	0	0	30
4		10	19	14	18	9		0	0	1	1		69
9		27	18	6	5	14		0	0	1	0		70
11		4	3	2	10	5		2	0	1	0	2	29
	mean	10.25	9.50	6.59	10.75	8.25		0.50	1.50	1.50	0.25	1.00	
3	DIFLUBENZURON	1	1	3	17	14		7	4	0	14		63
5		3	10	4	16	20		19	7	3	2	0	84
8		21	17	7	5	8		6	8	5	0		77
10		4	10	3	2	0		0	2	1	0	0	22
	mean	7.25	9.50	4.75	10.00	10.50		8.00	5.25	2.25	4.00	0.00	
2	UNTREATED	11	17	2	14	30	39	28	8	0	0		149
6		14	6	3	2	4	4	15	7	1	0	0	56
7		10	9	4	2	2	0	1	0	3	3		34
12		21	18	3	4	4	5	3	14	18	4		94
	mean	14.00	12.50	3.00	5.50	10.00	12.00	11.75	7.25	5.50	1.75	0.00	

ANNEX 3.4 (continued)

Nioro du Rip 1991

DIPTERA BOMBYLIIDAE													
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
FIELD	TREATMENT												
1	FENITROTHION	0	0	0	3	2		3	0	0	0	0	8
4		0	0	2	8	8		0	0	3	0		21
9		0	0	6	3	4		1	0	0	0		14
11		0	0	5	5	1		7	1	1	1	7	28
	mean	0	0.00	3.25	4.75	3.75		2.75	0.25	1.00	0.25	3.50	
3	DIFLUBENZURON	0	0	2	5	1		0	0	3	4		15
5		4	0	5	11	7		0	0	1	3	0	31
8		6	4	9	9	6		1	1	1	0		37
10		0	0	3	2	9		0	1	1	2	0	18
	mean	2.50	1.00	4.75	6.75	5.75		0.25	0.50	1.50	2.25	0.00	
2	UNTREATED	0	1	1	2	3	3	1	1	0	0		12
6		0	0	4	13	14	5	2	1	2	2	11	54
7		6	0	3	2	7	1	0	1	3	3		26
12		0	0	24	21	29	12	1	1	0	1		89
	mean	1.50	0.25	8.00	9.50	13.25	5.25	1.00	1.00	1.25	1.50	11.00	

DIPTERA ASILIDAE													
WEEK		1	2	3	4	5	6	7	8	9	10	11	total
							treatment						
	TREATMENT												
1	FENITROTHION	1	1	0	0	0		0	0	0	0	5	2
4		10	31	17	7	1		0	0	1	1		68
9		29	4	4	1	0		0	0	0	1		39
11		8	3	0	0	0		0	0	0	0	0	11
	mean	12	9.75	5.25	0.75	0.25		0.00	0.00	0.25	0.50	2.50	
3	DIFLUBENZURO	0	1	1	2	2		3	0	0	0		9
5		8	12	4	2	0		0	0	2	0		28
8		4	14	6	0	0		0	0	1	0		25
10		7	13	5	0	0		0	0	0	1	0	26
	mean	4.75	10.00	4.00	1.00	0.50		0.75	0.00	0.75	0.25	0.00	
2	UNTREATED	4	5	0	4	3	0	1	1	1	0		19
6		0	6	3	0	0	0	0	0	0	0	0	9
7		21	32	29	6	1	0	0	0	0	0		89
12		10	13	0	5	2	0	0	0	3	0		42
	mean	8.75	14.00	10.25	3.75	1.50	0.00	0.25	0.25	1.00	0.00	0.00	

CHAPTER 4 :

Impact of fenitrothion applications on natural mortality of grasshopper eggpods in Senegal (1991 treatments)

INTRODUCTION

Locust and grasshopper eggpods in Africa can be subjected to large natural mortality caused by arthropod predators and parasitoids. Percentage mormxlity caused by natural enemies varies according to country, type of habitat and species of grasshopper studied (table 4.1).

Table 4.1: Some examples of natural mortality of grasshopper eggpods in West-Africa.

Country	Species	% Mortality	Principal agent	Author
Mali, Niger	*Odaeleus senegalensis*	43 - 77	Tenebrionidae	Popov 1980
Mali	*Odaeleus senegalensis*	40 - 80	not determined	SNPV 1990
	others	0 - 80	not determined	
Niger	*Odaeleus senegalensis*	60 - 70	Bombyliidae	in Popov et al. 1990
Mali	*Locusta migratoria*	13	Scelionidae	in Popov et al. 1990
Senegal	mainly *Kraussaria angulifera*	0 - 43	not determined	Beye and Ndiaye 1992

Insecticide treatments against grasshoppers and locusts may kill predatory or parasitic arthropods. This could result in a reduction of natural mortality of eggpods and subsequently increase of grasshopper populations the following season.

This study investigates the effects of regular, non-experimental, fenitrothion applications against grasshoppers on natural mortality of eggpods in central Senegal.

MATERIALS AND METHODS

The study area

The study was carried out in central Senegal, within a triangle formed by the towns of Kaffrine, Kholkom and Koungheul. The area consists to a large extent of grazing land interspersed mainly with rainfed agriculture (pearl millet, groundnuts). Annual rainfall in the zone varies between 150 and 600 mm.

General study setup

Nine insecticide treatments against grasshoppers carried out by the Senegalese Crop Protection Service were monitored in detail with respect to principal application parameters. Treatments were carried out according to normal CPS practice and project staff did not intervene in this respect. The boundaries of all spray blocks were marked with white paint to facilitate them being revisited after several months. For every spray block a non-treated control area of approximately 4 ha was identified as close to the sprayed area as possible but always at least 500 m from the nearest edge to avoid possible contamination by drift from the treatment. Table 4.2 gives the location of each block.

Table 4.2. Location of study areas.

Block	Location	Coordinates	Description
I	Kholcom	14°34'N / 15°27'W	grassland with small shrubs
II	Kholcom	14°32'N / 15°28'W	grassland with small shrubs
III	Kholcom	14°31'N / 15°28'W	grassland with small shrubs
IV	Kholcom	14°35'N / 15°26'W	grassland with small shrubs
V	Santiou Paté	13°48'N / 13°40'W	cultivated fields (millet, maize) and fallow
VI	Kakaré	14°17'N / 15°30'W	cultivated fields (millet, peanuts) and fallow
VII	Guénté Paté	14°17'N / 14°55'W	cultivated fields (millet) and fallow
VIII	Thioyène	14°18'N / 14°54'W	cultivated fields (millet, maize, sorghum) and fallow
IX	Khaye Boubou	14°14'N / 14°53'W	cultivated fields (millet) and fallow

All sprayed blocks and their paired controls were visited mid December and detailed grasshopper eggpod surveys carried out. This was 2.5 to 3.5 months after treatment and about two months after the last rains in most zones. Blocks I, III and VI were visited a second time in June 1992. Percentages destruction of eggpods by natural enemies and densities of some groups of natural enemies were recorded.

Insecticide application

All treatments were carried out with the insecticide fenitrothion (Sumithion L-50, Sumitomo Chemical Corporation) in a 500 g a.i./l Ultra Low Volume formulation. The pesticide was applied using a Berthoud Super Puma sprayer, mounted on a small Unimog truck. The sprayer is of the airblast type. The spray head was about 3 metres above ground level. The drop spectrum for the sprayer at the operating settings was not known, but given the type of atomisation, it can be expected to be fairly wide.

In all cases the volume of pesticide at the start of the treatment and the volume left afterwards were measured back to the nearest 5 litres to allow for an estimate of the total volume applied. For blocks I-IV the size of the spray blocks was measured; for blocks V-IX this was not possible and the surface area treated was estimated based on mean vehicle speed, total time spraying, estimated mean track

spacing and number of tracks. Area dosage was calculated based on total volume of formulation applied and the estimated area treated.

Air temperature was measured at the start and end of the treatment using an aspired psychrometer. The range of windspeeds was measured during one minute at the start and one minute at the end of the treatment, with a cup anemometer held at approximately 1.75 m above the ground.

Grasshopper densities

Grasshopper densities were estimated for the predominant species in the blocks. Numbers of grasshoppers were counted in ten visualized 1 m² quadrats in the centre of each block. This was done just before the treatment. Numbers of live and dead grasshoppers were counted in ten 1-m2 quadrats two to four hours after each treatment to allow an estimate of acute mortality. During the last week of October all plots were visited again to assess reinvasion of the plots.

Soil sampling

Soil samples were taken to assess grasshopper eggpod densities, level of eggpod natural mortality and presence of certain natural enemies. This was done in all blocks during the first half of December 1991 and was repeated in blocks I, III and VI at the beginning of June 1992. No other blocks were sampled in this second round because of an early start of the rainy season in the area.

During the first sampling round, on each of the paired plots (both treated and untreated) a total of 90 soil samples were taken. Each sample measured 50 x 50 cm and was 20 cm deep. Of these samples 30 were situated under trees, 30 under and around bushes and 30 in low and open vegetation. Samples were layed out on 1 median and 2 diagonal transects in the central hectare of each plot.

During the second sampling round 40 samples were taken per plot, only under trees and shrubs. No sampling was carried out in bare soil since low eggpod densities were found in this habitat during the first round (see results below).

Every sample was carefully examined for grasshopper eggpods and certain natural enemies. All eggpods were counted and classified as "intact" or "damaged". The latter comprised both damage done by the predators such as larvae of Tenebrionidae which destroy a large part of the eggpod wall, as well as subtle damage such as small holes bored by certain fly larvae. Eggpods mechanically damaged by the digging were inspected for evidence of predators and if this was not present the eggpods were classified as "intact". Natural enemies of grasshopper eggs such as *Pimelia senegalensis* (Tenebrionidae) and larvae of Bombyliidae were counted whenever present in the sample.

Statistical analysis

All statistical comparisons of means between paired control and treated blocks were made with paired Student-t tests, after verifying for homogeneity of variances. Percentages were as a rule transformed using an arcsine transformation to improve normality of the data. Comparison of the linear regression coefficients was done by the Tukey-Kramer method (Sokal and Rohlf, 1981). Power analysis was carried out according to Fairweather (1991) and Steel and Torrie (1980).

RESULTS

Pesticide applications

Details of the pesticide applications are summarized in table 3.

Treatments in block I to IV were made under ideal meteorological conditions. Temperatures were below 30°C and windspeeds were above 2 m/s. Windspeeds during the last 5 treatments were somewhat lower, although still considered acceptable for ULV drift spraying. However, together with the higher temperatures during application, this may have resulted in slightly less deposition of the insecticide on the vegetation on these plots.

Table 4.3: Application parameters for the different spray blocks.

block	date of treatment	hour start - end	temperature start - end (°C)	windspeed (m/s)	volume applied (l)	area treated (ha)	dose applied (g m.a./ha)
I	26/08/91	09:45 - 10:25	28 - 28	2 - 4	100	40	1250
II	26/08/91	10:40 - 11:00	28 - 28	3 - 5	50	30	830
III	26/08/91	12:00 - 12:20	29 - 29	3 - 5	50	25	1000
IV	26/08/91	16:15 - 16:40	29 - 29	3 - 5	50	30	830
V	05/09/91	08:50 - 10:45	-	1 - 3	200	165	605
VI	17/09/91	17:20 - 18:25	33 - 34	1 - 2.5	100	90	555
VII	25/09/91	08:05 - 08:45	30 - 31	1 - 3	25	42	300
VIII	26/09/91	08:50 - 09:20	31 - 32	1 - 2	25	35	360
IX	04/10/91	-		1 - 2	20	28	360

The nominal application rate for all treatments was 350 g a.i./ha or 700 ml formulation/ha. As can be seen from the estimated actual dose rates, the appoximate nominal dose was only applied in 3 out of 9 cases. Actual rates varied from 85 % to 360 % of the intended rates, the average applied rate being twice the intended. This deviation was probably caused by insufficiently calibrated equipment and application practice.

Control efficacy and residual grasshopper populations

Almost all grasshoppers in the treated plots were in the larval stages, varying from 2nd to 5th instars (table 4). *Odaeleus senegalensis* was the predominant species, followed by *Kraussaria angulifera*. Mortality directly after the treatments varied from 88 % to 99 %. The higher dose rates did not result in a higher mortality. By the end of October, approximately one month after the last considerable rains, residual populations consisted mainly of *O. senegalensis* adults and *Kraussaria spp.*, most of which must have flown in from outside the plots. Densities of grasshoppers at the end of October were not significantly different in paired treated and control plots ($p = 0.18$; paired Student-t test).

Since at the time of the treatments most species were present only as larvae, it is most likely that the subsequent immigrant adults were responsable for the oviposited eggpods which were found during the eggpod survey. Although eggs in diapause of *O. senegalensis* can stay viable for longer than one year (Cheke, 1990), it is unlikely that this is the case given that sufficient rain fell in the area in 1991 for normal hatching. Therefore, most if not all eggpods in both the treated and the control plots are expected to have been laid after the dates of the treatment. In treated plots they were thus subjected to natural enemies which either survived the treatment while being exposed, or were protected from the spray (eggs or larvae in soil, for instance), or immigrated into the plots afterwards.

Table 4.4: Grasshopper densities and control efficacy in the spray blocks.

Block	Date	Density before treatment (#/m²)	predominant species [stage]	After treatment: # dead/m²	After treatment: # alive/m²	Mortality (%)	Density end of October (#/m²) (treated/ control)	Predominant species [stage] (treated/ control)
I	26/08/91	69	OSE,KAM [l]	64	3	95 - 96	4 / 6	OSE [ad]/ OSE [ad]
II	26/08/91	32	OSE,KAM [l]	14	2	88 - 94	6 / 11	OSE [ad]/ OSE,ONI [ad]
III	26/08/91	35	OSE,KAM [l]	25	1	96 - 97	4 / 4	OSE [?]/ OSE [?]
IV	26/08/91	31	OSE,KAM [l]	18	2	90 - 94	–	–
V	05/09/91	37	KAM,KAN [l]	34	2	99 - 99	6 / 6	OSE,ONI [?]/ OSE,ONI,KAN [?]
VI	17/09/91	24	OSE,KAM, KAN [ad,l]	26	1	96 - 96	4 / 4	OSE,KAM [ad]/ OSE,KAM [ad]
VII	25/09/91	37	OSE,ONI [l]	27	1	96 - 97	0.1/0.1	OSE,KAN [ad]/ OSE,KAN [ad]
VIII	26/09/91	18	OSE,KAM [l]	32	0.5	96 - 97	0.5/1	OSE,ONI,HDA [ad]/ OSE,KAN, CCY [ad]
IX	04/10/91	–	–	–	–	99 (estimation subjective)	0.5/0.5	OSE [?]/ OSE [?]

OSE: Odaeleus senegalensis, KAM: Krauseile amabile, KAN: Kraussaria angulifera, ONI: Odaeleus nigeriensis, HDA: Hieroglyphus daganensis, CCY: Catalopus cymbiferus, [ad]: adultes, [l]: larves.

Impact of the treatments on eggpod natural mortality

All raw data are given in Annex 4.1.

December 1991 sampling round

A total of 3275 eggpods were collected and examined for natural enemy damage. Eggpod density varied from 3.3 to 21 pods per square meter. On average eggpod densities in the treated blocks were 1.2 eggpods per square meter (or 14%) lower than their paired controls. This was not statistically significant (p=0.23; paired Student-t). The treatments in August and September therefore did not have any impact on residual eggpod densities at the end of the season. Far more eggpods were found under trees and bushes than from open micro-habitats (fig 4.1).

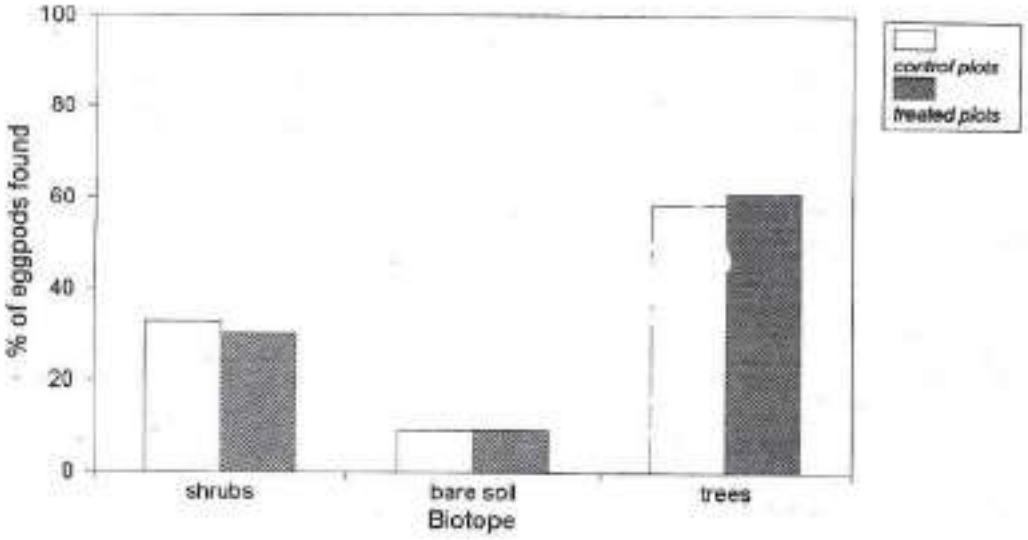


Figure 4.1. Percentage of eggpods found in three equally sampled biotopes: under shrubs, in bare soil and under trees.

In December 1991, eggpod mortality caused by natural enemies varied from 8 to 51 % (fig 4.2a). An 8.4 %, statistically significant, increase in natural mortality was observed in the treated plots (paired Student-t, p=0.014).

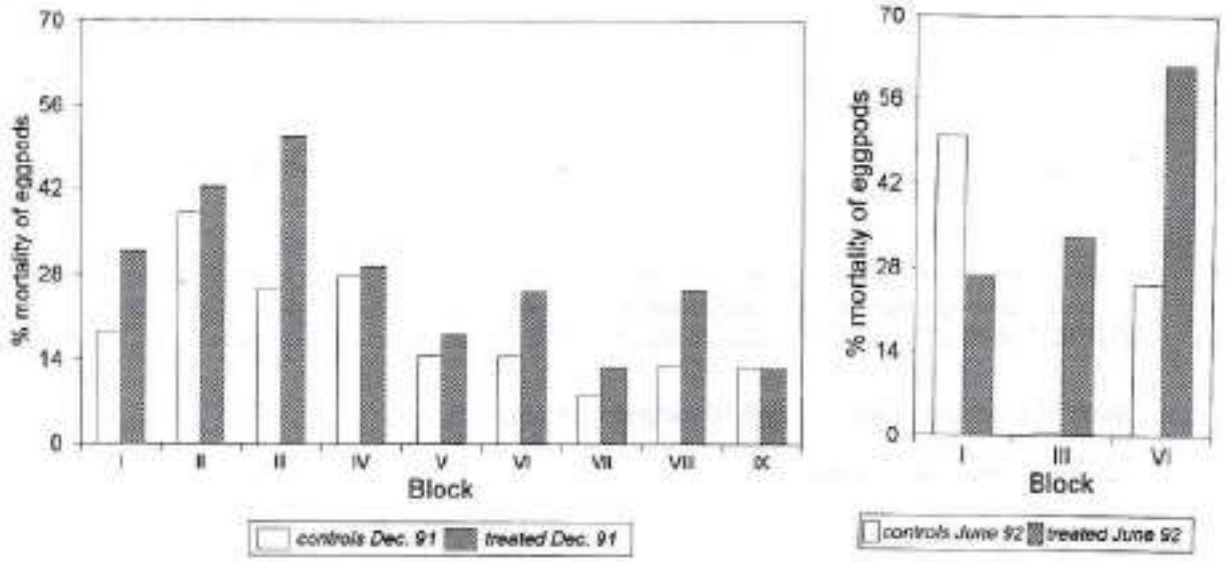


Figure 4.2. a) Natural mortality of eggpods in treated and paired control plots sampled in December 1991. b) Blocks I, III and VI were sampled a second time in June 1992.

June 1992 sampling round

A total of 472 eggpods were collected from 3 blocks (6 plots) and examined for natural enemy damage (see Annex 4.1 for raw data). Eggpod densities varied from 0.4 to 33 per square meter. In all but two samples, eggpod density was considerably lower in June than in the previous December, with a mean reduction of 33% (table 4.5). Since no hatching had taken place yet, this suggests that natural enemies which can destroy the whole eggpod, such as tenebrionid larvae, were still active after December. Eggpod densities were not significantly different between treated and control plots (n=3, paired Student-t, p=0.26).

Table 4.5: Comparison of densities of grasshopper eggpods between December '91 and June '92.

Block	Number of eggpods per 10 square meter			
	Control		Treated	
	Dec. 91	June 92	Dec. 91	June 92
I	32	4	34	34
III	27	9	31	15
VI	206	317	160	93

Eggpod mortality caused by natural enemies varied from 0 to 61% (fig 4.2b). In some cases the June mortality was higher than the December figure, in other cases lower. This apparent inconsistency may be caused by the fact that an unknown number of eggpods was destroyed beyond recognition as suggested above. The mean eggpod mortality in the treated blocks was not significantly different from the controls, but the number of repetitions was very low (paired Student-t, p=0.78).

Effect of natural enemies on eggpod mortality

Two groups of eggpod natural enemies were distinguished in this study: *Pimelia senegalensis* (Coleoptera, Tenebrionidae), of which both adults and larve were found in the soil samples, and larvae of Bombyliidae (Diptera), the latter being represented by one, presently unidentified, species or species complex.

During the first sampling round a total of 321 adults and 627 larvae of a large tenebrionid beetle, almost certainly *P. senegalensis* (final identification awaits further rearing studies being carried out in Dakar), and 159 bombyliid larvae were collected (Annex I). Only the larval stages of both groups of families of insects are known to be eggpod predators (Popov et al. 1990, Greathead 1963). During this study larvae of *P. senegalensis* were sometimes found inside partly destroyed grasshopper eggpods. The bombylid larvae were part of the soil sample but not found inside the eggpods. It is therefore not certain that this species actually attacks eggpods.

In fig. 3 it can be seen that both *P. senegalensis* and the bombyliid larvae tend to aggregate in the same biotopes as grasshopper eggpods. Figures 4.4 and 4.5 show the relation between predator density and eggpod mortality for these two groups. No significant correlation was found for bombyliid larvae ($r=0.20$, $df=16$, $p>0.05$). Larvae of *P. senegalensis*, however, were highly positively correlated with eggpod mortality ($r=0.77$, $df=16$, $p<0.01$; with slope of regression highly signicantly larger than zero: $p=0.001$).

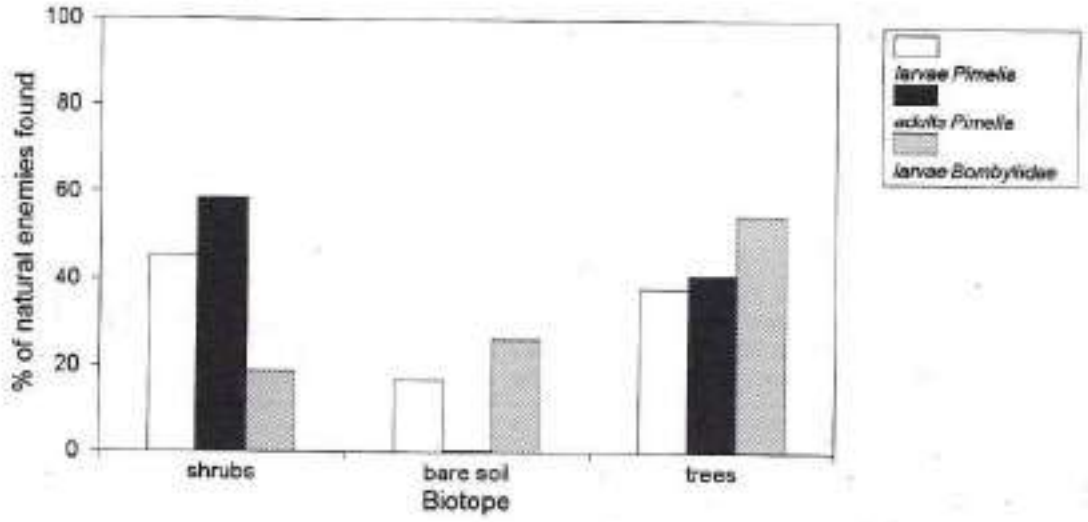


Figure 4.3. Distribution of *Pimelia senegalensis* and bombyliid larvae over the three equally sampled biotopes: under shrubs, in bare soil and under trees.

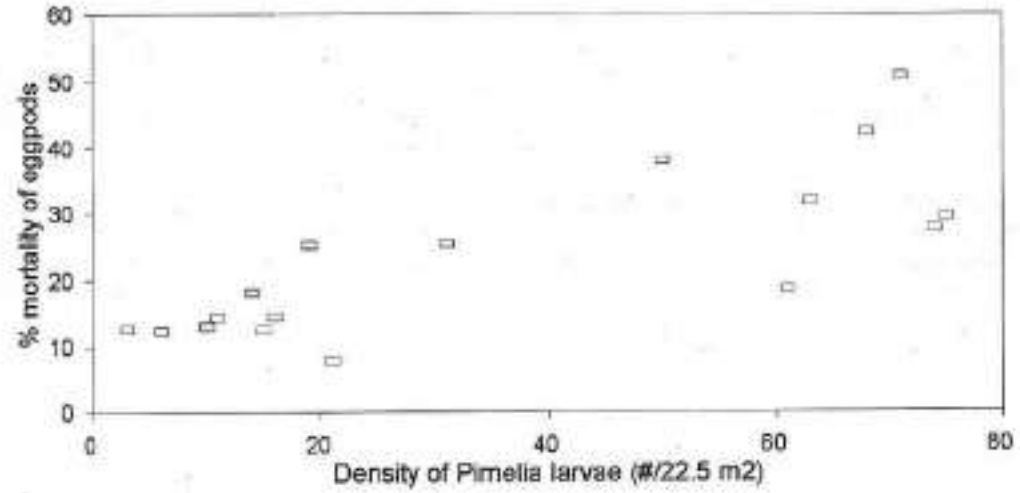


Figure 4.4. Relationship between the density of *Pimelia senegalensis* larvae and eggpod mortality (December 91 sampling round).

During the second sampling round in June 1992, 144 adults and 11 larvae of *P. senegalensis* were sampled as well as 6 bombyliid larvae. Table 6 shows the changes in population structure of *P. senegalensis* between December '91 and June '92. There is a clear reduction in larval density in all studied plots, while the number of adults on average has doubled. Also, most of the adults found still had very shiny cuticles, and thus were relatively young. This suggests that sometime towards the end of the dry season pupation has taken place as well as subsequent emergence of adults.

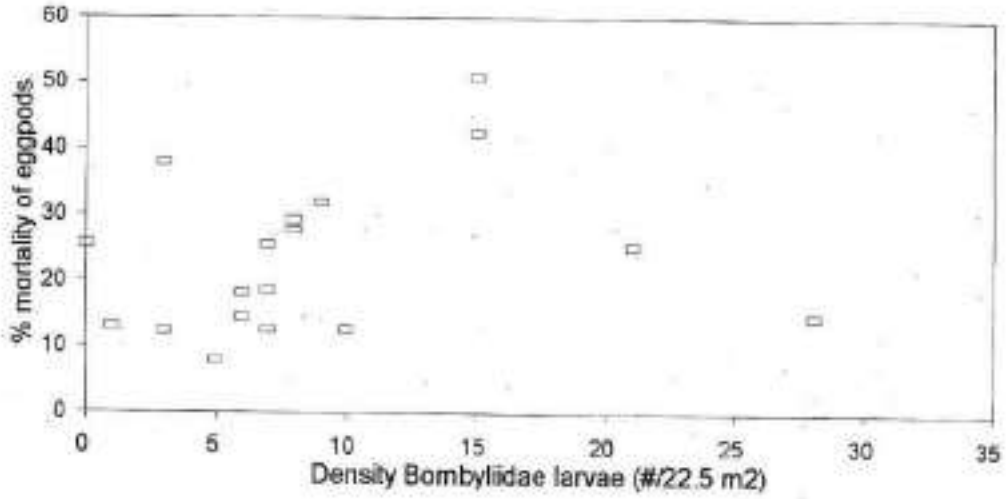


Table4. 6: Comparison of the number of *Pimelia senegalensis* in three spray blocks and paired controls in December 1991 and June 1992.

Block	Number of insects per 10 square meter							
	Control				Treated			
	Larvae		Adults		Larvae		Adults	
	Dec. 91	June 92	Dec. 91	June 92	Dec. 91	June 92	Dec. 91	June 92
I	24	3	14	54	24	0	23	19
III	14	3	16	7	26	1	20	34
VI	5	0	0.4	9	6	4	1	23
total	43	6	30.4	70	56	5	44	76

Effect of treatments on Pimelia senegalensis

No statistically significant difference was observed in densities of *P. senegalensis* larvae or adults between control and treatment plots (figs 4.6 and 4.7) during the first sample round, nor during the second (paired Student-t test) (table 4.6).

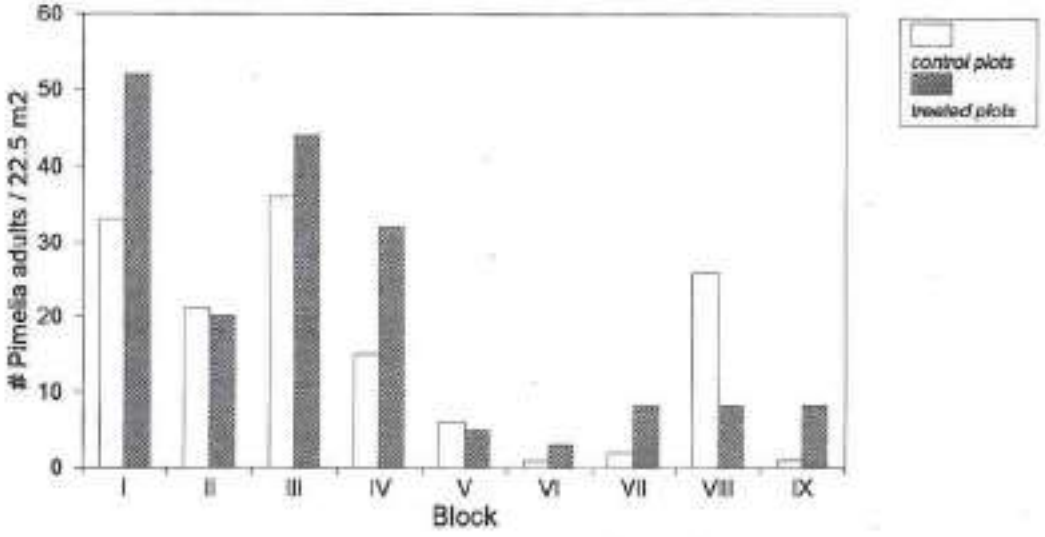


Figure 4.6. Densities of *Pimelia senegalensis* adults on treated and untreated plots. Treatments took place in August/September; densities measured in December.

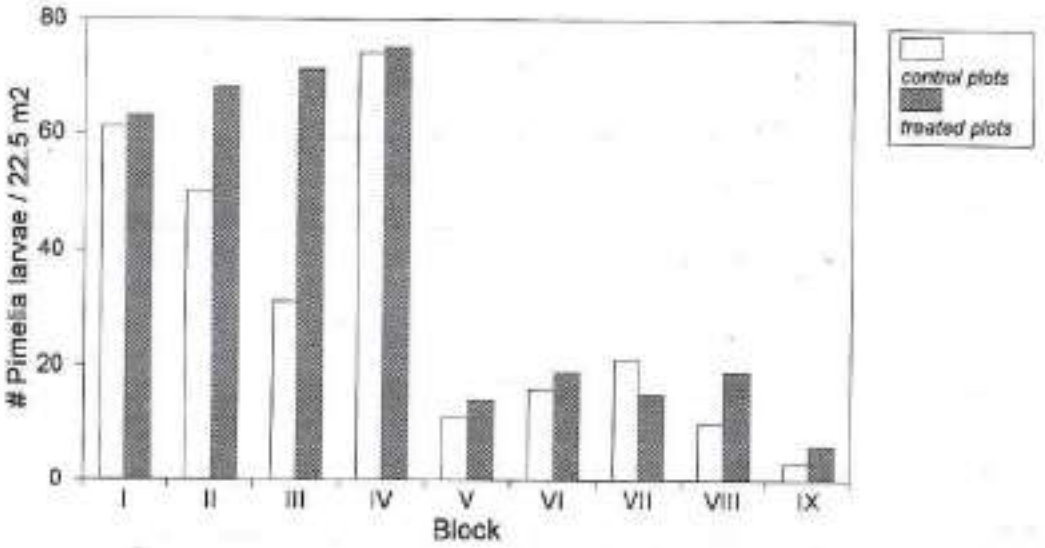


Figure4. 7. Densities of *Pimelia senegalensis* larvae on treated and untreated plots. Treatments took place in August/September; densities measured in December.

Confounding factors in the analysis

The effect of density

Factors which can influence the degree of eggpod mortality comprise not only the insecticide treatment but also eggpod and predator density. An increase in both prey and predator density will heighten the chance of prey encounter by the predator and increase natural mortality (see e.g. Begon et al, 1990 for different response types).

However, above it was found that the treated plots had a mean eggpod density which was not significantly different from the untreated plots. The same is true for Pimelia adults as well as larvae. Therefore, it can be assumed that the difference in eggpod mortality between treated and control plots has not been confounded by density effects of this predator or the prey, and was true.

Power analysis

While analysing the data of a study such as this one and deciding on the correctness of our hypotheses, we can make two type of mistakes. We can decide there exists a difference between treated and control plots while in reality there is not (Type I error). The chance of making such an error is the p-value mentioned regularly above. We have considered as not statistically different all comparisons in which there was a chance of more than 5% to make such a Type I error.

Hardly ever taken into account in ecological studies is the so-called Type II error (its probability often described as ß). This is the mistake one makes in deciding there exists no difference between treatments and controls while in reality there is. Obviously, in ecotoxicological studies making such a mistake underestimates the potential effect of a pesticide. The complement of ß is called the "power" of a test; the higher the power of a test the lower the chance of not being able to recognize a real difference between treatments (Fairweather, 1991).

As a rule, increasing the number of replicates in the study increases the power of the statistical test. An often used procedure in power analysis is to determine the minimum number of samples needed to be able to distinguish a preset difference between treatments. Such an analysis is carried out below for the sampled populations of *Pimelia senegalensis* adults. The study did not find an effect of fenitrothion treatments on these organisms. We need to determine if the study had enough replicates to be able to discern a biologically significant reduction in *Pimelia* adults. The power analysis follows Steel and Torrie's (1980) procedure for paired comparisons and is based on the following parameters: Probability of a Type I error (p) is 0.10; probability of a type II error (ß) is 0.20 (thus Power is 0.80). The minimum detectable effect size we have put at 50% of the mean *Pimelia* density in the control plots; In other words, we consider it ecologically important to find a change (both reduction or increase) in *Pimelia* adults density of 50% or more, compared to the control.

The appropriate equation is:

$$n \geq (S_D / \delta)^2 \, (t_{\alpha(df)} + t_{2\beta(df)})^2$$

with: α (is p, probability type I error) = 0.10
ß (probability type II error) = 0.20
δ (minimum detectible effect size) = 8 (is 50% of mean control density)

S_D (estimate of standard deviation of differences between paired control and treatment densities; calculated from Annex I) = 9.8
t (critical value of Student-t distribution for df degrees of freedom)0
df (degrees of freedom) = 8

The test is two-tailed since we found that fenitrothion applications do not necessarily cause a decrease in densities. The minimum number of replicates thus calculated is fourteen. This means that, for this specific study situation, our conclusion that fenitrothion treatments did not have an effect on *Pimelia* adults is invalid because the number of replicates used is lower than needed to draw such a conclusion.

A similar analysis can be carried out for the difference in Pimelia larvae between treated and control (with $\delta = 15$ since mean larval density in the controls is 31, and $S_D = 13.6$). Using the above equation, the minimum number of replicates needed to show a 50% change in larval density is 6. Therefore we consider the conclusion that fenitrothion did not cause a significant change in *Pimelia* larvae density valid.

DISCUSSION AND CONCLUSIONS

The results of this study suggest (but do not prove) that larvae of *Pimelia senegalensis* were the most important predators of grasshopper eggpods in central Senegal in 1991. The variation in eggpod mortality could be explained largely by the regression against the density of this predator. The fact that *P. senegalensis* larvae were found regularly inside eggpods strengthens the above correlation from a causal point of view. Larvae of Bombyliidae did not seem to be key predators of grasshopper eggpods under these conditions. It should be noted that no detailed assessment was made of densities of all Bombyliidae larvae, a potentially important group of eggpod predators, although these were definitely not present in large numbers.

Contrary to expectation, mortality caused by natural enemies increased statistically significantly, in the treated blocks. This increase could not be explained from differences in eggpod density. A possible explanation may be that the treatments have had more impact on natural enemies of eggpod predators than on the eggpod predators themselves. Rees and Onsager (1985) reported that removal of 3 species of robber flies (Asilidae), predators of dipterous parasitoids of grasshoppers, increased the parasitation rate among rangeland grasshoppers. In our study mean density of *P. senegalensis* was slightly higher in the treated blocks, though not significantly so. However, increased eggpod mortality has been observed before in Senegal in 1990 from the blocks which were treated at locust control dose rates by the LOCUSTOX pilot study. (Niassy and Diallo, 1990) This possible effect needs further study.

The treatments did not have a negative impact on *Pimelia* larvae, as was expected since they live in the soil and are thus not likely to be exposed. Adults densities in treated plots were not significantly lower than in control plots, but it was shown that the number of replicates in this study was insufficient to draw such a conclusion from a statistical point of view. Van der Valk (1990) did not observe a reduction in pitfall catches of adults of this species after an application of 485 g ai/ha of fenitrothion. However, when sprayed at 825 g ai/ha *Pimelia senegalensis* catches were reduced 75% for at least 4 weeks. In this study no reduction in adult densities were observed 2 months after treatment, not even in the plots treated at doses higher than 800 g ai/ha. This suggests that either the insecticide did not have a large initial effect or the populations were able to recover in the period between treatment and assessment (6-8 weeks).

We make the following suggestions with respect to further research in this field:

- A similar study should be repeated in Senegal to confirm that locust control applications with fenitrothion do not have a negative impact on eggpod mortality. In such a study, the presence of Bombyliid larvae and other fly parasitoids should be assessed as well to assure a more complete picture of the natural enemy fauna. The number of plots to be studied should preferably be increased.

- This study should be repeated outside Senegal, e.g. in Niger or Mali, to investigate if similar results are found. It should be noted that the natural enemy fauna of grasshopper eggpods in Niger seems to be completely different from the one in Senegal. Popov et al. (1990) report on a study in which eggpod mortality in Niger was caused principally (75-80%) by Bombyliid larvae, while Meloid larvae contributed to most of the rest. Results of this study in Senegal can therefore not necessarily be extrapolated directly to other Sahelian countries.

- It would be useful if more information is gathered on the natural enemies of grasshopper predators and parasitoids which may help in the interpretation of the results of this and similar studies.

- Further field observations on the life history of *P. senegalensis* are needed since no published data were found after carrying out a computerized literature search on this species using several major entomological databases.

REFERENCES

Begon M., J.L. Harper and C.R. Townsend (1990) Ecology. Individuals, Populations and Communites. 2n edition. Blackwell Scientific Publications. Boston etc. pp.945

Beye A. and C.T. Ndiaye (1992) Rapport de mission de prospection d'oothèques de sautériaux du 09/02/92 au 23/02/92. Direction de la Protection de Végétaux. Sénégal.

Cheke R.A. (1990) A migrant pest in the Sahel: the senegalese grasshopper Oedaleus senegalensis. Phil. Trans. R. Soc. Lond. B (328):539-553

Fairweather P.G. (1991) Statistical power and design requirements for environmental monitoring. Aust. J. Mar. Freshwater Res. 42:555-567

Greathead D.J. (1963) A review of the insect enemies of Acridoidea (Orthoptera) Trans. R. Ent. Soc. Lond. 114(14):437-517

Niassy A. and A.O. Diallo (1990). Etude de l'impact des traitements antiacridiens au fénitrothion, chlorpyriphos et diflubenzuron sur la prédation et le parasitisme des oothèques des populations acridiennes residuelles. Cas de Cataloipus cymbiferus (Krauss 1877) (Acrididae, Eyprepocnemidinae). Direction de la protection des végétaux. Senegal.

Popov G.B. (1980) Studies on oviposition, egg development and mortality in Oedaleus senegalensis (Krauss) (Orthoptera, Acridoidea) in the Sahel. Centre for Overseas Pest Research. London. pp.48

Popov G.B., M.H. Launois-Luong and J.J. van der Weel (1990) Les oothèques des criquets du Sahel. Collection Acridologie Opérationelle no.7. Département de Formation en Protection des Végétaux. Niamey. pp.153

Rees N.E. and J.A. Onsager (1985) Parasitism and survival among rangeland grasshoppers in response to suppression of robber fly (Diptera: Asilidae) predators. Environmental Entomology 14(1):20-23

SNPV (1990) Rapport de prospection des oothèques de sautériaux. Service National de la Protection des Végétaux. Bamako.

Sokal R.R. and F.J. Rohlf (1981) Biometry. The principles and practice of statistics in biological research. 2nd edition. Freeman and Co. New York. pp.859

Steel R.G.D. and J.H. Torrie (1980) Principles and procedures of statistics. A biometrical approach. Second edition. McGraw-Hill. New York etc. pp.633

van der Valk H.C.H.G. (1990) Beneficial insects. in: Environmental effects of chemical locust and grasshopper control. A pilot Study. J.W. Everts (ed). FAO. Rome. pp.171-224

ANNEX 4.1: RAW DATA OF THE STUDY

A DECEMBER 1991

CONTROL		No. OF EGGPODS / 22.5 M2									%
		INTACT				DESTROYED				TOTAL	MORTAL
BLOCK	biotope	A	B	C	total	A	B	C	total		
I		27	31	29	87	9	5	6	20	107	18.7
II		12	14	26	52	5	16	11	32	84	38.1
III		31	9	15	55	9	5	5	19	74	25.7
IV		21	10	34	65	6	16	3	25	90	27.8
V		54	13	121	188	10	3	19	32	220	14.5
VI		108	4	290	402	15	4	50	69	471	14.6
VII		169	8	154	331	15	0	14	29	360	8.1
VIII		38	11	129	178	8	6	13	27	205	13.2
IX		36	0	94	130	5	4	10	19	149	12.8
total		496	100	892	1488	82	59	131	272	1760	

TREATED		No. OF EGGPODS / 22.5 M2									%
		INTACT				DESTROYED				TOTAL	MORTAL
BLOCK	biotope	A	B	C	total	A	B	C	total		
I		27	6	31	64	5	11	14	30	94	31.9
II		20	7	26	53	6	24	9	39	92	42.4
III		11	20	21	52	1	17	36	54	106	50.9
IV		34	6	20	60	1	19	5	25	85	29.4
V		43	3	137	183	33	5	3	41	224	18.3
VI		58	6	63	127	14	6	23	43	170	25.3
VII		110	0	205	315	15	1	30	46	361	12.7
VIII		14	7	157	178	7	0	54	61	239	25.5
IX		45	1	80	126	10	0	8	18	144	12.5
total		362	56	740	1158	92	83	182	357	1515	

ANNEX 4.1: RAW DATA OF THE STUDY

A.DECEMBER 1991

CONTROL	No. PIMELIA / 22.5 M2									No. BOMBYLIIDAE / 22.5 M2			
	LARVAE				ADULTS				TOTA	LARVAE			
BLOCK	A	B	C	total	A	B	C	total		A	B	C	total
I	27	8	26	61	19	1	13	33	94	0	2	5	7
II	13	10	27	50	13	0	8	21	71	0	0	3	3
III	20	0	11	31	21	0	15	36	67	0	0	0	0
IV	38	14	22	74	8	0	7	15	89	4	0	4	8
V	7	2	2	11	2	0	4	6	17	1	1	4	6
VI	8	5	3	16	1	0	0	1	17	1	8	19	28
VII	7	6	8	21	2	0	0	2	23	0	4	1	5
VIII	2	2	6	10	10	0	16	26	36	0	1	0	1
IX	0	0	3	3	1	0	0	1	4	6	0	4	10
total	122	47	108	277	77	1	63	141	418	12	16	40	68

TREATED	No. PIMELIA									No. BOMBYLIIDAE / 22.5 M2			
	LARVAE				ADULTS				TOTA	LARVAE			
BLOCK	A	B	C	total	A	B	C	total		A	B	C	total
I	42	9	12	63	42	0	10	52	115	3	0	6	9
II	29	14	25	68	16	0	4	20	88	4	1	10	15
III	32	12	27	71	20	0	24	44	115	3	2	10	15
IV	29	8	38	75	9	0	23	32	107	4	3	1	8
V	8	3	3	14	5	0	0	5	19	0	2	4	6
VI	4	6	9	19	1	0	2	3	22	1	12	8	21
VII	5	1	9	15	4	0	4	8	23	0	4	3	7
VIII	8	5	6	19	7	0	1	8	27	2	2	3	7
IX	5	0	1	6	7	0	1	8	14	1	0	2	3
total	162	58	130	350	111	0	69	180	530	18	26	47	91

ANNEX 4.1 : RAW DATA OF THE STUDY

B:JUIN 1992

TEMOINS		No. OOTHEQUES / 10 M2							%	No. PIMELIA							MELOIDAE		
		INTACTES			DETRUITES			TOTA	MORTALITE	LARVES			ADULTES			TOTA	LARVES		
BLOC	habitat	A	C	total	A	C	total			A	C	total	A	C	total		A	C	total
I		0	2	2	0	2	2	4	50.0	0	3	3	17	37	54	57	0	0	0
III		3	6	9	0	0	0	9	0.0	1	2	3	4	3	7	10	0	0	0
VI		70	167	237	31	49	80	317	25.2	0	0	0	7	2	9	9	0	0	0
total		73	175	248	31	51	82	330		1	5	6	28	42	70	76	0	0	0

TRAITES		No. OOTHEQUES / 10 M2							%	No. PIMELIA							MELOIDAE		
		INTACTES			DETRUITES			TOTA	MORTALITE	LARVES			ADULTES			TOTA	LARVES		
BLOC	habitat	A	C	total	A	C	total			A	C	total	A	C	total		A	C	total
I		15	10	25	3	6	9	34	26.5	0	0	0	13	6	19	19	3	0	3
III		9	1	10	4	1	5	15	33.3	1	0	1	32	2	34	35	1	0	1
VI		20	16	36	52	5	57	93	61.8	0	4	4	20	3	23	27	2	0	2
total		44	27	71	59	12	71	142		1	4	5	65	11	76	81	6	0	6

CHAPTER 5 :

A laboratory toxicity test with *Bracon hebetor* (Say) (Hymenoptera, Braconidae) -first evaluation of Rearing and testing methods.

SUMMARY

Evaluation of the ecotoxicological effects of insecticides is partly based on laboratory toxicity tests for primary screening. This allows a selection to be made of insecticides to be further tested in field studies, which are generally much more expensive and time-consuming. Many of these laboratory screening tests have been developed for temperate European and American agro-ecosystems. However, very few, if any, exist for beneficial arthropods which are important in arid and semi-arid agro-ecosystems in Africa. The study described here was carried out within the framework of the assessment of the environmental side-effects of locust and grasshopper control in such semi-arid ecosystems.

In this report, rearing methods are described for the parasitoid *Bracon hebetor* Say (Hymenoptera, Braconidae), which is a natural enemy of the important millet pest *Heliocheilus albipunctella* De Joannis (Lepidoptera, Noctuidae), among others. The parasitoid is reared in the laboratory using *Ephestia kueniella* (Zeller) (Lepidoptera, Pyralidae) as a host.

Both the rearing of *B. hebetor* and *E. kuehniella* is done in the laboratory under ambient Sahelian conditions and doe's not require any specalized or expensive equipment. Results of more than one year of rearing suggest that the colonies of both host and parasitoid provide insects of continuous quality.

A method is decribed to rapidly screen the acute toxicity of insecticides on adults of *Bracon hebetor*. Simple glass test tubes are coated on the inside with the insecticide dissolved in acetone. Insects are subsequently exposed for 24 hours to different doses, and LC_{50}'s expressed in ng insecticide / cm^2 tube are calculated. Results of tests with fenitrothion showed a high similarity between tests when executed by different persons, using different populations of wasps, in varying environmental conditions.

Sublethal effects of the insecticide on reproduction parameters such as the number of viable offspring and longevity of surviving females were evaluated as well. Results of these observations were too variable, and a number of suggestions are made to improve this part of the toxicity test.

The 24-hour LC_{50} of fenitrothion ranged from 4 - 19 ng / cm^2 test tube. This is 15-130 times lower than initial residues measured in the field after desert locust control with fenitrothion at the recommended rate of 450 g a.i./ha. Consequently, there may be a high risk of killing a large fraction of a population of this parasitoid if present in millet during such treatments. Field data suggest, however, that fenitrothion degrades rapidly on millet and re-colonisation of sprayed areas may start within a few days after the treatment.

INTRODUCTION

Desert locust control at present only uses synthetic insecticides. Since most of these insecticides have broad spectrum activity, their potential environmental side-effects are recently receiving more attention (e.g Everts 1990). The knowledge about the risks of pesticide use in the arid and semi-arid regions in which locust control is carried out, however, is still very limited (van der Valk 1990, Matteson 1992). The FAO "LOCUSTOX Project" in Senegal attempts to fill in some of the gaps which exist in this knowledge. It does so by studying the ecotoxicological effects of insecticides on a limited number of non-target organisms. Furthermore, laboratory toxicity tests and field bio-assays are being developed to assist in the rapid evaluation of the risk of these insecticides on locally relevant organisms.

A large fraction of locust and grasshopper control in the African Sahel is carried out in pearl millet (*Pennisetum typhoides* (Burm.) Stapf and Hubt.). Recent studies have therefore particularly focussed on the impact of insecticides on beneficial insects in millet. A concern is that chemical locust control may kill natural enemies and so cause the development of secondary pests in millet. Van der Valk and Kamara (1993) and Kamara and van der Valk (in prep.) showed in a number of field studies that parasitic and predatory Hymenoptera were affected by late season locust control with fenitrothion in millet, and that this may have been the reason of an observed increase in the incidence of the millet headminer, *Heliocheilus albipunctella* De Joannis (Lepidoptera, Noctuidae).

However, field studies are expensive and because of the high variability encountered, need often to be repeated for several years before consistent results are obtained. Therefore, in pesticide registration schemes in much of Europe initial laboratory screening tests are carried out to limit the number of compounds for which field studies are deemed necessary (EPPO 1992). A number of standard screening tests for beneficial arthropods have been developed in this respect, e.g. by the International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC) (Hassan *et al.* 1985, IOBC 1988, Hassan 1992)

This report describes a number of experiments and observations carried out for the development of a standard toxicity test with the parasitoid *Bracon hebetor* (Say) (Hymenoptera, Braconidae). This braconid wasp is considered an important parasitoid of *Heliocheilus albipunctella*, the millet head miner or millet spike worm (Bhatnagar 1987), which is the most damaging millet pest in the western Sahel (Gahukar *et al.* 1986, Geddes 1990).

Braconid parasitoids are one of the major groups of natural enemies of Sahelian cereal pests (Bhatnagar 1987). However, only one test protocol for Braconidae is available under the IOBC scheme, on *Opius sp.*, out of a total of 10 parasitoid tests listed in the scheme. An analysis of literature data carried out by Theiling and Croft (1988) and Croft (1990) indicates that Braconidae tend to be extremely susceptible to most insecticides. Therefore, it appears that the choice of standard test organisms for temperate agro-ecosystems may not be representative for the situation encountered in the semi-arid Sahel.

A rearing method of *Bracon hebetor* will be described using *Ephestia kuehniella* Zell. (Lepidoptera, Pyralidae) as a host. The laboratory colonies of both the parasitoid and the host do not require climatized rearing rooms but can be set up under ambient laboratory conditions in a Sahelian climate such as that of Senegal. Most material needed for the rearing is available locally. The rearing methods described below are partly based on earlier work carried out by one of us (Diémé 1986,1989).

THE REARING OF *BRACON HEBETOR* ON *EPHESTIA KUEHNIELLA*

Rearing of the host

Introduction

Ephestia (=Anagasta) kuehniella (Zeller, 1879) (Lepidoptera, Pyralidae) is a virtually cosmopolitan pest of stored products, especially cereals and nuts (Weidner and Rack 1984). Adult moths are greyish (grey/brown or grey/blue) in color and have a wing spread of 20-22 mm. Larvae are creamy colored with a brown head capsule. In Senegal, *E. kueniella* is an important pest in stored millet, especially in traditional stores where millet is kept in bulk rather than in sacks, and access for the insect is easy.

Description of the standard rearing method

The laboratory rearing population was started up by collecting larvae and adults of *Ephestia kuehniella* from infested millet stores around Nioro du Rip, in west-central Senegal.

Adult moths are held in cages made from a transparent perspex cylinder covered by a lid and closed at the bottom by a fine iron mesh (fig. 5.1). Four small glass vials are suspended in the cage and filled with cotton soaked in 10 % (w/v) sugar water as adult food. Females deposite their eggs on carton strips suspended from the lid, or on the cage wall. The eggs subsequently fall through the iron mesh on a piece of black paper, which is placed permanently under the cage.

Every working day eggs are collected from the black paper under the cage and put in firmly closed, plastic petri dishes (diameter 15 cm), filled up to 3/4 with slightly moistened millet meal and millet grains (which further will be called "hatching dishes", fig. 5.1). All millet and millet flour is heated for approximately 30 minutes at 80-100 C to kill mites which may infest the millet and were found to cause high mortality both of *Ephestia* as well as *Bracon* eggs and young larvae. About 400-600 eggs are placed in each hatching dish.

Ephestia larvae are utilized as hosts for *Bracon*, as well as to maintain the moth population itself. In the first case, larvae are kept in the hatching dishes until 5th or 6th instar and then transferred to the *Bracon* rearing colony.

When larvae are to be used for maintenance of the *Ephestia* colony the millet and larvae (mostly 4th-5th instar) in the hatching dishes are, approximately 20-25 days after incubation of the eggs, transfered to larger, clear plastic "pupation containers" (fig. 5.1). The hatching dishes are too small for adult emergence, and often yield moths with malformations of the wings. Care has to be taken to transfer the larvae as a batch and not to individually manipulate each larva with, for instance a pair of soft forceps, since such handling reduces survival significantly. Two ventilation holes in the lid are covered by a double layer of fine mosquito netting to prevent stray *Bracon* wasps from parasitizing *Ephestia* larvae which wander on the netting. Three hatching dishes (approximately 1500 eggs) are transferred to one pupation container. The millet of the hatching dishes is supplemented with millet grains until an approximate density of about 1 larvae per gram of millet is reached.

Under the conditions described here, the first adults will start emerging 26-33 days after laying. The emerging adults are caught and transferred to the adult cage.

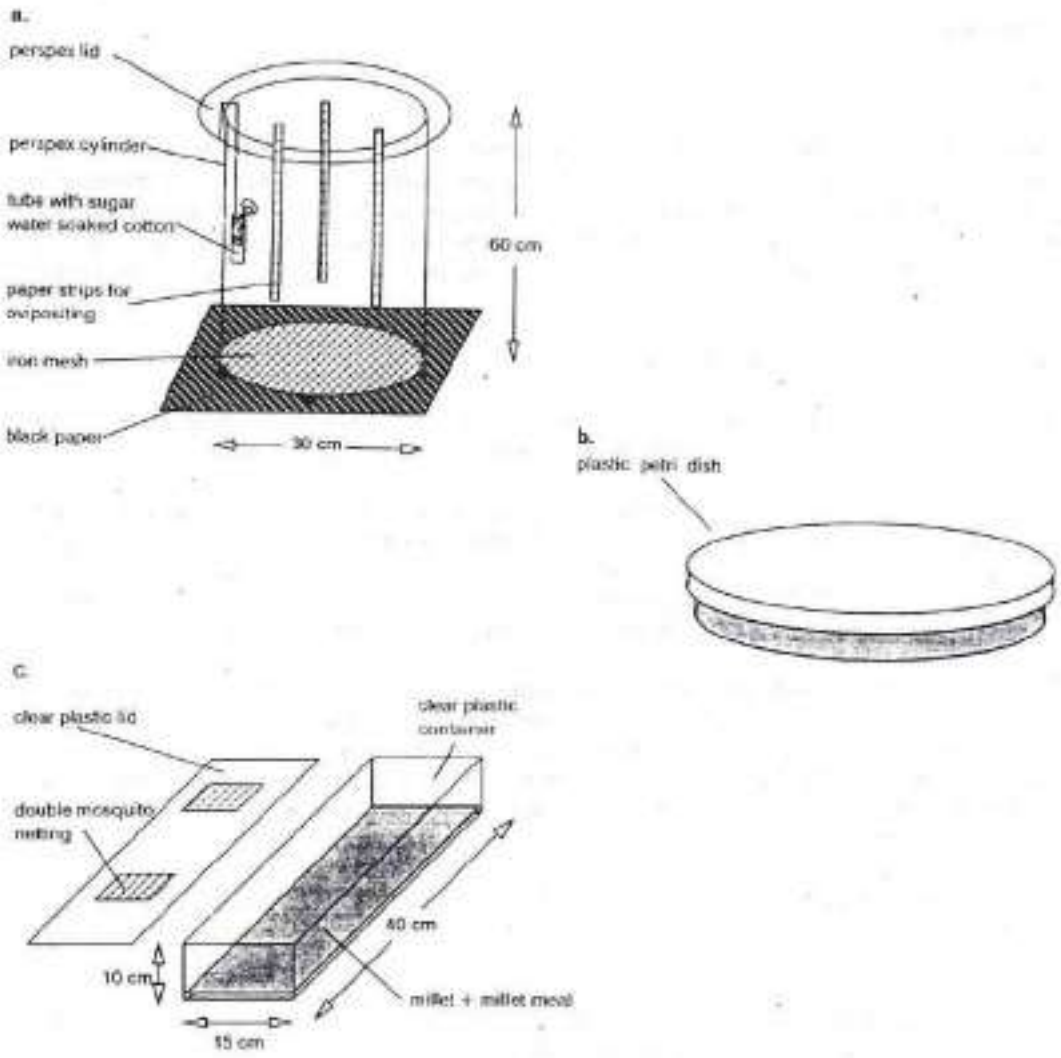


Fig. 5.1 Different types of cages used in the rearing of *Ephesta kuehniella*. a: adults moths are held in a perpex oviposition cage from under which the eggs are collected. b: eggs are subsequently transferred to hatching dishes filled with millet. c: late instar larvae are transferred to pupation containers, from which adults are collected.

Date of egg incubation and termination of each hatching dish as well as the initial egg number and final average larval stage are registered for each hatching dish. Date of incubation, code of the hatching dish(es) from which the larvae are taken as well as the daily number of emerging adults are registered for every pupation container.

The rearing is performed under ambient laboratory conditions (temp. 25-32 °C, relative humidity 30-80 %). A light regime of 8h(light) : 16h(dark) is maintained, using common neon lamps.

Incubating approximately 600 eggs daily is sufficient to harvest 250 late instar larvae every day by the time the colony is established. This is enough to keep both the host as well as the parasitoid population going. Slightly increasing the number of eggs incubated allows for a safeguard. Surplus eggs produced can then be discarded.

Factors influencing Ephestia development in the colony.

The egg stage

Eggs are white/creamy colored, but turn brown just before hatching due to the coloration of the larval head capsule. Under the conditions described above a large fraction of the eggs hatches within 24-48 hours after oviposition. No detailed studies on hatching rates were undertaken, but our observations suggest that nearly complete hatching is achieved under the described rearing conditions. This is in accordance with data from Daumal *et al.* (1975).

The larval stages

Larvae are creamy colored with a brown head capsule. They pass 5 or 6 stages before pupation. Survival from egg to last (5th and/or 6th) instar larvae in the hatching dishes averaged 39%, for egg densities ranging from 200 to 900 per petri dish (fig 5.2). No significant correlation was found between initial egg density and overall larval survival within this density range.

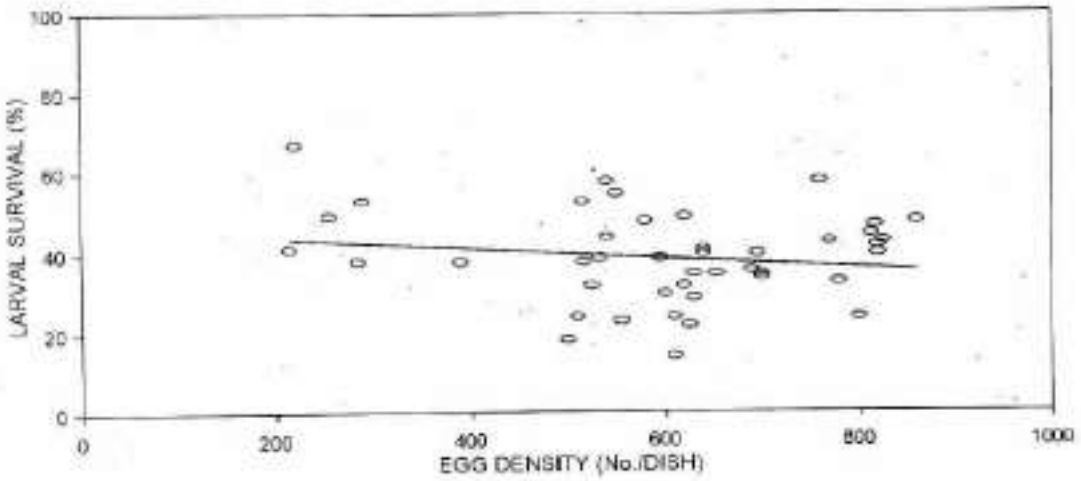


Fig. 5.2: Survival of *Ephestia kuehniella* from eggs to 5th/6th instar larvae or pupae in the hatching dishes as a function of initial egg density (n=43 cases, sept/oct 1992). Linear regression $R^2 = 0.02$ (not significant).

We also studied the influence of larval density on survival in the pupation containers. Densities of 3rd/4th instar larvae at the start of the experiment varied from 0.75 to 2.5 per gram of millet. Survival from 3rd/4th instar until last instar ranged from 66 to 77%, but there was no effect of density.

Combining the two studies mentioned above suggests that survival from the egg stage to 3rd/4th instar larvae is about 55%. We assume that mortality of early instar larvae causes this fairly low survival rate. Cannibalism may play a role as well.

In an earlier study (Diémé 1989) it was found that total larval development ranged from 15 to 25 days. In preliminary observations carried out while setting up the rearing methods, we found that total larval development time averaged 29 days, under ambient conditions (August-October).

Extreme care should be taken to avoid the *Ephestia* colony to become infected with *Bracon hebetor*. Any pupation container infected with *Bracon* may and often will be wiped out completely. The use of double mosquito netting on the aeration holes of the pupation containers, and the removal of aeration ridges in the lids of the plastic petri dishes used for hatching, reduces the risk of such attacks greatly. Under no circumstances should *Ephestia* and *Bracon* colonies be maintained in the same room. Similarly, infection with mites (not identified) occurred in the *Ephestia* colonies. This greatly reduced larval survival. Also, the mites are easily being transferred to the *Bracon* colony, where they both compete with and kill *Bracon* larvae. They were introduced with the millet, and therefore heating all millet to be used for the rearing at 80-100 °C for about 30 minutes is recommended. After this

procedure was introduced, no problems with mites were encountered anymore.

Pupae

The length of the pupal stage was found to be about 4 days. Pupal survival was estimated for 7 pupation containers (a total of 1275 pupae) during September 1992. Average survival to adult stage was 31% (ranging from 17 to 55%). Handling (counting and re-incubation) of the pupae may have reduced survival, however.

Adults

Average longevity of the adults was approximately 4 days for males and 6 days for females. This corresponds with earlier observations by one of us (Diémé 1989), when adult longevity ranged between 3 and 8 days. Average lifetime oviposition by individual females ranged from 160-240 eggs.

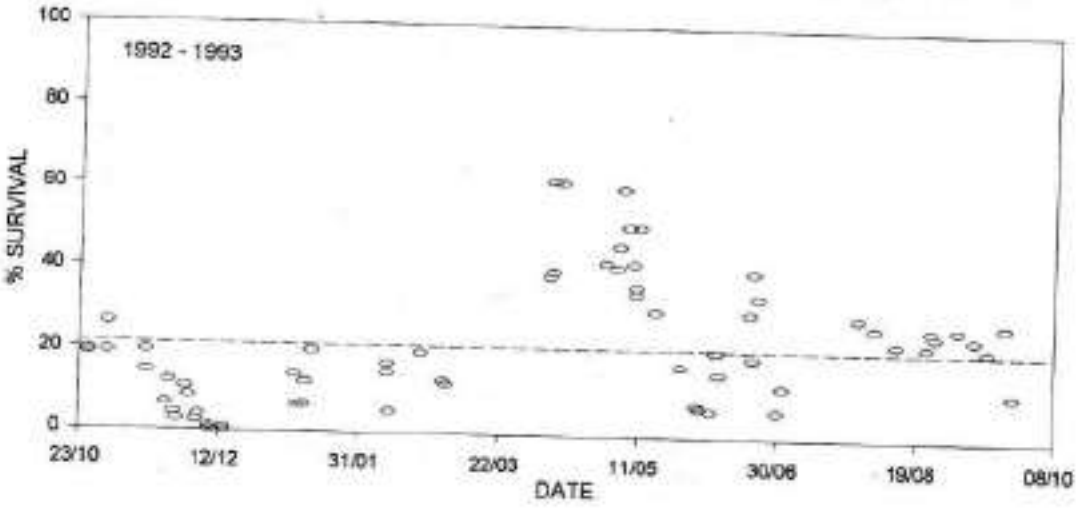


Fig. 5.3 Survival of *Ephestia kuehniella* from the egg stage until the adult stage over a one year period in the rearing colony at Nioro du Rip, Senegal. Dotted horizontal line is average.

Overall colony performance

Based on the initial rearing studies, we estimated that the development time from egg to egg for *Ephestia kuehniella*, under the above mentioned rearing conditions, would be approximately 35 days. Overall survival, from egg to adult, was estimated at about 16%. Highest mortality was expected to occur at early larval stages and during the pupal stage.

The rearing colony was subsequently maintained for a year, using the method described above. Survival from egg to adult is depicted in figure 5.3 for those populations which were transferred to pupation containers (i.e. the part of the *Ephestia* colony used to maintain itself). Those populations which remain in the, much smaller, hatching dishes until being used as host for *Bracon* are subjected to greater mortality and not representative of colony performance.

The colony all but crashed in early 1993 because of a mite infection, which explains the low survival of the *Ephestia* populations started up in the first half of December. The colony was cleaned up, but overall survival averaged only 10% until early March, when it was found that mites were still present at low densities. The laboratory colony was destroyed and a large number of adults and late instar larvae was brought in from millet stores to replenish the population in the first two weeks from March. Viewing figure 5.3 it seems that wild populations are more fit than several generations old laboratory reared ones. The high survival in April may be explained by such increased vigour. Average survival from egg to adult stage, including those periods in which mites were present was 22%, or 28% if we exclude this period. This is considerably better than our prediction based on the initial rearing studies

(see above). We contribute the increased survival in the colony to a strictly followed minimum of handling of the larvae during the rearing. Furthermore, pupal survival is probably higher than we estimated, as discussed above.

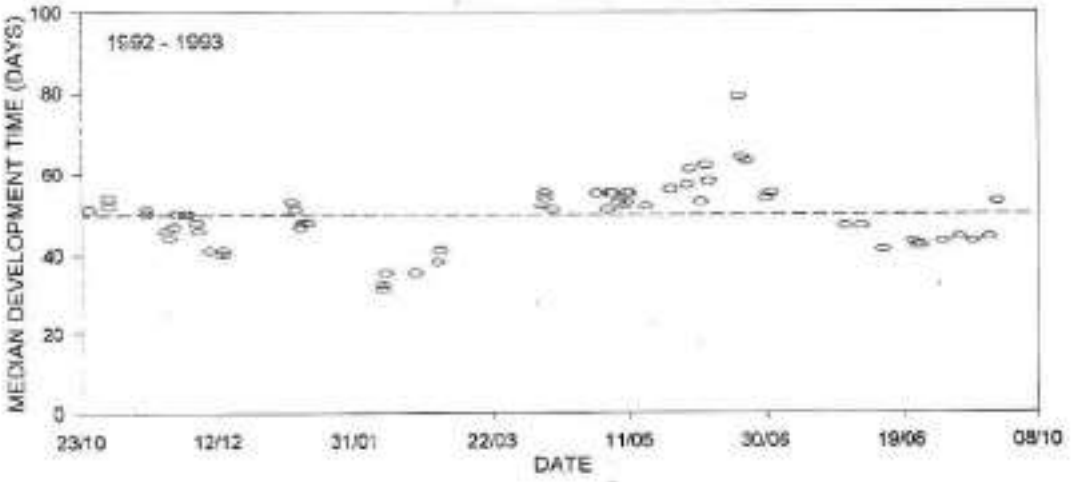


Fig.5.4 Median development time of *Ephestia kuehniella* in the rearing colony at Nioro du Rip, Senegal. Median development time is defined as the number of days between egg incubation (< 1 day after oviposition) and 50% adult emergence. Dotted horizontal line is average.

Median development time from egg to adult is shown in fig 5.4. We define the median development time as the period between egg incubation (less than 1 day after oviposition) and the date at which 50% of the adults have emerged. It averaged 50 days. This is considerably longer than predicted from controlled experiments mentioned above, and a possible explanation is given below.

A highly significant, positive correlation was found between median development time and transfer time (fig 5.5). The latter is the period between egg incubation and transfer of larvae from the incubation dishes to the pupation containers. The longer one waits with transfering the larvae, the longer the total development time becomes. However, as can be seen in fig 5.6, an increase in development time was not negatively correlated with survival. The increased development time caused by postponed larval transfer apparently
is a real reduction in development speed of the larvae and/or the pupae. Furthermore, we found that this is independent of initial density in the hatching dishes (fig 5.7). The cause for this apparent slow-down of development is unknown to us. It may be that some chemical factor, possibly a growth hormone or other semiochemical, is produced by the larvae. Such a chemical would build up in the hatching dishes since they are almost airtight. The moment that the larvae and pupae are transferred to the pupation containers, which are much bigger and better ventilated, its effect would diminish and development would speed up again.

Clearly, this observation needs further clarification, especially since the suggested process may have considerable impact on the turnover rate of the *Ephestia* colony.

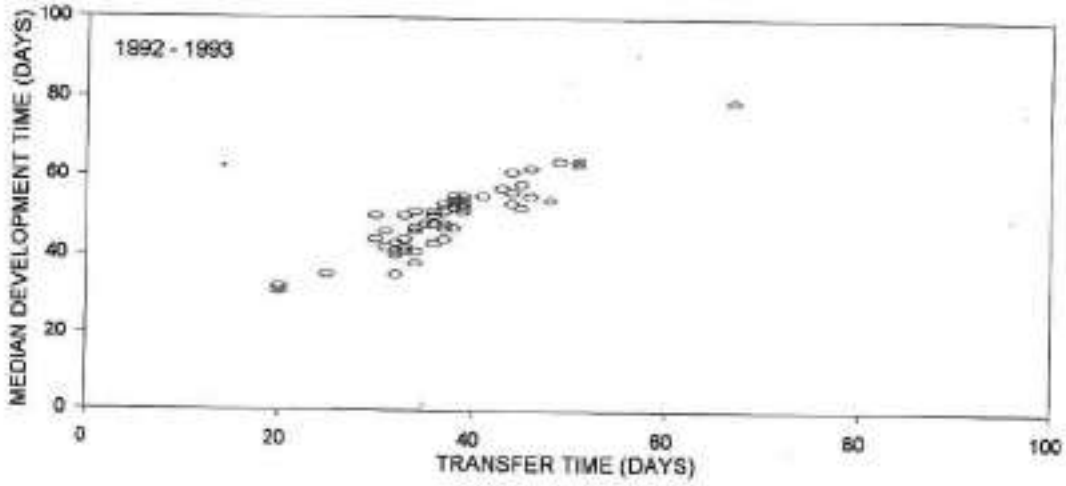


Fig.5.5 Relationship between transfer time of *Ephestia kuehniella* in a laboratory colony in Nioro du Rip, Senegal, and median development time. Transfer time is defined as the period between egg incubation and the transfer from the hatching dishes to the pupation containers.

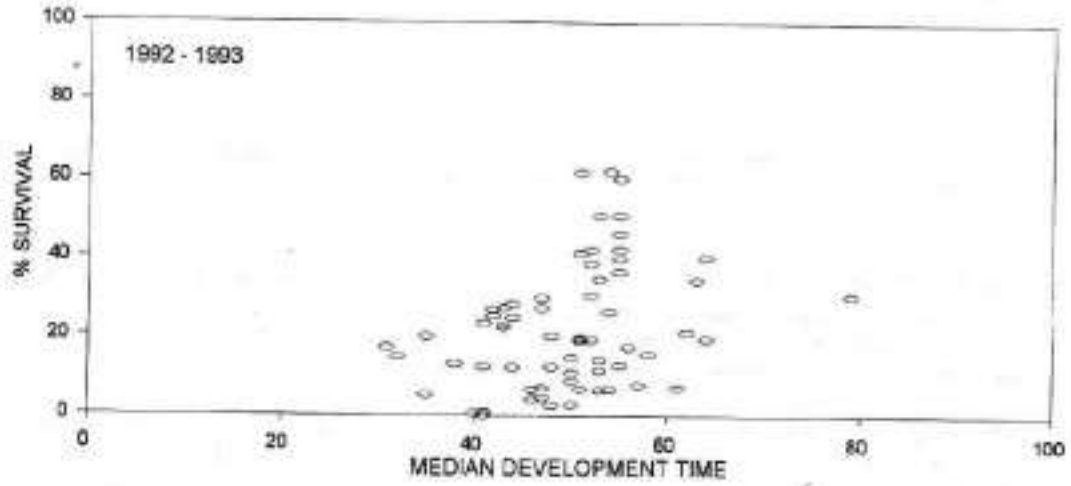


Fig.5.6 Relationship between development time of *Ephestia kuehniella* and survival from the egg stage to the adult stage, in a laboratory colony in Nioro du Rip, Senegal.

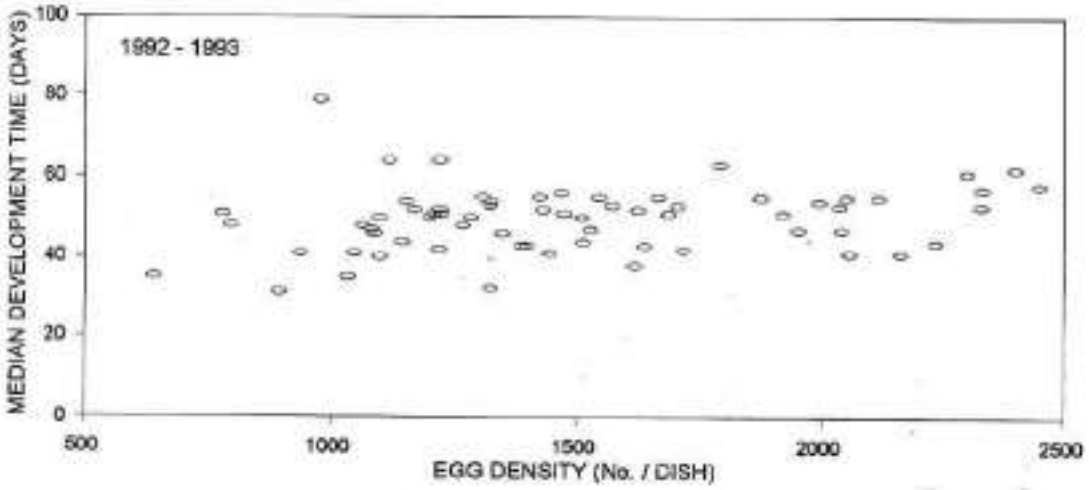


Fig.5.7 Relation between initial egg density in hatching dishes of *Ephestia kuehniella* and median development time, in a laboratory colony in Nioro du Rip, Senegal.

Rearing conditions

Average weekly temperature and relative humidity in the rearing room are given in Annex I. Average temperature remained fairly stable during most of the year and ranges from 26 to 32 °C. Average relative humidity dropped during the dry season to a minimum of 25%; during the rainy season it reached 75%. During the second half of the dry season the relative humidity was increased artificially to approximately 50%, to reduce dessication of the millet.

Since the low humidity period coincided largely with low colony survival due to predation by mites, we have refrained from trying to relate colony performance to humidity. Similarly, the low variability in temperature was not expected to greatly influence colony parameters such as fecundity and development time. Such relationships are better studied under more controlled circumstances.

Rearing of the parasitoid

Introduction

Bracon hebetor Say (Hymenoptera, Braconidae) (=*Habrobracon hebetor* Say, =*Habrobracon juglandis* (Ashm.)) is a cosmopolitan ectoparasitoid of a large number of lepidopteran species, as well as some Coleoptera. Risbec (1950) describes the species in his study of the entomofauna of Senegal and Mali as a parasitoid of the stored product pests *Corcyra sp.* and *Ephestia sp.*. Now we know that in this region *B. hebetor* also parasitizes *Heliocheilus albipunctella* and *Helicoverpa armigera* (Bhatnagar, 1987). From other regions in the world the species is known to attack such important crop and storage pests as *Spodoptera littoralis*, *Heliothis virescens* and *Ephestia cautella*, among many others (Fry 1989).

Adult wasps are up to 4 mm long and have a red or creamy coloration. Females are easily recognised by their ovipositor and their slightly bigger abdomen; males also have clearly longer antennas than females. Before ovipositing, *B. hebetor* paralyses its host. The elongated eggs (0.5 mm) are laid on late instar caterpillars. The young larvae attach themselves to the outside of the caterpillar, feeding on the haemolymphe of the host. The white coloured larvae develop fast, forming their cocoons after a few days. *Bracon hebetor* is a haploid species: Female offspring only results from fertilized eggs, while unfertilized ones produce male progeny.

The wasp has a short life cycle without diapause. Therefore its hosts must be available during the whole year. This situation is provided in the western Sahel by 2 hosts *Ephestia kuehniella* and *Heliocheilus albipunctella*, both related to the same crop (millet). *Heliocheilus* is important during the

time of the year when the millet is on the field. *Ephestia* in millet stores functions as an alternate host during the other part of the year (Bhatnagar, 1987). Potential other alternate hosts during the dry season are *H. armigera* on tomatoes, other vegetables and cotton, and *Phtorimaea operculella* (potato tuber moth) on potatoes. Their importance as hosts in Senegal is not known, however.

Description of the rearing method

The laboratory rearing culture can be started up by inducing parasitism of *Ephestia* larvae by wild *Bracon* in the dry season, or by collecting cocoons of the wasp when millet is maturing at the end of the rainy season.

During the dry season and the beginning of the rainy period controlled parasitism is stimulated around traditional millet stores. Open plastic containers filled with a layer of millet and corrugated cardboard, and containing up to 200-300 late instar *Ephestia* larvae (fig. 5.8), are suspended below the thatch of a traditional millet store. The container is covered by mosquito netting with a mesh size of approximately 1 mm such as to avoid *Ephestia* to escape but allow *Bracon* to enter the containers. The *Ephestia* larvae tend to hide in the corrugated cardboard, making them an easy target for *Bracon* to paralize and oviposite on. The container is suspended from the store thatch for 2 to 4 days, closed off, and subsequently transferred to the laboratory. If *Ephestia* is parasitized, adult wasps will emerge after approximately 6-10 days.

When millet heads are maturing in the field, *Heliocheilus* larvae parasitized by *Bracon* as well as *Bracon* cocoons can be collected by inspecting millet heads showing attacks of *Heliocheilus*. The heads are then transferred to the laboratory and kept in a container with humid soil or a thick layer of humid filter paper placed on the bottom to keep the head fresh a long as possible. The container is than covered with fine-mesh mosquito netting and adult wasps may be found up to 10-14 days after incubation. This method is much more labour intensive than the first one described, however.

Adult wasps are subsequently held in plastic cages (fig. 5.8), which are made from a 20 litre bucket with a tight fitting lid. A large hole is cut in the lid and covered by a piece of glass to facilitate manipulation of the insects in the cage. Part of the bucket wall is replaced by a nylon mesh tube for access to the cage. Another part of the wall is replaced by nylon netting to increase aeration. Four small glass vials are suspended in the cage, filled with cotton soaked in 10 % sugar water as adult food.

Several hundreds of wasps can be held in such a cage at a time. Females are caught individually in small glass tubes for transfer to parasitization dishes (fig. 5.8). These are made of plastic petri dishes with close fitting lids to avoid *Bracon* from escaping. Every dish contains 100 large *Ephestia* larvae (5th or 6th stage) and 10 *Bracon* females which have spent 1 day in the mating cages.

After about 6 days the adult wasps (parents) are released, outside the laboratory. Offspring adults start emerging about 8-10 days after incubation of the parasitization dish. Newly emerged adults are released in the adult cages every working day from different parasitization dishes, where they can further mix before females are being used to parasitize hosts for a next generation.

The date of incubation, the dates of emergence and the total number of emerging adults as well as their estimated sex ratio are registred for each parasitization dish.

The rearing is performed under ambient laboratory conditions (temperature 25-30 °C, RH 50-85 %). The colony can be kept under natural light conditions, in a room with a window.

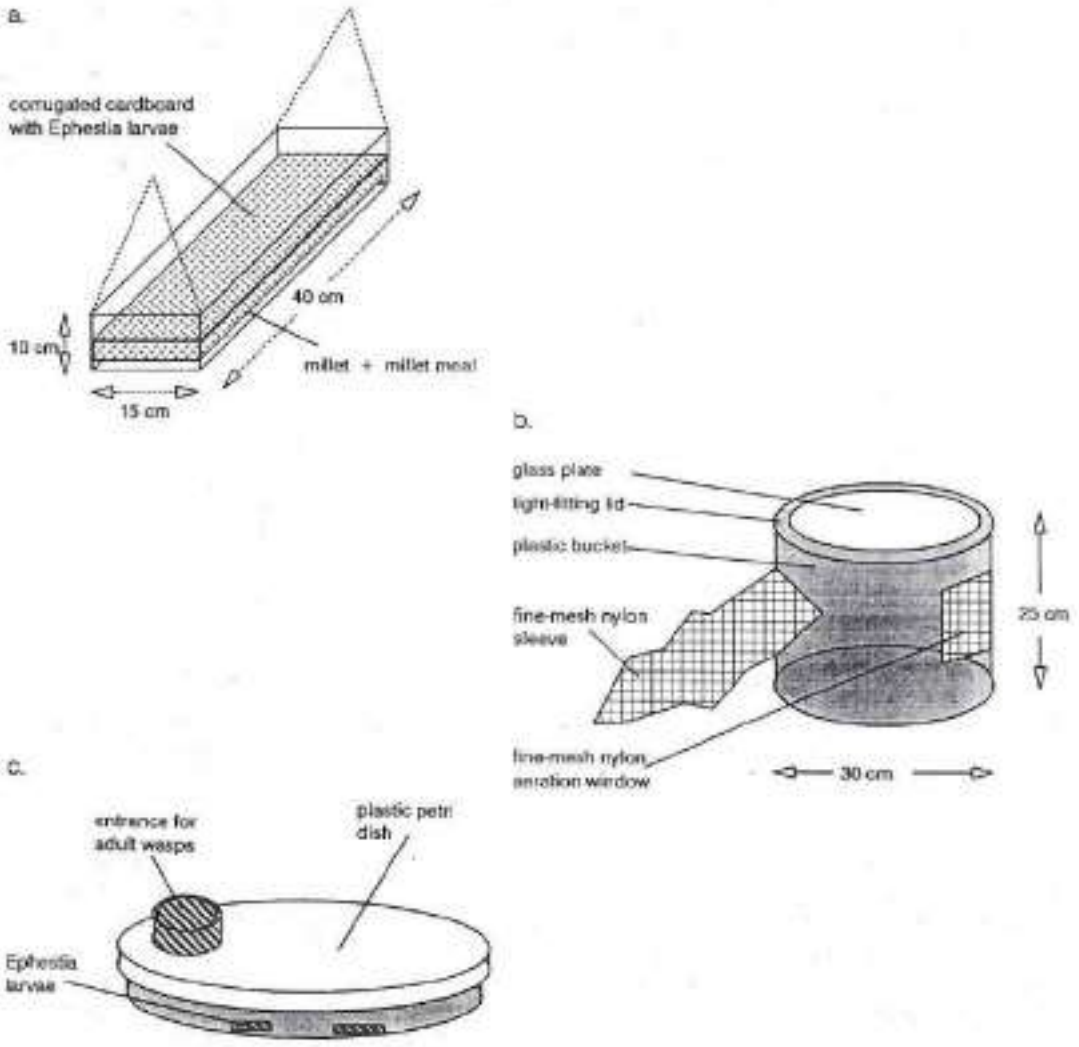


Fig.5.8 Different cages used in the laboratory rearing of *Bracon hebetor*. a: containers used to for controlled parasitization of *Ephestia kuehniella* by *B. hebetor*, to be suspended in (traditional) millet stores. b: cages for adult parasitoids. c: parasitization dishes in the laboratory.

Factors influencing Bracon development

No detailed assessment of the functional or numerical reponse of the parasitoid to host density was made. We will evaluate below the data routinely collected during colony maintenance. It should be noted that these are data pooled from the rearing colony logbook over a period of one year, under various environmental conditions. Conclusions drawn from these data can therefore not be considered experimental proof.

Adult fecundity

In the rearing colony as a rule 10 females of *Bracon* are presented with 100 larvae of *Ephestia* in each parasitization dish (the "standard" host-parasitoid ratio), but in a few cases other ratio's were used. Figure 2.9 shows the relationship between host/parasitoid ratio and number of offspring surviving until the adult stage. No significant correlation between host/parasitoid ratio and offspring was found for the 1992 series. For the 1993 series offspring per female increased significantly with the number of hosts available. However, it is clear from both graphs that the variability at the standard host-parasitoid ratio is large, and the number of non-standard ratios was relatively small. Even though these results may suggest that the standard ratio may not be optimal use of *Bracon* females, we do not recommend increasing the number of hosts per female unless absolutely necessary: A reduced number of females producing the following generation of the colony would only increase the speed of inbreeding.

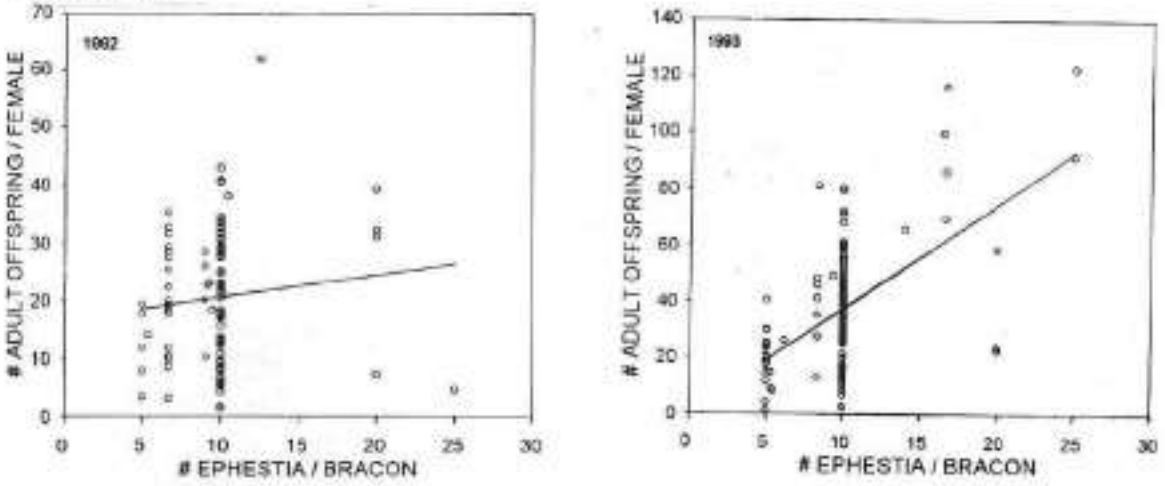


Fig.5.9 Relationship between host/parasitoid ratio (no. *Ephestia* larvae / no. *Bracon* ♀) and the number of adult offspring per *Bracon* female, for the laboratory colony in Nioro du Rip, Senegal, in 1992 and 1993. Slope of regression line not significantly different from zero for 1992; for 1993 slope significantly larger than 0 ($p<0.05$).

The average number of offspring per female which survive until the adult stage is 22 for the 1992 series and 34 for 1993 (fig 5.10). The number of offspring varies greatly from one case to another, but no pattern could be detected. There is no apparent decrease of fecundity over time for the 1992 series, which are continuous descendants of one and the same starting population. In the 1993 colony wild adults were introduced into the rearing population several times to reduce any inbreeding which might take place. It is not clear if this is the reason that fecundity is about 50% higher than in 1992. It should be noted that the observed number of offspring per female in the colony is very much lower than can be attained by *B. hebetor* during its lifetime (20-40 days for a *Bracon* female). When *Bracon* females are provided with unparasitized host every other day until death, 200 offspring per female are obtained regularly in our laboratory, with a maximum of over 400 offsping per female. Similar high life-time fecundities are given by Benson (1974), Antolin and Strand (1992) and Nikam and Pawar (1993).

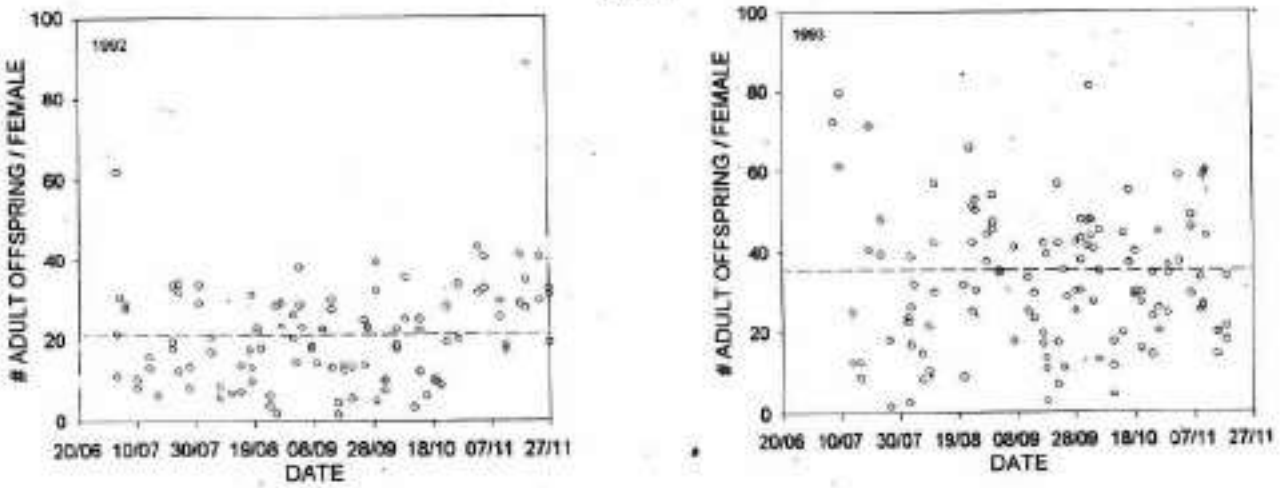


Fig.5.10 Female fecundity of *Bracon hebetor* in the laboratory colony in Nioro du Rip, Senegal, in 1992 and 1993. Fecundity is expressed as the number of adult offspring per female. Horizontal dashed line is the average fecundity over the observation period.

Egg / larval development

Median egg/larval development time of *B. hebetor* was defined as the period between incubation of the parasitization dish with wasp females (which is generally also the day of peak oviposition) and the middle of the adult emergence period. It averaged 11.5 days and varied little (fig. 5.11). Benson (1973) reports a comparable life-cycle of 12-14 days at 25 °C.

Sex ratio

On average 45% of the emerging wasps were female. This is comparable to figures found in the literature for laboratory populations (Benson 1974, Nikam and Pawar 1993, Rotary and Gerling 1973) Similar to Benson's (1974) observations, the variation in sex ratio of consecutive generations is high, ranging from as low as 3% to 80% in our case (fig.5.12). No clear pattern emerges, however, variability being equally spread during the season. There is no obvious change in sex ratio over time either.

Mating

Bracon hebetor females accept a mate immediately after emergence from the cocoon, and copulate only once. In the laboratory, mean times to mating between virgin females and males were found to vary from 160 to 280 seconds. Mothers mated significantly faster with sons than with brothers or unrelated males. Copulation is brief, only lasting 5-20 seconds (Petters et al. 1985). We did not find any difference in number of offspring and sexratio of females taken from mating cages in which they had spend less than 24 hours compared to cages in which they had spend more. This confirms the detailed observations by Petters et al. (1985).

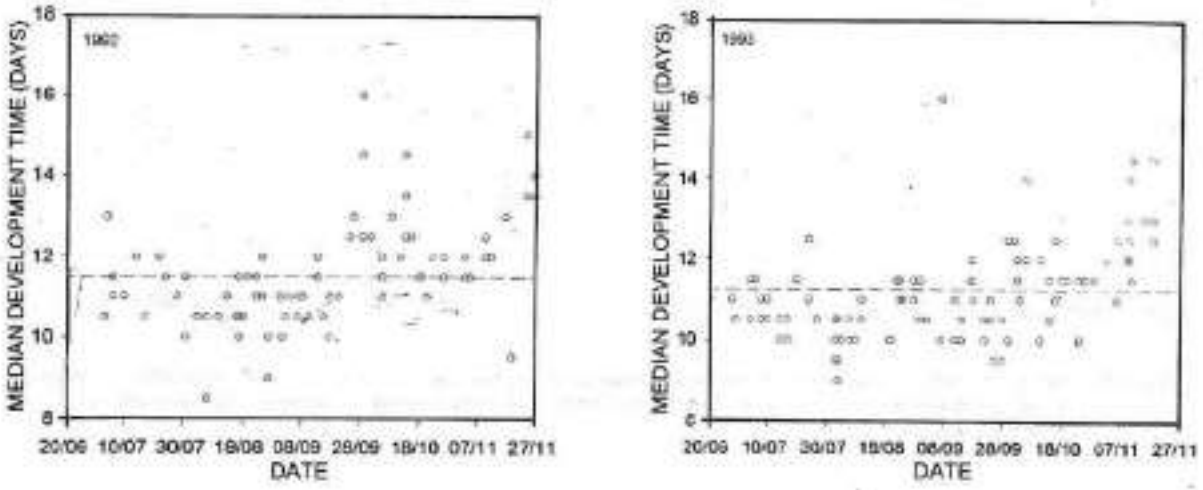


Fig. 5.11 Median egg/larval development time of *Bracon hebetor* in the laboratory colony in Nioro du Rip, Senegal, in 1992 and 1993. Median development time is defined as the time between incubation of the parasitization dishes with female wasps and the middle of the adult emergence period.

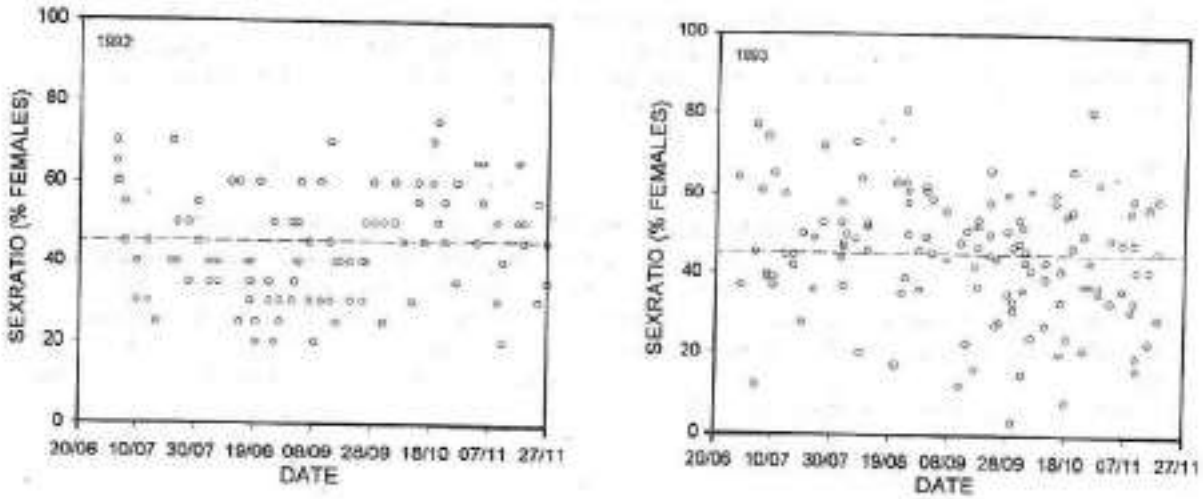


Fig.5.12 Sex ratio (% females) of emerging adult *Bracon hebetor* in the laboratory colony in Niaro dur Rip, Senegal in 1992 and 1993.

Since mating takes place so soon after adult emergence, the risk of inbreeding is higher if insufficient females are allowed to parasitize hosts in the same parasitization dish. Inbreeding may result in reduced performance of the colony (Antolin and Strand 1992), and thus in unintentional changes in susceptibility to insecticides between laboratory and field populations. To assess if any change in

fitness occurred because of possible inbreeding we compared two lines of wasps in the colony. Line 1 was started off on 29 June 1992 and no wasps from outside the colony were added after 10 August. Line 2 was started off on 7 October. Table 5.1 shows the differences in median development time, sex ratio and average number of offspring per female between line 1 (wasps reared for approximately 12 generations) and line 2 (during the first 2 generations after introduction). No significant differences were found for any of the above parameters.

Table 5.1 : Differences in colony performance of two independent lines of *Bracon hebetor*. Observations on Line 1 were taken after 10-12 laboratory generations (1-14 December); on Line 2 directly after introduction from the field (26 October - 11 November). Five repetitions per line. Differences were tested using a Student-t test (two-tailed), after confirmation of homogeneity of variances.

Parameter	Line	Average	Standard deviation	Significance
# adult offspring / female	1	36.0	4.6	0.86
	2	34.8	14.0	
sex ratio (% ♀)	1	46.0	7.4	0.58
	2	42.0	13.5	
median development time (days)	1	13.0	2.1	0.31
	2	11.8	1.4	

THE EFFECT OF FENITROTHION ON *BRACON HEBETOR*

Testing the acute toxicity of fenitrothion on *Bracon hebetor*

Material and methods

The acute toxicity test used here is based on the "adult vial test" used in pesticide resistance monitoring (Micinki *et al.*, 1991), originally developed by Plapp and Vinson (1977). It is based on coating the inside of a glass vial with the insecticide and exposing the insects to this surface for a fixed period of time. Toxicity is expressed as an amount insecticide per unit exposed surface area, which can be extrapolated to field situations. This method was chosen since it does not require expensive laboratory spraying equipement.

Fenitrothion was obtained from a commercial batch of Ultra Low Volume insecticide (Sumithion® 500 g a.i./l ULV; batch number 4853Y11; Sumitomo Corporation, Japan). It was dissolved in acetone and diluted to the required concentrations using glass volumetric pipettes and volumetric flasks. All solutions were kept in a refrigerator at approximately 4 °C, in the dark.

Borosillicate glass test tubes (diameter 11-13 mm; length 100-125 mm) were used as exposure chambers. They are washed with detergent, rinsed with distilled water and then dried completely in the full sun or in a laboratory stove, on the day before or the day of the test itself. This is necessary to completely remove any greasy substances from the inside of the tubes which will interfere with the homogeneous evaporation of the acetone.

One ml of each acetone solution is pipetted in the exposure tube; ten replicate tubes per concentration. The tubes are subsequently placed in an automatic rotator (Roto-Torque™), which is set under such an angle that the liquid level reaches just under the rim of the tube (fig. 3.1) (Note: a simple manual multi-tube rotator can be made from plywood; a construction note is available on request). The rotator turnes slowly so that the acetone evaporates off, leaving only a dry coating of the insecticide on the inside of the glass tube. After complete evaporation of the acetone in the rotator (10-15 minutes at 25-30 °C), the tubes are air-dried for another hour before wasps are introduced. Control tubes are treated with acetone only.

Bracon wasps are caught from the rearing colony in small glass vials. One male and one female are introduced per exposure tube. The tubes are closed off with a small piece of fine mesh mosquito netting, held in place by a plastic ring. The exposure tubes with the insects are then placed, horizontally and in random order, in a stand so that the bottoms of the tubes are turned to the light. The latter in order to stimulate the phototactic *Bracon* to stay on the treated walls of the tubes as much as possible. A ventilator turning at slow speed is placed perpendicular to the rack to increase ventilation and reduce a possible build up of insecticide fumes in the tubes. The tests are performed in a well aerated room. After 24 hours of exposure the number of surviving males and females are noted for each concentration.

Temperature and relative humidity are measured continuously during the test, using a thermo-hygrograph.

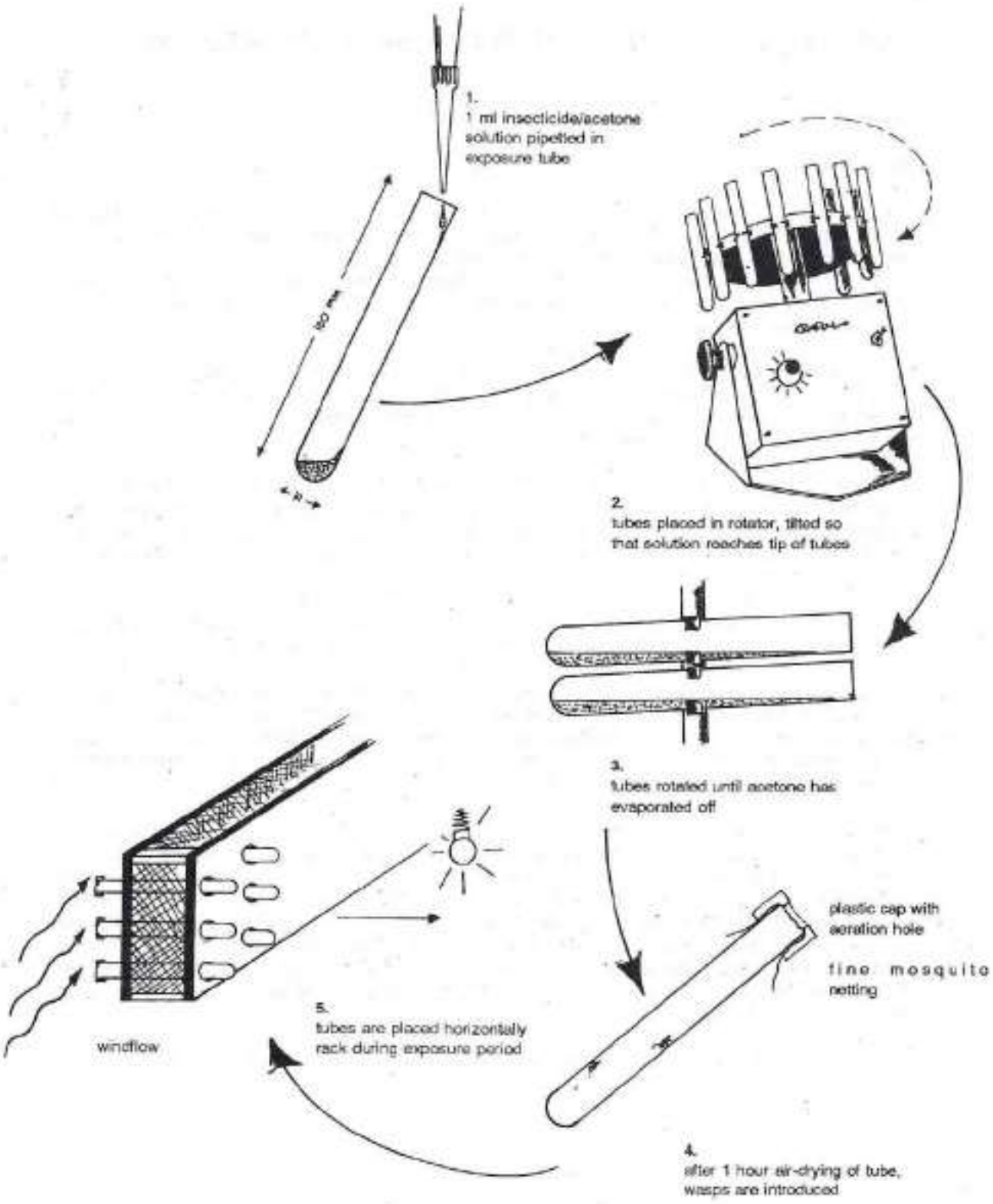


Fig.5.13. Different stages of the acute toxicity test with *Bracon hebetor*.

Test doses are expressed in ng active ingredient/cm² glass. 24-hour LC_{50} values and their 95% confidence limits are then calculated using the method described by Kooijman (1980). It is based on a log-logistic dose-effect relationship. Calculations were made using a computer programme developed by the Netherlands' Institute for Inland Water Management and Waste Water Treatment (RIZA, Lelystad, The Netherlands).

Results

Eight acute toxicity tests were performed with fenitrothion, during different periods of the year and using different lines and ages of *Bracon*. Three different persons carried out different tests. Test conditions are given in table 5.1.

Table 5.2. Conditions during the acute toxicity tests with fenitrothion and state of *Bracon hebetor* wasps.

Test no.	*Bracon*		Test conditions		
	# generations in colony	age at test (days)	date	average temperature (°C)	average rel. humidity (%)
1	5	0-4	14 Aug 1992	n.d.	n.d.
2	10	1-2	10 Oct 1992	30	58
3	11	1-3	21 Oct 1992	24	50
4	1	1-4	02 Nov 1992	30	55
5	1	1-3	07 Nov 1992	29	36
6	–[1]	0-2	14 Aug 1993	31	70
7	–	0-3	20 Aug 1993	29	68
8	–	0-2	03 Sep 1993	28	73

[1]: not known since wild wasps were at the time regularly introduced in the colony; n.d.: not determined.

Table 5.2 lists the LC_{50}'s for males and females seperately, and for both sexes combined. (raw data of all tests in Annex II). Control mortality was never more than 20%, and in most cases no control mortality occured at all. This suggests that the solvent acetone does not negatively affect the wasps, nor does handling and caging during the test. Females were always less susceptible to fenitrothion than males, although this was only twice statistically significant. This slight difference in susceptibility may be due to the larger size of the female compared to the male. LC_{50}'s between the various tests showed a Coefficient of Variation of 43% in case of the combined sexes. Highest and lowest LD_{50}'s varied at most by a factor of 5. There did not seem to be any correlation between LC_{50} and age or generation of the wasps, nor with temperature and relative humidity during the test.

Testing the effect of fenitrothion on the fecundity of *Bracon hebetor*

Female wasps surviving the acute toxicity test were further observed for the effects of the insecticide on fecundity and longevity.

Materials and methods

Bracon females which survived the acute toxicity tests were individually held in glass tubes closed by a plug consisting of iron mesh and mosquito netting. The fine-mesh mosquito netting avoids escaping of *Bracon* while the iron mesh prevents *Ephestia* larvae from eating holes through the mosquito-netting. A cotton-tip soaked in 10 % sugar water is suspended in each tube as food.

Table 5.3. 24 hour LC_{50}-values (ng/cm²) and 95% confidence intervals (between brackets) of the acute toxicity tests with fenitrothion. Coefficient of Variation calculated over the LC_{50} values.

test no.	males	females	total
1	3.0 (1.8 - 5.0)	6.0 (4.4 - 8.3)	4.4 (3.2 - 5.9)
2	3.4 (2.4 - 4.8)	15.0 (7.2 - 31.0)	6.5 (4.7 - 9.0)
3	5.3 (3.2 - 8.8)	18.9 (13.4 - 26.8)	13.7 (10.2 - 18.1)
4	7.5 (4.6 - 12.1)	18.0 (12.1 - 27.0)	12.1 (8.1 - 18.1)
5	10.2 (7.1 - 14.5)	12.6 (9.4 - 17.3)	18.0 (13.0 -25.0)
6	4.6 (2.3 - 9.5)	21.9 (8.1 - 59.1)	11.2 (5.7 - 22.0)
7	7.1 (5.3 - 9.7)	10.7 (7.9 - 14.5)	9.4 (7.6 - 11.7)
8	15.5 (11.0 - 21.7)	25.0 (13.5 - 46.2)	18.9 (14.0 - 25.6)
C.V.	59%	39%	43%

Five late instar *Ephestia* larvae are provided as hosts for each female. Every second day the female is transferred to a new tube with 5 unparasitized larvae. This procedure is repeated until the female dies.

Longevity is registered for every female. The number of offspring males and females and the number of larvae which died in their cocoon were counted for every tube with parasitized larvae.

Fecundity was assessed for four independent tests.

Analysis

Average number of offspring per surviving female is calculated for each insecticide dose as:

$$\sum_{x} M_x$$

with: M_x = average number of adult offspring per surviving female raised from eggs laid between time x-1 and x

Combining the data from the acute toxicity test and the observations on female longevity and fecundity, a population reproduction factor R^{Tox} can now be calculated for each dose:

$$R^{Tox} = \sum_{x} (L_x M_x)$$

with: R^{Tox} = reproduction factor
L_x = fraction of total number of females initially incubated in the acute toxicity test surviving until time x
M_x = average number of adult offspring per surviving female laid between time x-1 and x

This reproduction factor R^{Tox} is the average number of adult offspring surviving until adulthood which are born to a female exposed to the insecticide. The reproduction factor is calculated for each dose of the insecticide, as well as for the untreated control. R^{Tox} is calculated in a similar way as the Basic Reproductive Rate "R" (also called Net Rate of Increase) often used in population dynamics equations (e.g. Begon, Harper and Townsend 1990), but is not identical to it. However, R^{Tox} is a good parameter to assess potential insecticide impact at population level, especially since it includes sublethal effects influencing reproduction.

Calculation of the above reproduction factors from the raw data sets has been programmed in computer spreadsheet format, which is available on request.

Results

Annex III lists the results of these observations. Longevity, fecundity and R^{Tox} are plotted in fig. 5.4 as percentage of their average control values.

Average longevity of females surviving the acute toxicity test is determined as from the start of the fecundity test. Longevity was just as often higher than the control as it was reduced (fig. 5.4a). No consistent reduction in longevity is seen with increasing doses. A high variability in data was observed.

Average fecundity is expressed as the average number of offspring which reach adulthood per surviving female. This underestimates "real" fecundity, which tends to be expressed as the number of eggs laid per surviving female. It does cover, however, any effects the insecticide may have on early lifestages of the offspring generation and is as such a more relevant parameter for insecticide impact on parasitoid population dynamics (Elzen 1989). It is clear from figure 3.4b that fecundity is not consistently reduced by fenitrothion. Again, results showed considerable variability.

The effect of fenitrothion on the reproduction factor R^{Tox} is shown in figure 3.4c. At doses higher than approximately 15 ng/cm^2 the reproduction factor drops below control levels in all tests. The LC_{50}'s calculated for fenitrothion are of the same order of magnitude. This reduction in R^{Tox} is therefore brought about by increased acute mortality, rather than reduced fecundity or survivor longevity (see above).

A note of caution applies to the relatively high variablity observed in the reproduction tests. These are partly caused by the low number of females on which the results are based, especially at the higher dose rates. It is necessary in future to increase the number of females in the acute toxicity tests to have sufficient survivors for the subsequent observations on reproduction. Another factor which may have increased variability in fecundity data is the handling of the hosts. *Bracon* larvae attach themselves to the outside of the *Ephestia* larvae, rather than being well protected inside their host as is the case of endoparasitoids. Handling during transfer of the *Bracon* female from one host tube to the other may have caused the eggs or larvae to dislodge from their hosts. The degree to which *Bracon* eggs and larvae are prone to dislodge from their hosts needs to be further evaluated under the conditions of the test. Until this is known, the results from these reproduction tests need to be viewed with certain caution.

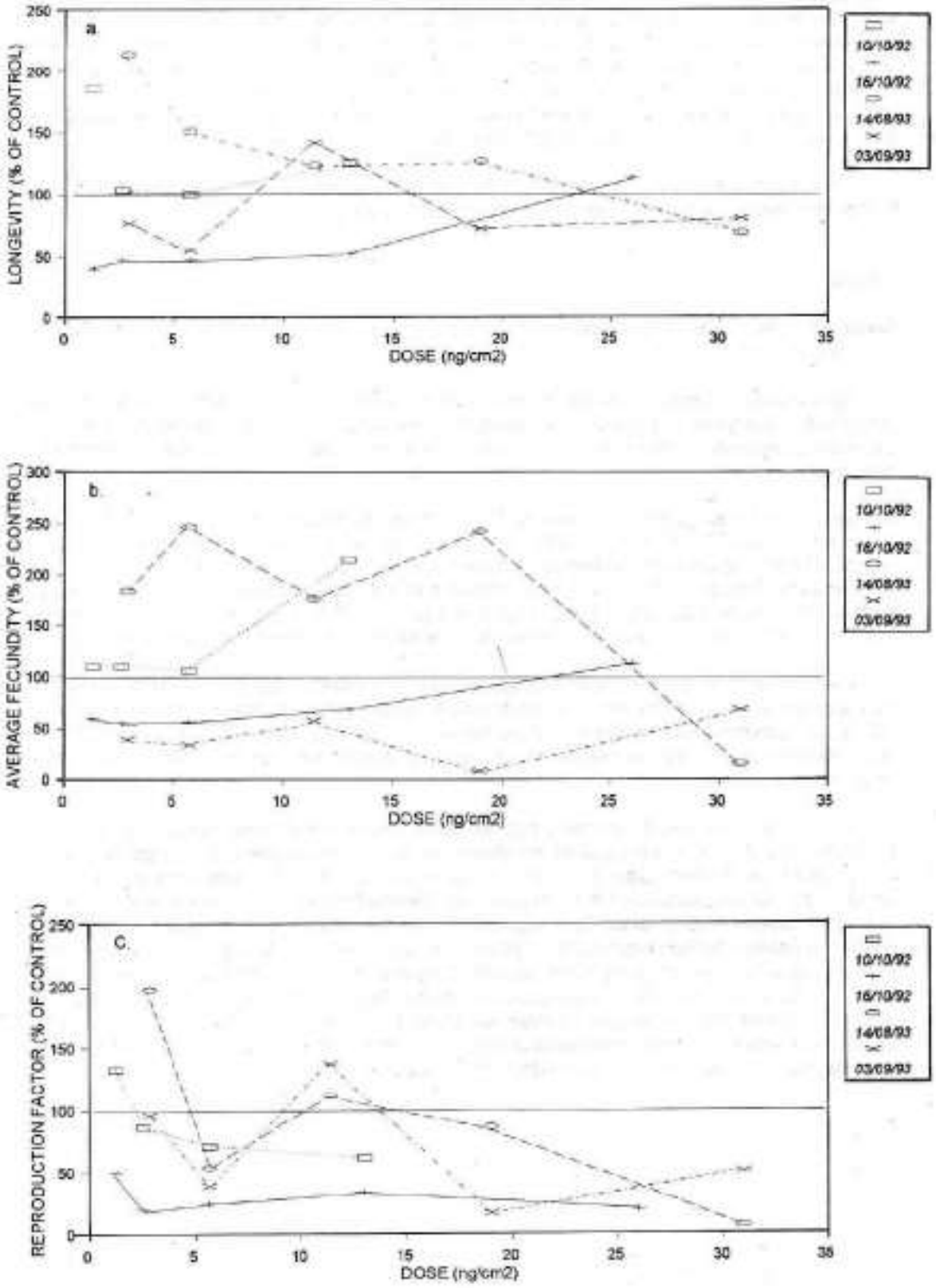


Fig. 5.14 a: Average longevity of surviving females, b: average fecundity and c: the reproduction factor (R^{Tm}) of *Bracon hebetor* exposed to fenitrothion in the laboratory toxicity test. All parameters expressed as percentage of the untreated controls. For absolute data see Annex III.

DISCUSSION AND CONCLUSIONS

Rearing of *Ephestia kueniella*

The laboratory colony of *Ephestia kueniella* can be maintained easily under Sahelian ambient conditions. Survival from egg to adult is slightly more than 20%, with most mortality occuring in early larval stages and possibly the pupal stage. Much higher survival rates have been reported by Daumal *et al.* (1975) for an automated rearing facility where *E. kueniella* was reared on wheat semolina. A number of their techniques could be tested under Sahelian conditions to improve our rearing method. However, given the high fecundity of the female moths, even now a relatively small standing population of adults provides a sufficiently large number of larval offspring to be used as hosts for the *Bracon* colony. Care has to be taken not to transfer *Ephestia* larvae too late from the hatching dishes to the pupation containers since, for unknown reasons, this seems to slow down larval development time considerably. Surplus eggs produced from the *Ephestia* colony could be used in future to rear such egg parasitoids as *Trichogramma spp.* (Trichogrammatidae) and *Chelonus spp.* (Braconidae), both of which are encountered regularly in millet fields in Senegal and for which rearing protocols on this host are already available.

Rearing of *Bracon hebetor*

The rearing of *Bracon hebetor* on larvae of *E. kueniella* as described above was shown to pose no specific problems under ambient Sahelian conditions. The very short generation time of the wasp ensures that large numbers can be produced on short notice for toxicity testing. The fitness of the laboratory colony, measured in parameters as fecundity, sex ratio and development time, did not seem to change in any clear pattern over time. This means that, on average, wasps of comparable fitness are produced, which helps to reduce variability in the results of the toxicity tests. We recommend, however, that regular introduction of wild wasps in the colony be made to reduce the speed of any inbreeding. Indeed, hybrid vigour may have been the reason why the 1993 population showed a higher average fecundity. However, the better results in 1993 may also have been caused by increased experience with the rearing techniques.

Acute toxicity test

The toxicity of fenitrothion to *Bracon hebetor* was fairly constant, irrespective of certain differences in the laboratory population, environmental conditions during the test or the person carrying out the tests. This strengthens the above remarks about the similarity in the quality of wasps produced by the colony. Also, it shows the good degree of repetitivity of the acute toxicity test itself. The fact that no expensive equipment is needed to carry out the test, only increases its value for standard screening of insecticides in Africa.

Internationally, many laboratory toxicity tests tend to be standardized to the extreme with respect to the organisms used and the environmental conditions under which the tests need to be executed. This often requires expensive rearing and testing facilities, such a climate chambers, in which environmental parameters can be precisely controlled. At present only a limited number of institutions in Africa dispose of such facilities, and the possibilities of on site screening according to this type of protocols on locally relevant beneficial arthropods are thus limited. We would, therefore, support the approach taken in this study, of developing tests which can be executed under less fixed environmental conditions (i.e. ambient conditions during most of the year). Given the fact that climatic variation in sub-saharan Africa tends to be rather less pronounced than, for instance in northern Europe, this approach may not be unrealistic. Of course, for the test results to be reliable one would need to determine the ranges of environmental conditions within which the variability in results would be acceptable. The data from the acute toxicity test with fenitrothion presented here seem to suggest that variability in LC_{50}'s can be acceptable over a range of test conditions encountered in the western Sahel.

The toxicity test decribed above determines a dose-effect relationship, evaluating mortality of the wasp at different insecticide doses. This is different from the standard screening procedures for

beneficial arthropods recommended by IOBC and EPPO. These organizations prescribe the testing of the highest operational concentrations of the insecticide formulation at a standard rate of 1.5 mg of liquid / cm^2 of exposure surface (Hassan 1992). This procedure has two major drawbacks: The volume application rates recommended by IOBC may be relevant for high volume pesticide application in Europe, but would result in vastly overdosed test surfaces (approx. 150x) when applied to the highly concentrated ULV formulations standardly used in locust control in Africa. Furthermore, a single dose screening test as recommended by IOBC does not provide nearly the same possibilities for extrapolation of laboratory data to actual field circumstances as dose-effect data do. Unfortunately, the large body of screening data gathered under the IOBC scheme is impossible to extrapolate to field circumstances other than those for which the tests were conducted, and almost useless for pesticide use in, for instance, Africa. Furthermore, since formulations rather than active ingredients are tested, simple comparisons of toxicity between different insecticides become virtually impossible.

We, therefore, strongly prefer the "classical" toxicological approach of determining dose-effect relationships and calculating, for instance, LD_{50}'s, even though this is a more elaborate approach than the IOBC recommended one. When, furthermore, toxicity is expressed as a quantity of insecticide per unit surface area, as is the case in the described test, comparisons to field situations where insects tend to be predominantly exposed by residual contact, become relatively straightforward (see below).

Fecundity observations

The results from the fecundity observations on females surviving the acute toxicity test were too variable. The effects of increasing the number of females in the test, as well as the impact of handling on larval survival of *Bracon*, need to be evaluated. This may result in results which can be replicated better.

The additional information on insecticide impact provided by the fecundity observations, especially at sublethal doses, clearly warrants further development of this part of the toxicity test.

The toxicity of fenitrothion to *Bracon hebetor*

The 24-hour LC_{50} of fenitrothion for males and females combined ranged from 4 to 19 ng / cm^2 of glass surface. Males were slightly more susceptible than females, as was also observed by O'Brien *et al.* (1985) for *Bracon mellitor* exposed to the organophosphate azinphosmethyl. This may be related to size differences. Soultanopoulos and Broumas (1979) report a topical LD_{50} of 2.1 ng fenitrotion / insect. The small absolute difference between their topical and our residual LD_{50} suggests that fenitrothion is transferred very efficiently from the glass surface to the insect.

Gadji (1993) measured initial fenitrothion residues on millet leaves (Souna variety) after experimental applications of approximately 450 g a.i. / ha, as recommended for desert locust control. These ranged from 30 - 55 mg fenitrothion / kg fresh vegetation. Souna millet leaves have a relative weight of about 190 g / m^2 (freshweight, SD=20; n=10). Therefore, on average, initial fenitrothion residues will range from 285 - 525 ng / cm^2 of millet leaves, assuming that both sides of the leaves will have received the insecticide. Initial residues are thus at least 15 - 130 times higher than the LC_{50}'s measured in the toxicity test.

Bio-availability of the insecticide in the laboratory test, however, with the fenitrothion coated on glass, will be higher than in nature where it will be bound more strongly to the leaf. For a same dose, effects of fenitrothion on leaves can therefore be expected to be lower than on glass. However, given the high levels of insecticide measured in the field, we estimate that acute mortality of *Bracon* immediately after treatment will be high. This was indeed observed in bioassays using freshly sprayed vegetation. More than 95% of the wasps died after being exposed to leaves harvested within 4 hours after spraying. However, no increased mortality was observed anymore after exposure to leaves harvested 50 hours after treatment. Gadji (1993) found that fenitrothion residues had dropped well below 0.1 mg a.i. / kg fresh vegetation (or 1.9 ng / cm^2) three days after treatment. It appears thus that although highly toxic to *Bracon* immediately after

locust control applications, the impact of fenitrothion decreases very rapidly with time. This would allow recovery of affected wasp populations through immigration or emergence from cocoons to occur fairly soon after treatment, thus reducing long term impact of the insecticide.

Grosch (1975) showed that the carbamate insecticide carbaryl after a single sub-LC_{50} topical application reduced egg production of *B. hebetor*. O'Brien *et al.* (1985) found a reduction in fecundity of *Bracon mellitor* after 20 days of exposure to the LC_5 dose of azinphosmethyl, an organophosphate insecticide. They used a vial test similar to ours. In our study no clear effects could be observed on reproduction at sub-LC_{50} doses of fenitrothion, although this may have partly been due to the high variability in the data. Given the fact that reproductive effects of insecticides may have at least as large an impact on parasitoid population dynamics as acute mortality, further development of this part of the toxicity test with *B. hebetor* needs to be pursued.

REFERENCES

Antolin M.F. and Strand, M.R. (1992) Mating system of Bracon hebetor (Hymenoptera: Braconidae). Ecological Entomology 17:1-7.

Begon M., Harper J.L. and Townsend C.R. (1990) Ecology. Individuals, populations and communities. Second Editon. Blackwell, Boston etc. pp.945.

Benson J.F. (1973) Intraspecific competition in the population dynamics of Bracon hebetor Say (Hymenoptera: Braconidae). Journal of Animal Ecology 42:105-124

Benson J.F. (1974) Population dynamics of Bracon hebetor Say (Hymenoptera: Braconidae) and Ephestia cautella (Walker) (Lepidoptera: Phycitidae) in a laboratory ecosystem. Journal of Animal Ecology 43:71-86

Bhatnagar V.S. (1987). Recherche et developpement de la lutte intégrée contre les ennémis des principales cultures vivrières dans les pays du Sahel. Senegal. Programme de Lutte Biologique. Rapport Technique Final GCP/RAF/128/CLS. Organisation des Nations-Unies pour l' alimentation et l'agriculture (FAO), Rome, pp.168

Croft B.A. (1990) Arthropod biological control agents and pesticides. John Wiley & Sons, New York2 etc., pp.723

Daumal J., Voegele J. and Brun P. (1975) Les trichogrammes II. - Unité de production massive et quotidienne d'un hôte de substitution Ephaestia kuehniella Zell. (Lepidoptera, Pyralidae). Annales de Zoologie (Ecologie animale) 7(i):45-59 (Abstract only)

Diémé E. (1986) Etude biologique au laboratoire de Bracon hebetor (Say), parasite de Raghuva albipunctella (De Joannis) et d'Ephestia kuehniella (Zell) au Sénégal. République du Sénégal, Ministére du développement rural. 28 pp.

Diémé E. (1989) Etude biologique de Bracon hebetor. Sahel PV INFO 12:9-17

Elzen G.W. (1989) Sublethal effects of pesticides on beneficial parasitoids. pp. 129-150 in: Jepson P. (ed.) Pesticides and non-target invertebrates. Intercept, Wimborne.

EPPO (1992) Decision making scheme for the environmental risk assessment of plant protection products. Chapter 9, working document 92/3745. European and Mediterranean Plant Protection Organization, Paris.

Everts J.W. (ed.)(1990) Environmental effects of chemical locust and grasshopper control - A pilot study. Project Report ECLO/SEN/003/NET. Food and Agriculture Organization of the United Nations (FAO), Rome, pp.277.

Fry J.M. (1989) Natural enemy databank 1987. CAB International, Wallingford, pp.185

Gadji B. (1993) Déposition et dégradation du fénitrothion et du diflubenzuron sur végétation et dans les sols au Sénégal & suivi des résidus dans les stockage de mil en monde rural (campagne 1992). Rapport LOCUSTOX 93/5. Organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO), Dakar, pp.39.

Gahukar R.T., Guevremont H., Bhatnagar V.S., Doumbia Y.O., Ndoye M. and Pierrard G. (1986) A review of the pest status of the millet spike worm, Raghuva albipunctella De Joannes (Noctuidae: Lepidoptera) and its management in the Sahel. Insect Science and its Application 7(4):457-463.

Geddes A.M.W. (1990) The relative importance of crop pests in sub-Saharan Africa. Natural Resources Institute Bulletin No. 36. NRI, Chatham, pp. vi.+89

Grosch D.S. (1975) Reproductive performance of Bracon hebetor after sublethal doses of Carbaryl. Journal of Economic Entomology 68(5):659-662

Hassan S.A. *et al.* (1985). Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/ WPRS Working Group 'Pesticides and Beneficial Organisms'. EPPO Bulletin 15:214-255.

Hassan S.A. (ed.) (1992) Guidelines for testing the effects of pesticides on beneficial organisms: description of test methods. IOBC/WPRS Bulletin 1992/XV/3: 1-186

IOBC (1988) Working group "pesticides and beneficial organisms". IOBC/WPRS Bulletin 1988/XI/4: 1-143

Kamara O. and van der Valk H. (in prep.) Side-effects of fenitrothion and diflubenzuron on beneficial arthropods in millet in Senegal (the 1992 study). LOCUSTOX Report. Food and Agriculture Organization of the United Nations (FAO), Dakar.

Kooijman S.A.L. (1980) Parameter analysis of mortality rates in bioassays. Water Research 15:107-119

Matteson P.C. (1992) A review of field studies of the environmental impact of locust/grasshopper control programmes in Africa. pp. 347-356 in: Lomer, C.J. and Prior, C. (eds.) Biological control of locusts and grasshoppers. Proceedings of a workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April-1 May 1991. CAB International, Wallingford.

Micinski S., Luttrell R.G., Leonard B.R. and Graves J.B. (1991) Relationship of adult vial test data on pyrethroid resistance to field control of tobacco budworm in cotton. Southwestern Entomologist suppl. 15:173-186.

Nikam P.K. and Pawar C.V. (1993) Life tables and intrinsicc rate of natural increase of Bracon hebetor Say (Hym., Braconidae) population on Corcyra cephalonica Staint. (Lep., Pyralidae), a key parasitoid of Helicoverpa armigera Hbn. (Lep., Noctuidae). Journal of Applied Entomology 115:210-213

O'Brien P.J., Elzen G.W. and Vinson S.W. (1985) Toxicity of azinphosmethyl and chlordimeform to parasitoid Bracon mellitor (Hymenoptera: Braconidae): Lethal and reproductive effects. Environmental Entomology 14(6):891-894.

Petters R.M., Kendall M.E., Taylor R.A.J. and Mettus R.V. (1985) Time required for mating and the degree of genetic relatedness in the parasitoid wasp, Bracon hebetor Say (Hymenoptera, Braconidae). Melsheimer Entomological Series 35:21-27

Plapp F.W. Jr. and Vinson, S.B. (1977) Comparative toxicities of some insecticides to the tobacco budworm and its ichneumonid parasite, Campoletis sonorensis. Environmental Entomology 6:381-384

Rotary N. & Gerling, D. (1973) The influence of some external factors upon the sex ratio of Bracon hebetor Say. Environmental Entomology 2(1):134-138.

Risbec J. (1950) La faune entomologique des cultures au Sénégal et au Soudan Français. Station expérimentale de M'Bambey. Gouvernement général de l'Afrique occidentale française, Paris, pp.508

Soultanopoulos C. and Broumas Th. (1979) Comparative toxicity of various insecticides to Habrobracon hebetor and Chelonus eleaphilus (Hymenoptera: Braconidae). Med. Fac. Landbouww. Rijksuniv. Gent 44(1):179-183

Theiling, K.M. and Croft B.A. (1988) Pesticide side-effects on natural enemies: a database summary. Agriculture, Ecosystems and Environment 21:191-218

van der Stoep J. (1992) The development of laboratory toxicity tests with Bracon hebetor (Say) for the study of the environmental effects of locust control. Department of Entomology report no. 13-93. Wageningen Agricultural University, Wageningen, pp.45.

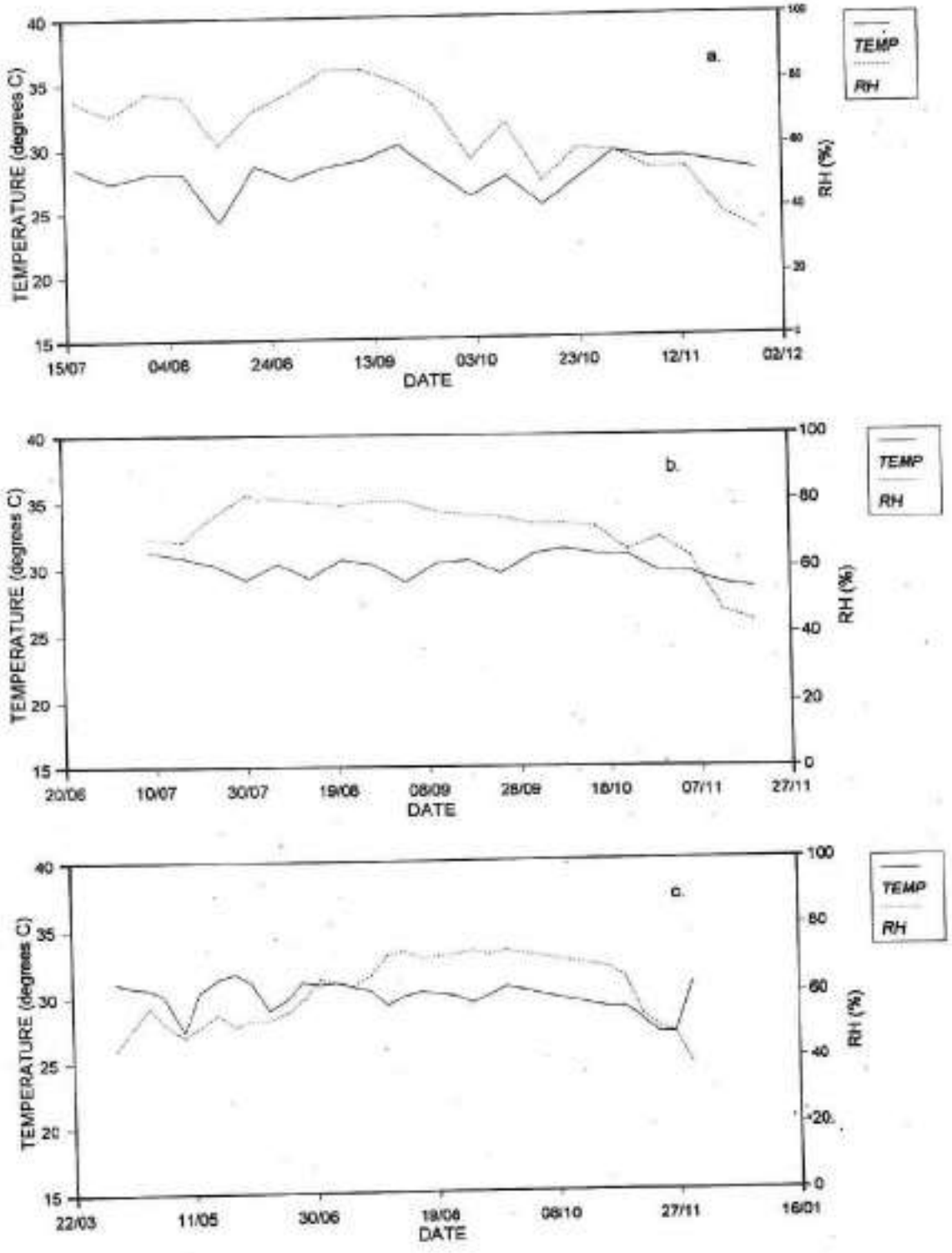
van der Valk H.C.H.G. (1990) Environmental impact studies of chemical locust and grasshopper control - A review. Report to the Scientific Advisory Committee of the Coordinating Group on Locust Research (CGLR). FAO, Rome, pp.37

van der Valk H. and Kamara, O. (1993) The effect of fenitrothion and diflubenzuron on natural enemies of millet pests in Senegal (the 1991 study). LOCUSTOX Report No. 93/2. Food and Agriculture Organization of the United Nations (FAO), Dakar, pp.34.

Weidner H. and Rack G. (1984) Tables de détermination des principaux ravageurs des denrées entreposées dans les pays chauds. Schriftenreihe no. 129. Deutsche Gesellschaft fur Technische Zusammenarbeit (GTZ), Eschborn, pp.148.

ANNEX 5.1

Average weekly temperature and relative humidity in the rearing rooms at Nioro du Rip, Senegal. a: *Bracon* colony - 1992. b: *Bracon* colony - 1993. c: *Ephestia* colony - 1993. TEMP = temperature; RH = relative humidity; both measured using a laboratory thermohygrographe.



ANNEX 5.2. Number of *Bracon hebetor* alive and dead after 24 hours of exposure to fenitrothion.

test no.	concentration (ng/cm²)	males		females		total	
		alive	dead	alive	dead	alive	dead
1	0	10	0	9	0	19	0
	1.1	6	4	9	0	15	4
	2.8	7	3	8	2	15	5
	5.7	5	5	7	3	12	8
	11	0	10	1	9	1	19
	28	0	10	0	10	0	20
2	0	10	0	10	0	20	0
	0.26	9	1	10	0	19	1
	0.57	10	0	10	0	20	0
	1.3	10	0	10	0	20	0
	2.6	6	4	10	0	16	4
	5.7	3	7	8	2	11	9
	13	0	8	5	3	5	11
3	0	10	0	9	1	19	1
	1.3	8	2	7	3	15	5
	2.6	7	3	8	2	15	5
	5.7	6	4	10	0	16	4
	13	3	7	7	3	10	10
	26	0	10	2	8	2	18
4	0	10	0	10	0	20	0
	1.3	7	3	9	1	16	4
	2.6	10	0	10	0	20	0
	5.7	7	3	10	0	17	3
	13	3	7	7	3	10	10
	26	1	9	3	7	4	16
5	0	10	0	10	0	20	0
	1.3	10	0	10	0	20	0
	2.6	10	0	10	0	20	0
	5.8	7	3	10	0	17	3
	13	4	6	9	1	13	7
	26	1	9	6	4	7	13
6	0	7	3	9	1	16	4
	2.9	2	8	9	0	11	8
	5.7	2	8	3	7	5	15
	11.4	4	6	9	0	13	6
	19	1	9	5	5	6	14
	31	0	10	5	5	5	15

test n°	concentration (ng/cm²)	males		females		total	
		alive	dead	alive	dead	alive	dead
7	0	9	1	10	1	19	1
	2.9	9	0	9	1	18	1
	5.7	7	2	9	0	16	2
	11.4	1	9	2	8	3	17
	19	1	9	2	7	3	16
	31	0	10	2	8	2	18
8	0	10	0	10	0	20	0
	2.9	10	0	10	0	20	0
	5.7	8	2	9	1	17	3
	11.4	8	2	6	4	14	6
	19	3	7	6	4	9	11
	31	2	8	5	5	7	13

ANNEX 5.3

Results from the fecundity observations on females of *Bracon hebetor* surviving 24 hours of exposure to different doses of the insecticide fenitrothion. Fecundity is expressed as the average number of offspring reaching adulthood per surviving female. Average longevity (days) of *Bracon* females surviving the acute exposure test is calculated starting 1 day after exposure, when the fecundity observations started. Calculation of the reproduction factor R^{Tm} is explained in the text. It is expressed as the average number of offspring reaching adulthood per female originally introduced in the acute toxicity test.

Date of test	Dose (ng fenitrothion / cm²)									
Parameter	0	1.3	2.6	2.9	5.7	11.4	13	19	26	31
FECUNDITY (M_x)										
10 Oct 1992	214	235	235		227		460			
16 Oct 1992	230	138	128		130		157		259	
14 Aug 1993	130			239	320	229		315		20
03 Sep 1993	205			80	69	116		16		141
LONGEVITY										
10 Oct 1992	14.0	26.0	14.5		14.0		17.5			
16 Oct 1992	16.8	6.6	8.0		8.0		8.7		19.0	
14 Aug 1993	7.5			16.0	11.3	9.3		9.5		5.2
03 Sep 1993	6.9			5.3	3.8	9.8		5.0		5.6
REPRODUCTION FACTOR (R^{Tm})										
10 Oct 1992	119	158	103		85		74			
16 Oct 1992	128	64	26		32		44		26	
14 Aug 1993	73			143	39	81		63		5
03 Sep 1993	32			30	12	43		6		16

CHAPTER 6

Toxicity tests with fenitrothion on *Pimelia senegalensis* and *Trachyderma hispida* (Coleoptera, Tenebrionidae).

SUMMARY

Toxicity tests with fenitrothion, an insecticide used in locust control, were carried out on two Tenebrionidae (*Pimelia senegalensis* and *Trachyderma hispida*) in order to study its impact on these Coleoptera. Two types of tests were used: topical and ingestion tests.

The LD_{50}'s found for *Pimelia senegalensis* reveal a real risk of locust control for this species. *Trachyderma hispida* is less sensitive to fenitrothion and the LD_{50}'s by ingestion are far higher than residue levels found on vegetation after locust control treatments.

The degree of similarity among repeated tests is discussed and a number of suggestions concerning the methodology are proposed in order to reduce the variability observed in some experiments.

INTRODUCTION

Coleoptera belonging to the family of Tenebrionidae are of great importance in the arid and semi-arid ecosystems, both because of their number and biomass as well as for their ecological function.

Crawford (1991b) quotes some estimates of tenebrionid densities and their biomass in deserts that may reach 100000 individuals per hectare in an American desert, or more than 190 kg per hectare in a Kazakhstan steppe. In desert dune field habitats, about 30 % of all insect species are Tenebrionidae (Seely 1991). Gillon (1983) showed the importance of Tenebrionidae in the semi-arid sahelian pastures in northern Senegal, where 19 to 58% of the total arthropod biomass is composed of species belonging to this group. He considered the Tenebrionidae, together with scorpions and Solifuga, as characteristic of arid savannas.

Tenebrionidae of arid zones are generally considered to be omnivores, eating primarily litter and plant matter, but sometimes also living animal prey. This is the case for adults of *Pimelia* which we observed in our laboratory colony attacking their own larvae and debilitated adults. Tenebrionid larvae are clearly omnivorous, attacking grasshopper eggpods in the western Sahel (Popov 1980, Niassy *et al.* 1993). In the Namibian desert in South Africa, Tenebrionidae are the major consumers of plant litter (or "macro-decomposers"), the absence of humidity being a limiting factor for microbial decomposition (Louw and Seely 1982). Crawford (1991a) suggests even that tenebrionids may regulate organic matter breakdown, especially in arid dune field habitats.

Considering their importance, the impact of locust and grasshopper control on Tenebrionidae has recently received more attention. Van der Valk (1990) showed that fenitrothion, applied at dose rates used against the desert locust, reduced the catches of *Pimelia senegalensis* and *Vieta senegalensis*, two abundant tenebrionid species in the northern savanna of Senegal.

Standard toxicity tests have not been developed for this group of non-target organisms. These tests could contribute to the early evaluation of insecticide risk. In temperate ecosystems, tenebrionids are mainly considered as pests of stocks (e.g. *Tribolium spp.*). Toxicity tests developed for these pests species, however, are based on modes of exposure to insecticides which are less applicable to non-target species.

Below, we present a number of toxicity tests carried out with two species of Tenebrionidae, *Pimelia senegalensis* Ol. and *Trachyderma (=Ocnera) hispida* (Forsk). The larvae of the first species are predators of grasshopper eggpods. The second species was included in a limited number of tests because of its abundance in central Senegal, and in the arid zones of Africa and the Middle-East in general. Abushama (1984) and Ayyad and Ghabbour (1986) report the presence of *Trachyderma hispida* in the desert of Sudan, and Abushama and Al-Salameen (1989) in Kuwait. The use of two different species will also allow us to have a first idea of the differences in susceptibility in this group of desert tenebrionids.

The study below has two objectives: First, to assess the toxicity of fenitrothion, the insecticide most used in locust and grasshopper control in Africa, for these two non-target Tenebrionidae. We will try to compare the laboratory toxicity data with the doses and residues found in the field during control operations. The second objective is to get an idea of the repeatability of the methods used. Wild insects were used in the tests rather than insects reared in the laboratory, the latter being nearly impossible because of the very long life-cycle of the beetles. However, their susceptibility to the insecticide may be influenced by differences in the time or location of capture. Therefore, comparisons were made between populations caught in two different zones, during two different periods of the year.

MATERIAL AND METHODS

Insects used

Test were carried out on adults of *Pimelia senegalensis* and *Trachyderma hispida* (Coleoptera, Tenebrionidae). Insects were caught during three periods: in June 1992 in the Kaffrine area (14°05'N - 15°35'W) in the central Senegal; in November 1992 in the same area; and in July 1993 around Linguère (15°15'N - 15°25'W) in the north of the country. These populations were kept separated in the laboratory in Dakar on a varied diet (millet bran, bread and cabbage) up to the time they were used for the toxicity tests. Only healthy insects were drawn from these populations. No sexual distinction could be made before the test for either of the two species. Although it is possible to determine the sex after the insect dies, we didn't do it in this first set of tests. All LD_{50} calculations were thus based on the total number of insects which were submitted to the treatments. The freshweight of *P. senegalensis* was measured using an electronic scale. It averaged 1.5 grammes per insect. For the calculation of the LD_{50}'s this average weight was used for *P. senegalensis* as well as for *T. hispida*, a tenebrionid of comparable size.

Preparation of the fenitrothion solutions

All the solutions were prepared from a fenitrothion parent solution of 500 g /l ULV (SUMITHION® 500 ULV, Sumitomo Corporation, Japan, lot number 4853Y11). Dilutions were made with pure acetone, using volumetric flasks and pipettes. Doses to be applied were established according to series of logarithms of 10 (^{10} log) as is usually done in toxicological tests (Bulkema *et al.* 1982).

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

Topical exposure test

The application of the solutions was carried out with a manual micro-applicator (Burkhard®) equipped with a micro-syringe, the droplet size of which was calibrated in the laboratory before the tests. A 2 µl volume was adopted as the droplet size to be applied on each insect. During the treatments, the insects were placed on a petri dish and the droplet was deposited at the juncture between the thorax and the abdomen (a relatively supple part of the insect where the droplet can quickly penetrate). The applications were done following increasing concentrations, starting with the test solution (acetone). Between two applications the syringe was rinsed with the next solution to be tested.

In general, ten insects were exposed per concentration and placed in a plastic tray (30 cm diameter x 12 cm high) covered with a cardboard sheet. They were fed with millet bran. Mortality observations were made 24, 48, 72 and 96 hours after treatment. The insects were considered "dead" either if they did not react any more with their legs or antennae to a slight stimulation with a pair of tweezers, or if they were moribund and didn't recover during the days of observation (Leeper *et al.* 1982).

Ingestion test

The insects were fed on millet bran mixed with different fenitrothion test solutions diluted in acetone. Ten millilitres were taken from each solution to be homogeneously applied to 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 of the acetone. A control treated with acetone only was included in each test. The different petri dishes were subsequently placed in plastic trays (20 cm diameter x 12 cm high) containing each ten insects, which were covered with a cardboard sheet.

The fenitrothion-treated food was renewed every 24 hours and mortality evaluated, for a 96-hour period. For the calculation of the LD_{50}'s the insects were considered "dead" either if they did not react any more with there legs or antennae to a slight simulation with a pair of tweezers, or if were

moribund and didn't recover during the days of observation (Leeper *et al.* 1988).

Analysis

LD_{50} calculations were carried out according to a "log-logit" iterative model (Kooijman 1980), using a software package developed by RIZA ("Institute for Inland Water Management and Waste Water Treatment", the Netherlands). Two LD_{50} values were considered statistically identical if their 95% confidence intervals overlapped.

Ambient conditions

The tests were carried out in the ambient conditions of the laboratory. The temperature and relative humidity during the tests were measured with a thermo-hygrograph (Annex 1).

RESULTS

Tables 6.1 to 6.4 show the different results obtained during the tests.

Table 6.1: Number of survivors of *Pimelia senegalensis* in the topical exposure tests with fenitrothion. Ten insects were exposed per concentration for tests 1 and 2, and 15 for test 3.

Series	Solution concentration (g a.i./l)	Dose (µg a.i./ g insect)	Time after exposure (hours)			
			24	48	72	96
Test 1 (23/09/92)	25	33	3	0	0	
	7	9	8	7	5	
	2.5	3.3	10	9	8	
	0.7	0.9	10	10	10	
	0.25	0.33	10	10	10	
		0	10	10	10	
Test 2 (14/12/92)	25	33	0	0	0	0
	10	13	5	2	0	0
	4	5.33	7	7	7	7
	1.6	2.13	10	10	10	10
	0.64	0.85	10	10	9	9
		0	10	10	9	9
Test 3 (26/08/93)	25	33	3	0	0	0
	10	13	6	5	3	3
	4	5.33	12	9	9	9
	1.6	2.13	14	11	11	10
	0.64	0.85	15	14	14	14
		0	15	15	15	15

Table 6. 2: Number of survivors of *Trachyderma hispida* in the topical exposure tests with fenitrothion. Ten insects were exposed per concentration.

Series	Solution concentration (g a.i./l)	Dose (µg a.i./ g insect)	Time after exposure (hours)			
			24	48	72	96
Test 1 (15/12/92)	25	33	8	8	8	8
	10	13	10	9	8	8
	4	5.33	10	10	10	10
	1.6	2.13	10	9	9	9
	0.64	0.85	10	10	10	9
		0	10	10	10	10

Table 6.3: Number of survivors of *Pimelia senegalensis* in the ingestion tests with fenitrothion. Ten insects were exposed per concentration for tests 1 and 2, and 15 for tests 3 and 4.

Series	Solution concentration (g a.i./l)	Dose (mg a.i./kg brain)	Time of exposure (hours)			
			24	48	72	96
Test 1 (08/01/93)	10	10000	0	0	0	0
	1	1000	0	0	0	0
	0.1	100	6	2	1	0
	0.01	10	8	7	6	6
	0.001	1	10	10	10	9
		0	10	10	10	10
Test 2 (21/01/93)	0.2	200	1	0	0	0
	0.05	50	9	5	5	3
	0.014	14	10	9	9	9
	0.004	4	9	9	9	9
	0.001	1	10	10	10	10
		0	10	10	10	10
Test 3 (27/08/93)	1	1000	0	0	0	0
	0.03	30	0	0	0	0
	0.010	10	0	0	0	0
	0.003	3	11	6	6	1
	0.001	1	14	14	14	14
		0	15	15	15	15
Test 4 (14/09/93)	0.01	10	13	10	10	3
	0.006	6	15	15	15	15
	0.003	3	15	14	14	14
	0.002	2	15	15	15	15
	0.001	1	15	15	15	15
		0	15	15	15	15

The data in tables 6.1 to 6.4 were used to calculate the LD_{50}'s, the lethal doses for 50% of the population of tenebrionids in question.

The topical tests with *T. hispida* (table 6.2) did not cause sufficient mortality. The data collected did not allow to calculate an LD_{50}. This tests was not repeated due to a lack of test insects.

Table 6.4: Number of survivors of *Trachyderma hispida* in the ingestion tests with fenitrothion. Ten insects were exposed per concentration.

Series	Solution concentration (g a.i./l)	Dose (mg a.i./kg bran)	Time of exposure (hours)			
			24	48	72	96
Test 1 (13/01/93)	10	10000	2	0	0	0
	1	1000	4	1	1	1
	0.1	100	10	9	9	8
	0.01	10	10	10	10	10
	0.001	1	10	10	10	10
		0	10	10	10	10
Test 2 (21/01/93)	10	10000	0	0	0	0
	2.5	2500	0	0	0	0
	0.7	700	3	0	0	0
	0.2	200	8	6	3	1
	0.05	50	8	7	7	7
		0	10	10	10	10

Table 6.5: LD_{50}'s of fenitrothion for *Pimelia senegalensis* and *Trachyderma hispida* after topical and ingestion toxicity tests. The LD_{50} is expressed as µg a.i./g insect for the topical tests, and mg a.i./kg bran for the ingestion tests. For the topical tests, LD_{50}'s are for a given number of hours (h) after exposure; for the ingestion tests LD_{50}'s are for a given time (h) of exposure.

Species	Type of test	Series	LD_{50} (95 % confidence interval)			
			24 h	48 h	72 h	96 h
Pimelia senegalensis	Topical	1	21.9 (13.2-36.2)	11.1 (7.1-17.4)	7.7 (4.9-11.9)	
"	"	2	10.7 (7.2-16.1)	8.0 (5.4-11.9)	5.3 (3.6-7.9)	5.3 (3.6-7.9)
"	"	3	12.2 (7.8-18.9)	5.8 (3.9-8.6)	5.1 (3.4-7.7)	4.8 (3.2-7.2)
Pimelia senegalensis	Ingestion	1	86.7 (36.7-204.7)	29.4 (12.6-68.6)	17.8 (7.6-41.8)	10.6 (4.4-25.2)
"	"	2	92.8 (53.8-159.9)	41.6 (25.3-68.5)	41.6 (25.3-68.5)	32.2 (19.5-53.3)
"	"	3	3.6 (2.8-4.6)	2.7 (2.2-3.4)	2.7 (2.2-3.4)	1.9 (1.4-2.5)
"	"	4	12.9 (9.9-16.9)	10.7 (9.2-12.5)	10.7 (9.2-12.5)	8.5 (7.4-9.8)
Trachyderma hispida	Ingestion	1	1384 (595-3214)	315 (137-724)	315 (137-724)	237 (102-547)
"	"	2	358 (209-614)	176 (102-303)	117 (65.9-207)	82.3 (44.6-152)

DISCUSSION AND CONCLUSIONS

We noted that maintaining the tenebrionid population in the laboratory or the insectarium was very easy. Adult mortality is low. We kept adults of *P. senegalensis* for more than a year in the colony. Oviposition was regularly observed. This will allow to add later a reproduction toxicity test to the series of screening tests with this group of organisms. A standard operation procedure for the colony maintenance is in preparation and will be available on request. The toxicity tests are easy to carry out and do not require equipment or which is expensive or difficult to maintain.

The results of the topical application tests (table 6.5) show little variation between the LD_{50}'s for *P. senegalensis*. The three tests were composed of insects caught during two periods of the year and in two different localities. Yet the confidence intervals overlap without exception, and therefore we consider that the LD_{50}'s are not statistically different.

The LD_{50} could not be calculated for *T. hispida*. A comparison of tables 1 and 2 shows, however, that this species is less sensitive to fenitrothion than *P. senegalensis*, having an LD_{50} which appears to be higher than 33 µg a.i. /g insect.

The result of the ingestion tests with *P. senegalensis* show considerable differences between calculated LD_{50}'s (table 6.5). The first two series, using insects caught late November 1992, are not statistically different. This is also the case for the two series with *T. hispida*, caught during the same period. However, the Pimelia's caught in July 1993 (series 3 and 4 in table 6.5) were considerably more sensitive to the insecticide than those of the two first series, particularly during the first 48 hours of exposure. This difference becomes smaller with increasing exposure time.

The reason why we observe this difference in susceptibility between populations of the beetles in the ingestion tests and not in the tropical tests is unknown. One explanation could be the physiological state of the beetles, particularly the size of the fat body.

The fat body of *Trachyderma hispida* adults is relatively thin in young insects, but it becomes yellowish and thicker with age. (in: Abushama and Abdallah 1986). The toxicity of lipophilic insecticides, such as fenitrothion, may be reduced if the organism has an large fat body (Geyer *et al.* 1993) The lipids may absorb the insecticide which therefore cannot reach the sites of biological action in the body. We do not know the age of the tenebrionids used in the tests, but we noticed that the elytra of the insects caught in July 1993 were less scratched and had less lesions than those of the population caught in November 1992. It is therefore possible that insects in series 3 and 4 were younger, and thus more sensitive to fenitrothion.

The second reason why the fat body of the insects caught at the beginning of the rainy season might be less important than at its end, does not depend on the age of the insects. *Pimelia* seems to be relatively inactive during the dry season, when they take refuge, probably in the soil. This is the case for most species of Tenebrionidae in the northern savanna of Senegal (Gillon 1983). A prolonged period of food deprivation will reduce the fat body of the insects. Abushama and Abdallah (1986) have shown in this respect that the lipid and glycogen levels in the fat body of some tenebrionids (*T. hispida*, *P. grandis* and *Adesmia antiqua*, all desert species) decreased with 50% after 35 days without food. *Pimelia* caught at the beginning of the rainy season could, for that reason, be more susceptible to lipophilic insecticides, regardless of there age.

The fact that this difference in susceptibility is not that clear after the tropical application is not necessarily in contradiction with what we just elaborated above. If the insecticide is applied at the level of the thorax it is intercepted to a lesser degree by the fat body before reaching the site of biological action (the nervous system). However, the ingested product must go through the digestive system in the abdomen before reaching the haemolymph and be carried towards the nervous system. During its distribution throughout the body of the organism, the insecticide could be temporarily incorporated in the fat body in the abdomen, which will reduce its toxicity. In this respect, it is worth noting that the differences in the LD_{50}'s between the different populations of the beetles become smaller with increasing exposure time.

The variability in the results of the ingestion toxicity tests means that additional investigations are necessary in order to clarify its cause before we can standardise the methodology of the test.

In the ingestion tests as in the tropical ones *Trachyderma hispida* is clearly less susceptible to fenitrothion than *Pimelia senegalensis*.

The topical LD_{50}'s of fenitrothion for *Pimelia senegalensis* are comparable to those found for the desert locust (*Schistocerca gregaria*). MacCuaig (1983) mentions median lethal doses varying from 5.9 to 7.8 µg a.i./g insect for the fifth instar larvae of *S. gregaria*, and from 3.7 to 8.4 µg /g for the adult. Consequently, supposing a similar degree of exposure, we can expect that the recommended dose against the desert locust will have harmful effects on this group of non-target organisms.

To assess the risk of intoxication by ingestion, we have made a comparison with the fenitrothion residues found after locust and grasshopper treatments. Figure 1 shows the LD_{50}'s for *P. senegalensis* and *T. hispida* in relation to the residues measured by Gadji (1993) on vegetation after treatments in an arid savanna in the north of Senegal. The hatched area in the figure represents the range of residue levels measured. All the tenebrionid populations with LD_{50}'s below this hatched zone are likely exposed to residues in the field which can cause high mortality. However, populations with LD_{50}'s just above the hatched area can also be subject to mortalities of the insecticide, since the LD_{50}'s are calculated assuming exposure to a constant level of insecticide. In nature, however, residues decrease from a maximum reached just after the treatment.. Consequently, insects with LD_{50} above the hatched area in figure 1, at for instance 96 hours of exposure, were exposed to higher residues 24 or 48 hours after the treatment. If we would want to take into account such dynamics a more detailed modelling of the behaviour of the insecticide and the insects would be required. Such models are available (Goodman 1982, Salt and Ford 1984, Wiles and Jepson, in press). Additional data concerning the fate of the insecticide and the biology and the behaviour of tenebrionids are presently being collected by the LOCUSTOX Project.

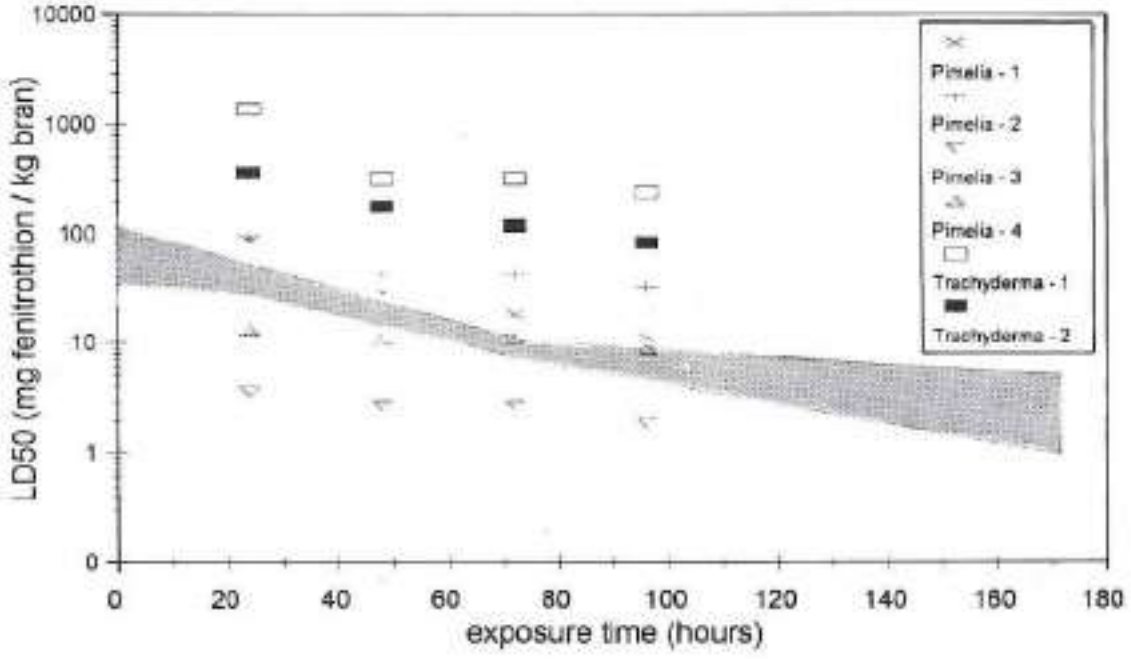


Figure 6.1: Median lethal doses (LD_{50} ; mg a.i./kg of millet bran) for different times of exposure to fenitrothion by ingestion for *Pimelia senegalensis* (4 series) and *Trachyderma hispida* (2 series). The vertical lines are the 95% confidence limits (the slight horizontal shifting between the series of the same exposure time is to allow for easier reading). The hatched area represents fenitrothion residue levels found on *Boscia senegalensis*, a small shrub, in the arid savannah in the north of Senegal at various moments after treatments with doses varying from 440 to 980 g a.i./ha (residue levels according to Gadji 1993).

We hope to be able to implement this type of modelling for the evaluation of ecotoxicological risks in the near future.

The rough comparison between LD_{50} and the residue levels carried out above suggests, however, that *P. senegalensis* is at high risk. It can be affected after a treatment with fenitrothion, which is unlikely for *Trachyderma hispida*. This difference shows again that representatives of the same taxon, which are also ecologically similar, do not necessarily have a comparable susceptibility to an insecticide.

We suggest that similar experimentations be carried out with other insecticides used in locust and grasshopper control, in order to answer some of the questions raised above, especially the impact that the capture period and the physiological state of the insect may have on the toxicity of the insecticide.

REFERENCES

Abushama F.T. (1984) Epigeal insects. pp. 129-144 in: Sahara desert. Cloudsley-Thompson, J.L. (ed). Key Environments Series. IUCN. Pergamon Press, Oxford, pp.348.

Abushama F.T. and Abdallah F.H. (1986) The effect of starvation on the utilisation of food reserves in desert beetles. Journal of Arid Environments 10:187-191

Abushama F.T. and Al-Salameen M.A. (1989) Temperature reactions of desert tenebrionid beetles from Kuwait. Journal of Arid Environments 16:293-304

Ayyad M.A. and Ghabbour I. (1986) Hot deserts of Egypt and the Sudan. pp. 149-202 in: Evenari, M. et al. (eds.) Hot deserts and arid shrublands. Ecosystems of the World, part 12B. Elsevier, Amsterdam. pp.451

Bukema A.L., Niederlehner B.R. and Cairns J. (1982) Biological monitoring - Part IV: Toxicity testing. Water Research 16:239-262

Crawford C.S. (1991a) Animal adaptations and ecological processes in desert dunefields. Journal of Arid Environments 21:245-260

Crawford C.S. (1991b) The community ecology of macroarthropod detrivores. pp. 89-112 in: Polis, G.A. (ed) The ecology of desert communities. The University of Arizona Press, Tuscon. pp.456

Gadji B. (1993) Déposition et dégradation du fénitrithion et du diflubenzuron sur végétation et dans les mares temporaires en milieu sahélien (campagne 1991). Rapport Project Locustox 93/4. Organisation des Nations-Unies pour l'Alimentation et l'Agriculture (FAO), Dakar, pp. 35

Geyer H.J., Scheunert I., Rapp K., Gebefügi I., Steinberg C. and Kettrup A. (1993) The relevance of fat content in toxicity of lipophilic chemicals to terrestrial animals with special reference to dieldrin and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Ecotoxicology and Environmental Safety 26:45-60

Gillon Y. (1983) The invertebrates of the grass layer. pp.289-311 in: Bourlière F. (ed.) Tropical Savannas. Ecosystems of the World, part 13. Elsevier, Amsterdam. pp.730

Goodman E.D. (1982) Modeling effects of pesticides on populations of soil/litter invertebrates in an orchard ecosystem. Environmental Toxicology and Chemistry 1:45-60

Kooijman S.A.L. (1980) Parameter analysis of mortality rates in bioassays. Water Research 15:107-119

Leeper J.R., Raffa, K.F. and Bruhn J.A. (1988) Analysis of mortality characterization in developing LD values from topical application test results. Tropical Pest Management 34(4):404-406

Louw G. and Seely M. (1982) Ecology of desert organisms. Longman Scientific and Technical, Harlow. pp.194

MacCuaig R.D. (1983) Insecticide index (2nd edition) - An index giving details of tests made to assess the usefulness of particular insecticides for control operations against locusts. Food and Agriculture Organization of the United Nations (FAO), Rome, pp.191

Niassy A., Beye A. and van der Valk H. (1993) Impact of fenitrothion applications on natural mortality of grasshopper eggpods in Senegal (1991 treatments). FAO/Locustox report No.93/1. Food and Agriculture Organization of the United Nations, Dakar. pp.17

Popov G.B. (1980) Studies on oviposition, egg development and mortality in Oedaleus senegalensis (Krauss)(Orthoptera, Acridoidea) in the Sahel. Centre for Overseas Pest Research, London, pp.48

Salt D.W. and Ford M.G. (1984) The kinetics of insecticide action. Part III: the use of stochastic modelling to investigate the pick-up of insecticides from ULV-treated surfaces by larvae of Spodoptera littoralis Boisd. Pesticide Science 15:382-410

Seely M. (1991) Sand dune communities. pp. 348-382 in: Polis, G.A. (ed) The ecology of desert 8 communities. The University of Arizona Press, Tuscon. pp.456

van der Valk H.C.H.G. (1990) Beneficial arthropods. pp. 171-224 in: Everts, J.W. (ed.) Environmental effects of chemical locust and grasshopper control - A pilot study. Project report ECLO/SEN/003/NET. Food and Agriculture Organization of the United Nations, Rome, pp.277

Wiles J.A. and Jepson P.C. (in press) An index of the intrinsic susceptibility on non-target invertebrates to residual deposits of pesticides.

ANNEX 6.1

Temperature and relative humidity during the toxicity tests at the entomology laboratory of the Crop Protection Directorate in Dakar, Senegal.

DATE	TEMPERATURE (°C) min-max	RELATIVE HUMIDITY (%) min-max
15-18/12/1992	25 - 26	64 - 74
07-12/01/1993	23 - 24	36 - 55
13-19/01/1993	24 - 26	50 - 64
22-24/01/1993	24 - 25	60 - 72
15-17/09/1993	28 - 30	60 - 72

CHAPTER 7 :

Toxicity tests with *Metarhizium flavoviride* (Deuteromycetes, Moniliales) on *Bracon hebetor* (Hymenoptera, Braconidae), *Pimelia senegalensis* and *Trachyderma hispida* (Coleoptera, Tenebrionidae).

SUMMARY

Metarhizium flavoviride is an entomopathogenic fungus which attacks primarily locusts. Its use as a myco-insecticide is currently being developed with a view to reducing the widespread use of synthetic insecticides in locust control. Although in nature it is found almost exclusively on Orthoptera, the specificity of action of this fungus is little down. Thus some toxicity trials with *Metharhizium flavoviride* (strain IMI 331089) were carried out on two Tenebrionidae (*Pimelia senegalensis* and *Trachyderma hispida*) and one Braconidae (*Bracon hebetor*). Tenebrionds are insects that attack the eggpods of locusts; they are also important agents in the degradation of organic matter in dry ecosystems. The Braconid is a natural enemy of several Sahelian crop pests.

Among the three species, only *B. hebetor* showed mortality in the insects treated, which could be attributed to the effect of *M. flavoviride*. This was confirmed with the percentage of sporulation observec on the treated insects. On the other hand, none of three modes of exposure enabled us to show the effect of the product on the two species of Tenebrionidae. Even with the ingestion of the dead *Zonocerus variegatus* treated with M. flavoviride, it was not possible for us to show an effect on the product on *P. senegalensis*. It appears seem that the two tenebrionids are not sensitive to *M. flavoviride* strain IMI 3311089.

INTRODUCTION

The partial destruction of the natural beneficial organisms of crops, predators, parasitoïds, or entomopathogenic agents, as a result of phytosanitary treatments, has often had the consequence of introducing imbalances, not to mention the increase in the populations of the target pests or changes in dominance. To counter this, integrated pest management seems to be the only judicious way. Thus, the establishment of integrated protection strategies includes the study of the toxicity of pesticides on beneficials.

The product studied here is *Metarhizium flavoriride* W. Gams & J. Rozsypal (Deuteromycetes, Moniliales), a pathogenic fungus of insects. This product, used as an insecticide in the form of ULV spray had been developed by Chris Prior and his colleagues at IIBC (International Institute of Biological Control) in Silwood Park, England (Prior et al. 1992). *M. flavoviride* is a fungus which grows only on insects and it appears fairly specific to locusts and grasshoppers. When the spore of the fungus attacks the insect, it germinates by sprouting upwards to reach tubes of 20 µm long and 1 to 2 µm thick in the cuticle. The end of the tubes blows up and using the combination of enzymatic digestion and brute force, the fungus penetrates the cuticle. It then forces its way into the insect, breaking into little fragments, which disperse in the insect's circulatory system. The pathogen kills the locust by blocking the circulatory system as soon as the insect is full of fungi, even though toxins ("destruxins") can also play a role in the process (Charnley 1992). There are several strains of *M. flavoviride* and it is the IMI 331089 strain which was tested here on two Tenebrionidae and one Braconidae.

The methodology described here concerns the laboratory test. Field experimentation, which is generally costly and laborious is implemented only for compounds which proved to be toxic during rigorous laboratory test (Sauphanor *et al.* 1992). The species tested are *Pimelia senegalensis, Trachyderma hispida* Forskal and *Bracon hebetor* Say. The first two species belonging the Tenebrionidae family, are of great importance in arid and semi-arid ecosystems (Crawford 1991). The larvae of these tenebrionids (for instance *P. senegalensis*) attack the eggpods of grasshoppers or locust in the Sahel. (Popov 1980). The adults are important detritivores. The last species, *B. hebetor*, is a hymenopteran belonging to the Braconidae family. The species is cosmopolitan and is present in the entire West African subregion. (Diémé 1985). It is a parasitoïd which has caught the attention of researchers due to its efficacy and its easy rearing (Bal 1993). Bhatnagar (1987) advocated the use of *B. hebetor* for the control of *Heliocheilus albipunctella* De Joanis (Lepidoptera, Noctuidae), the millet headminer.

STUDY METHODOLOGY

The insects

The effect of *Metarhizium flavoriride* was studied on two Tenebrionidae (*Pimelia senegalensis* and *Trachyderma hispida*) and one Braconidae (*Bracon hebetor*). The tenebrionids were collected during two periods: in July 1993 in Linguère (15° 23'N - 15° 08'W) in Northern Senegal ; and in May 1994 in Kaffrine (14° 05'N - 15° 35'W) in central Senegal. *Bracon* was taken from those reared in the laboratory at Nioro du Rip (13° 45'N - 15° - 46'W).

In addition, three locust species were used as reference controls bearing in mind the fact that *M. flavoviride* has an effect on them. These are *Kraussaria angulifera* (Krauss) (Ortheoptera, Acrididae), *Schistocerca gregaria* (Forskal) (Orthoptera, Acrididae) and *Zonocerus variegatus* (Linné) (Orthoptera, Pyrgomorphidae). The first species (KAN) was collected in the form of eggpods at the same time and in the same area as the tenebrionids. The second (SGR) was collected as adults at Saly (14° 26'N - 17° 01'W) in the coastal area of Senegal in January 1994. The third (ZVA) was collected in larval form at Kédougou (12° 31'N - 12° 10'W) in South-Eastern Senegal. All the insects, except *Bracon hebetor*, were maintained at the insectarium of the Plant Protection Directorate in Dakar up to the time of their use in the tests. *B. hebetor* was transported each time in the form of cocoons from its rearing colony to Dakar where the emergence of the adults took place.

Insecticide

The insecticide tested is *Metarhizium flavoriride*, strain IMI 331089, isolated originally from *Omithacris furbida cavroisi* in Niger. Its multiplication was carried out at IITA (the International Institute of Tropical Agriculture) in Cotonou, Benin. The insecticide is in the form of spores soluble in oil. The currently recommended dose is 100 g of spores dissolved in 2 litres of a mixture of kerosene (70 %) and groundnut oil (30 %) to treat one hectare. One hundred grams contains about 5×10^{12} spores.

Treatment and exposure methods

Solutions of *M. flavoriride* in kerosene: groundnut oil (70: 30) were prepared several tims for the tests at a concentration of 2.5×10^{12} spores per litre. The solutions were stored at 5°C between the tests but for not more than 12 days. In all cases, the controls were treated with the formulation without spores.

Applications were made according to three modes of exposure.

Topical exposure

The application of solutions was done with a manual micro-applicator (Burkhard®), equipped with a micro-syringe 25 whose droplet size was calibrated. A volume of 1 µl was adopted as the size of droplet to be applied to the tenebrionids and locusts and 0.25 µl on the bracons. The droplet was deposited at the juction between the thorax and the abdomen for the tenebrionids and under the pronotum for the locusts. No form of anaesthesia was used during the treatment of coleoptera or locusts. The bracon were stored in a refrigerator (5°C) a few minutes before treatment in order to reduce their mobility.

It is worth noting that 0.25 or 1 µl or the applied formulation presents a high overdosage in relation to the doses that insects could receive by direct contact during field treatment of the dose currently recommended.

For the tests with tenebrionids, ten insects were treated and placed in a plastic container (30 cm diameter x 12 cm high), covered with a cardboard. The tenebrionids were fed with millet bran during the test. For the locusts also, ten individuals were treated and placed in breeding cages (25 x 25 x 50 cm, made of wire gauze). They were fed with millet or mango leaves during the period of the tests. As for *Bracon*, twelve insects were treated and put in plastic bottles of 5.5 cm diameter and 6.5 height. The caps of the bottles were perforated in the centre and a piece of mosquito netting was glued over the hole to allow aeration. A cotton "eartip" soaked in sugar water served as food.

Residual exposure

Application of the solutions was carried out by means of three methods :

- with the help of the micro-applicator 1 µl droplets were deposited in 1 cm^2 squares drawn on a piece of blotting paper, cut into pieces of 6 x 18 cm

- with a Micro-ULVA® battery driven spayer, 1 hectare of fallow land was treated at the recommended dose, within which blotting papers (42 x 51 cm) were placed. These blotting papers were then cut into pieces of 6 x 18 cm for the locust and *Bracon* tests while 10 cm diameter blotting papers were used for the tests with *Pimelia*.

- with a laboratory spray tower (Burkhard® type Potter Tower), 6 x 18 cm plastic overhead sheets or Canson millimetre paper were treated. The spraying pressure was 3lb/m^2. The equipment was calibrated in order to measure the formulation losses in the Tower, about 80 % of the volume sprayed. A volume of 250 µl was sprayed each time, being drawn from a solution prepared at a dose of 6.7 g of spores/litre (= 3.35 x 10^{11} spores/l). The dose per surface area thus obtained corresponds to the recommended dose of 5 x 10^{12} spores per hectare. After drying at room temperature for one hour in the case of the millimetre and blotting papers, and two hours in the case of the plastic overhead sheets, the tested surfaces were placed in the bottles and the insects introduced.

The sheets were placed with the treated side facing inwards in the bottles. *Pimelia* were exposed in plastic containers of 10 cm diameter and 11.5 cm high. The blotting papers treated in the field were cut and placed at the bottom of the containers. There were two containers per treatment (treated and control) and five *Pimelia* per container. The locusts were arranged individually in containers of 8 cm high, whose opening is 6.5 cm in diameter while the base is 5 cm. There were ten containers per treatment. Twenty bottles were used per treatment for the ZVAs. Identical bottles were used in residual exposure tests for *Bracon*. About fifty individuals were exposed for each treatment or control.

Exposure by ingestion

This exposure method was used exclusively for *P. senegalensis* . The aim was to study the effect of toxins (destruxins) which could be produced by *M. flavoviride* after infection of the locust. Even if the entomopathogen does not succeed in infecting certain non-target insects directly, the consumption of infected locusts could, in the case of toxin being produced, affect scavengers.

Dead *Zonocerus variegatus* (ZVA), tested with *M. flavoviride* were given as food to *Pimelia*, 24 hours after the locust's mortality had been observed. For the ingestion test, 10 or 15 *Pimelia* were placed with the carcasses of ZVA placed in plastic containers.

Experimental conditions

The experiments were set up on a bench in the laboratory under ambient conditions :

- temperature between 21 and 25°C
- relative humidity between 60 and 70 %.

Observations

Mortality

The insects were exposed for one to two weeks. Mortality was assessed every 24 hours throughout the entire exposure period. It is evaluated in terms of all the treatments carried out during the period. The experiment ends as soon as total mortality among the individuals treated with *M. flavoviride* is obtained, or after an exposure period of maximum fifteen days.

Sporulation

Following death, the carcasses are incubated in peter dishes to obtain sporulation of *M. flavoriride* on the treated insects. A filter paper moistened with distilled water is placed at the bottom of the dish on which the insects are arranged, and then the dishes are closed with parafilm. For the tenebrionids and the locusts, only one insect was incubated per dish. As for *Bracon*, 5 to 10 individuals are incubated per dish.

RESULTS

The results of the observations are presented in Table 7.1 and 7.2 and the raw data of the texts in Annexes 7.1 to 7.5. The overall effect of the treatment was evaluated as the number of survivors and the percentage of sporulation of incubated dead insects.

Only the first evaluation criterion was used for the tenebrionids, given the low mortality rate and the non-sporulation of the incubated carcasses. No infection of the two tenebrionids exposed *M. flavoviride* (strain IMI 331089) could be demonstrated. A very low mortality was observed in all tests (Table 7.1). A secondary effect caused by the production of toxins could not be demonstrated either. This suggests that the product had no effect on these two species. Besides, no sporulation could be obtained on the few carcasses of the treated individuals.

On the other hand, *Bracon* seemed to have suffered the effect of the fungus: a virtually total mortality was observed in the individuals treated after a maximum exposure of 8 days (Table 7.2). The high percentage of sporulation on the carcasses after incubation seems to confirm this (Annex 7.4). Only the results of residual exposure are evaluated, since topical treatment is not adapted to the size of the insect. The droplets generated by the available equipment were too large, thus given rise to too high control mortalities. The mortality of *Bracon* in the control experiments is fairly high, reflecting probably a secondary effect of the formulation. This is supported by the fact that sporulation has never been observed for these controls.

The effects of *M. flavoviride* strain IMI 331089 on the three species of locusts used as reference controls was confirmed. Total mortality was observed in treated individuals after maximum exposure of 8 days (Annexe 7.2). Sporulation of incubated carcasses from 60 to 100 % was obtained (Annex 7.5).

Table 7.2 : Effect of *Metarhizium flavoviride* on *Bracon bebetor*.

Series	Mode of exposure (residual)	Duration of test (days)	Mortality (%)		Sporulation (%)	
			Controls	Treated	Controls	Treated
Test 1 (11.02.94)	Filter paper treated with micro-applicator	4	52	84	0	0
Test 2 (15.02.94)	Filter paper treated with Micro-ULVA	8	48	100	0	32
Test 3 (08.07.94)	Plastic treated with POTTER of Tower	5	69	100	0	100
Test 4 (12.07.94)	Millimetre paper treated with POTTER Tower	6	50	100	0	84
Test 5 (14.07.94)	Plastic treated with POTTER Tower	5	74	100	0	78
Test 6 (15.07.94)	Millimetre paper treated with POTTER Tower	5	78	100	0	88

Table 7.1: Effect of *Metarhizium flavoviride* on two tenebrionids: *Pimelia senegalensis* and *Trachyderma hispida* (non-targets), together with locusts as reference controls (targets).

Series	Mode of exhibition	Duration of test (days)		Mortality (%)				
				Non-targets (Tenebrionids)		Targets (locusts)		
		Non-target	Targets	Controls	Treated	Controls	Treated	Species
P. senegalensis Test 1 (17.01.94)	topical (micro-applicator)	15	11	20	20	10	100	KAN[1]
Test 2 (19.01.94)	"	12	7	10	20	0	100	SGR
Test 3 (20.01.94)	"	11	8	20	20	10	100	SGR
Test 5 (26.02.94)	"	9	-	0	10	-	-	-
Test 4 (15.02.94)	residual (Micro-ULVA)	10	5	0	10	20	60	SGR
Test 7 (19.06.94)	ingestion (ZVA treated micro-applicator)	15	-	0	0	-	-	-
Test 8 (13.7.94	"	15	-	0	0	-	-	-
T. hispida Test 6 (16.09.94)	topical (micro-applicator)	13	12	0	0	0	100	ZVA

[1]KAN: *Kraussaria angulifera*; SGR = *Schistocerca gregaria*, ZVA = *Zonocerus variegatus*; no = no locust used as reference

DISCUSSION AND CONCLUSION

Three exposure methods were used for the application of myco-pesticide solutions. The results indicate that the method of treatment with the Potter Tower, for the residual exposure, seems to be the most effective, especially for small insects. The use of glossy millimetre paper as a support allows for rapid drying of the formulation as well as a high transfer of spores to the insects. With large insects, topical treatment with a Micro-applicator gives also good results.

None of the three modes of exposure shows an effect of the product on *P. senegalensis* and *T. hispida*, two coleopteras of the same size. Even in the tests by ingestion, *Pimelia* seems not to be susceptible. Therefore, it may be that there are no toxic toxins produced by this strain. However, it is worth noting that the acute toxicity of destruxins may vary according to the taxonomic group. Diptera and Lepidoptera being very susceptible (James *et al.* 1993).

B. hebetor showed a high mortality after treatment which seems to be attributable to the effect of *Metharhizium*. This is supported of sporulation observed. Nevertheless, we used only one strain of *M. flavoviride*, and therefore this would not mean that the other strains would give the same results.

At present, there is very little information published concerning the effect of *M. flavoviride* on non-target arthropods (Goettel *et al.* 1990). In a laboratory test with the honey bee (*Apis mellifera*), a mortality of 29 % was observed after a treatment with twice the field dose. This was confirmed by the sporulation observed on the carcasses (Lomer *et al.* 1993). Some tests on Coreida (Hemiptera), Formicidae (Hymenoptera) and larvae of Scarabaeidae (Coleoptera) did not show their infection by the entomopathogen (Lubilosa 1994).

It may be that Hymenoptera are relatively sensitive to *Metarhizium flavoriride*, and tests on this taxon shoud be continued in order to better determine the specificity of the fungus. Some field bio-assays should also be carried out in order to evaluate the real risk of these Hymenoptera getting infected in more natural conditions of exposure, since the laboratory tests have relatively high exposure levels.

REFERENCES

Bal AB (1993) Etude du parasitisme naturel d'*Heliocheilus albipunctella* De Jannis (Lepidoptère: Noctuidae) par *Trichogrammatoidea* sp. (Hymenoptère : Trichogrammatidae) à Bambey. Insect Sci. Applic. 14(2) : 221 - 223.

Bhatnagar VS (1987) Recherche et développement de la lutte intégrée contre les ennemis des principales cultures vivrières dans les pays du Sahel. Sénégal. Programme de lutte biologique. Rapport Technique final GCP/RAF/128/CLS, FAO, Rome, pp. 168.

Charnley AK (1992) Mechanisms of fungal pathogenesis in insects with particular reference to locustx. p. 181-190 in: Lomer CJ and Prior C (eds). Biological control of locusts and grasshoppers. Proceedings of a workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April - 1 May 1991. CAB International, Wallingford, pp. 394.

Crawford CS (1991) Animal adaptation and ecological process in desert dunefields. Journal of Arid Environments 21: 245-260.

Diémé E (1985) Aperçu sur la biologie de *Bracon hebetor* Say (Hyménoptère, Braconidae) parasite de *Raghuva albipunctella* Joanis et *Ephestia kuehniella* Zell. Communication présentée à la Réunion Technique Annuelle des Groupes de Travail, Projet CILSS de Lutte Intégrée. Ouagadougou 25 Fév. - 1 Mars.

Goettel MS, Poprawski TJ, Vanderberg JD, Li Z et Roberts DW (1990) Safety on non-target invertebrates to fungal biocontrol agents. Chapter 15. pp. 209-232 in: Laird M et al. (eds) Safety of Microbial Insecticides. CRC Press Inc, Boca Raton FL.

James PJ, Kershaw MJ, Reynolds SE et Charnley AK (1993) Inhibition of desert locust (*Schistocerca gregaria*) malphigian tubule fluid secretion by destruxins, cyclic peptide toxins from the insect pathogenic fungus *Metharizium anisopliae*. J. Insect Physiol. 39(9) 797-804.

Lomer CJ, Shah P, Paraison A, Godonou I, Douro-Kpindou OK, Thomas M, Kooyman C, Moore D, Ball B, Pye B and Langewald J (1993) Ecological studies on grasshoppers and effects of formulations on non-target organisms, pp. 129-132 in: The IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. Final report for phase I.

LUBILOSA (1994) *Metarhizium flavoriride*. Biological insecticide for experimental use. Technical and safety information sheet. Draft 07 March 1994. IIBC/IITA/DFPV Locust and Grasshopper Biological Control Project.

Popov GB (1980) Studies on oviposition, egg development and mortality in *Oedaleus senegalensis* (Krauss) (Orthopera, Acridoidea) in the Sahel. Centre for Overseas Pest Research, London, pp. 48.

Prior C, Lome CJ, Herren H, Paraiso A, Kooyman C & Smit JJ (1992) The IIBC/IITA/DFPV collaborative research programme on the biological control of locusts and grasshoppers. p. 8 - 18 in: Lomer CJ and Prior C (eds). Biological control of locusts and grasshoppers. Proceedings of a workshop held at the International Institute of Tropical Agriculture, Cotonou, Republic of Benin, 29 April - 1 May 1991. CAB International, Wallingford, pp. 394.

Sauphanor B, Blarsinger B et Sureau F (1992) Méthode de laboratoire pour évaluer l'effet des pesticides sur *Forficula auricularia* L. (Dermaptera: Forficulidae). Bull. SROP/WPRS 1992/XV/3: Pesticides and Beneficial Organisms: Guidelines (test methods), pp 117 - 121.

ANNEX 7.1 : Effect of *Metarhizium flavoviride* on *Pimelia senegalensis* and *Trachyderma hispida*. Number of survivors in tests by topical application and by ingestion

Series	Equipment	Mode of exhibition	Treatment	Time after exposure (days)																% mortality
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Test 1 (17.01.94)	*P. senegalensis* treated with micro-applicator	Topical	Treated	10	10	10	8	8	8	8	8	8	8	8	8	8	8	7	7	30
			Controls	10	10	10	10	9	9	9	8	8	8	8	8	8	8	8	8	20
Test 2 (19.01.94)	"	"	Treated	10	10	10	10	10	9	9	9	9	9	9	9	8				20
			Controls	0	10	10	10	10	10	10	10	10	10	10	10	9				10
Test 3 (20.01.94)	"	"	Treated	10	10	10	10	10	10	10	10	10	10	10	8					20
			Controls	10	10	10	10	10	9	9	9	8	8	8	8					20
Test 4 (15.02.94)	Filter papers spread on the ground of a field treated with micro-ULVA ; on which are placed *P. senegalensis*	Residual	Treated	10	10	10	10	10	10	10	10	10	10	9						10
			Controls	10	10	10	10	10	10	10	10	10	10	10						0
Test 5 (28.02.94)	*P. senegalensis* treated with micro-applicator	Topical	Treated	10	10	10	10	10	10	10	10	10	10							0
			Controls	10	9	9	9	9	9	9	9	9	9							10
Test 6 (16.06.94)	*T. hispida* treated with micro-applicator	Topical	Treated	10	10	10	10	10	10	10	10	10	10	10	10	10	10			0
			Controls	10	10	10	10	10	10	10	10	10	10	10	10	10	10			0
Test 7 (19.06.94)	*P. senegalensis* on carcasses of ZVA treated (test of 16.06.94 Table IV) and ZVA killed naturally (without treatment)	Ingestion	Treated	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
			Controls	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0
Test 8 (13.07.94)	*P. senegalensis* on carcasses of ZVA treated (test of 6.07.94, Table IV) and ZVA naturally	Ingestion	Treated 1	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0
			Treated 2	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0
			Controls com.	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	0

NB : Time 0 indicates the number of individuals incubated per treatment. The few carcasses of *P. senegalensis* obtained were incubated for observation of sporulation. However, none of the carcasses sporulated. This makes us believe that the dead *Pimelia* were not infected by *M. flavoviride*.

ANNEX 7.2 Effect of *Metarhizium flavoviride* on *Kraussaria angulifera*, *Schistocerca gregaria* and *Zonocerus variegatus*. Number of survivors in tests by topical and residual exposure

Series	Equipment	Mode of exhibition	Treatment	Time after exposure (days)													% mortality
				0	1	2	3	4	5	6	7	8	9	10	11	12	
Test 1 (17.01.94)	*K. angulifera* treated with micro-applicator	Topical	Treated	10	10	10	9	9	9	9	9	8	5	3	0		100
			Controls	10	9	9	9	9	9	9	9	9	9	9	9		10
Test 2 (19.01.94)	*S. gregaria* (SGR) treated with micro-applicator	"	Treated	10	10	8	8	8	5	3	0						100
			Controls	0	10	10	10	10	10	10	10						0
Test 3 (20.01.94)	"	"	Treated	10	10	10	10	9	7	5	5	0					100
			Controls	10	10	10	10	10	10	10	10	9					10
Test 4 (11.02.94)	Filter papers treated with micro-applicator on which SGR is exposed	Residual	Treated	10	10	10	3	3	0								100
			Controls	10	10	10	9	9	9								0
Test 5 (15.02.94)	Filter papers spread on the ground in th field treated with micro-ULVA on which SGR is placed	"	Treated	10	10	10	10	8	8	7	7	5	4				60
			Controls	10	10	10	10	9	9	9	8	8	8				80
Test 6 (16.06.94)	ZVA treated with micro-applicator	Topical	Treated	10	10	10	10	9	5	5	5	3	3	3	2	0	100
			Controls	10	10	10	10	10	10	10	10	10	10	10	10	10	0
Test 7 (17.06.94)	ZVA exposed on plastic and millimetre paper treated with a POTTER Tower	Residual	Treated plastics	20	20	20	18	18	17	9	5	5	5	4	0		100
			Treated millimetre paper	20	20	20	20	20	20	18	15	10	3	0			100
Test 8 (06.07.94)	ZVA treated with micro-applicator	Topical	Treated A	10	10	10	10	10	8	7	7	4	2	0			100
			Treated B	10	10	10	10	10	8	8	6	4	1	0			100
			Treated C	10	10	10	10	9	7	6	4	2	0				100
			Common Controls	10	10	10	10	10	10	10	10	10	10	10			0

NB : Time 0 indicates the number of individuals incubated per treatment. The few carcasses of *P. senegalensis* obtained were incubated for observation of sporulation. However, none of the carcasses sporulated. This makes us believe that the dead *Pimelia* were not infected by *M. flavoviride*.

ANNEX 7.4 : Effect of *Metarhizium flavoviride* on *Bracon hebetor*. Evaluation of the sporulation of incubated carcasses

Series	Treatment	Number			% sporulation	Time to emerge of spores
		Tested	dead and incubated	sporulated		
Test 1 (11.02.94)	Treated	25	21	0	0	-
	Controls	25	13	0	0	-
Test 2 (15.02.94)	Treated	25	25	8	32	2 to 4 days
	Controls	25	16	0	0	-
Test 3 (06.07.94)	Treated	50	50	50	100	3 to 4 days
	Controls	50	40	0	0	-
Test 4 (12.07.94)	Treated	50	50	42	84	4 to 5 days
	Controls	50	25	0	0	-
Test 5 (14.07.94)	Treated	50	50	39	78	3 to 5 days
	Controls	50	37	0	0	-
Test 6 (15.07.94)	Treated	50	50	43	86	3 to 4 days
	Controls	50	39	0	0	-

ANNEX 7.5 Effect of *Metarhizium flavoviride* on *Kraussaria angulifera*, *Schistocerca gregaria* and *Zonocerus variegatus*. Sporulation evaluation of incubated carcasses

Series	Treatment	Number			% sporulation	Time to emerge of spores
		Tested	dead and incubated	sporulated		
Test 1 (17.02.94)	Treated	10	10	6	60	2 to 3 days
	Controls	10	1	0	0	-
Test 2 (19.01.94)	Treated	10	10	8	80	2 to 4 days
	Controls	10	1	0	0	-
Test 3 (20.01.94)	Treated	10	10	7	70	2 to 3 days
	Controls	10	1	0	0	-
Test 4 (11.12.94)	Treated	10	7	0	0	-
	Controls	10	1	0	0	
Test 5 (15.02.94)	Treated	10	8	2	20	2 days
	Controls	10	2	0	0	-
Test 6 (16.06.94)	Treated	10	NI*	-	-	-
	Controls	10				
Test 7 (17.06.94)	Treated (plastic)	20	20	20	100	2 to 4 days
	Treated (paper)	20	20	20	100	2 to 3 days
Test 8 (08.07.94)	Treated A	10				
	Treated B	10	NI*	-	-	-
	Treated C	10				
	Controls	10				

NI* = Not incubated. Carcasses are given to *P. senegalensis* (Test 7 and 8, Table 1).

HAPTER 8

Deposition and degradation of fenitrothion and diflubenzuron on vegetation and in temporary ponds in a sahelian area (1991 Campaign).

SUMMARY

Plots of millet at maturation stage, temporary ponds at Nioro du Rip (Centre of Senegal), and plots of *Boscia senegalensis* at Richard-Toll (North of Senegal) were treated with fenitrothion and diflubenzuron.
Samples were taken from millet leaves, from millet ears, from water of ponds in Nioro du Rip and from leaves of *Boscia senegalensis* in Richard-Toll over a period of one week (fenitrothion) and of two weeks (diflubenzuron). The residues were analyzed.

Subjected to micro-ULVA at simple nominal dose (450 g a.i./ha) the fenitrothion initially settled (one hour after treatment) at 50 to 70 mg a.i./kg of wet millet leaf (mg a.i./kg). On the ears a variable deposit was found of 1 and 6 mg a.i./kg. In the ponds the average found was 80 µg a.i./l. Nominal double doses made on the *Boscia senegalensis* resulted in 100 ppm deposit on average.

At a dose of 50 g a.i./ha on average, the deposition of the diflubenzuronwas 40 mg a.i./kg on millet leaves, less than 20 µg a.i./l in pond water; on leaves of the *Boscia senegalensis* average deposition was 31 mg a.i /kg.

The half-life of fenitrothion on millet leaves rarely reaches 18 h, and varies between 20 and 45 h on *Boscia senegalensis* and can go up to 60 hours in the pond water. The half-life of the diflubenzuron found range from 100 to 150 hours on the millet leaf and generally remain inferior to 50 hours on the *Boscia senegalensis*.

INTRODUCTION

In 1991, the LOCUSTOX Project conducted a experimental campaign in the field which lasted 3 months (August to October). This campaign took place in two different areas whose climate and vegetation are very different.

- Nioro du Rip is a rainy zone with a three-month rainy season; it is an area where plots of millet and temporary ponds had been chosen as study sites.

- Richard Toll represents arid savannah, poor in rainfall and also characterized by a very thin grass carpet; on the plots selected as experimentation sites, the dominant vegetation is constituted by trees with the marked presence of the *Boscia senegalensis*.

The experiment represents one stage in a study with fenitrothion and diflubenzuron, to identify their impact on the environment. After treatment, samples of vegetation (millet or Boscia senegalensis leaves) of millet ears and pond water are taken from the sites, to later serve for the analysis of pesticide residues.

Two main objectives are targeted in this study:

- The determination of the initial rate of deposition which constitutes valuable data in the interpretation of results of ecotoxicological research.

- Research on pesticide persistence in semi-arid areas.

GENERAL ELEMENTS OF THE TREATMENT

Products used during the treatment

Two products were targeted during this first experimental campaign: fenitrothion and diflubenzuron.

Fenitrothion

Fenitrothion is a pesticide of the organophosphorous group. It is sold under the name of Sumithion. The batch number of the one used was 485 3Y11. The IUPAC name is O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate. From its physical and chemical properties, it has the advantage of being a pesticide of limited persistence in time. This reduced persistence is compensated by a rapid mode of action. In fact, fenitrothion acts on contact, through ingestion or inhalation on its targets. Fenitrothion has been for ten years the most used chemical agent in locust control.

Diflubenzuron

Diflubenzuron is a pesticide of the benzoylurea family. The IUPAC name is N(((4-chlorophenyl)amino)carbonyl)-2,6-difluorobenzamid.

Contrary to fenitrothion, it is a product with notable persistance. It is a growth regulator, which means that it attacks the target at larvae stage and deregulates moulting. It is an inhibitor of chitine synthesis which gives it the qualities of a relatively selective pesticide. Diflubenzuron is not in fact very toxic to warm-blooded animals

This characteristic makes it a product which is used more and more in the crop pest control.

Table 8.1 : Technical data of treatment

Product		Nioro du Rip, Micro-ULVA		Richard-Toll ULVAMAST
		Millet plots	Temporary water	Savannah plots
FENITROTHION	Concentration of formulation (g/l)	500	50	500
	Rate (ml/mn)			
	Width of passes (m)	50	55	410
	Speed of movement (m/s)	11	17.5-18	40
	Frequency of Disk (t/mn)	50	30	115
	Droplet size (µm)	6000-6400	6000-6400	
	Height of sprayer (m)	80-100	80-100	
		1.8-2.0	0.5-1.00	2.5
DIFLUBENZURON	Concentration of Formulation (g/l)	450 g/l diluted with gaz-oil to 60 g/l	450 g/l diluted with gaz-oil to 60 g/l	450 g/l diluted with gaz-oil to 60 g/l
	Rate (ml/mn)	50	50	450-480
	Width of passes (m)	11		40
	speed of movement (m/s)	50	30	115
	Frequency of disk (t/mn)	6000-6400	6000-6400	
	Droplet size (µm)	80-100	80-100	
	Height of sprayer (m)	1.8-2.0	1.82-2.00	2.5

Equipment and technique of treatment have been described in detail in chapters 2, 3 and 4.

The treatment of the two sites of Nioro du Rip in the millet plots and the temporary ponds and that of Richard-Toll on an area dominated by the *Boscia senegalensis* was made in downward direction. To better appreciate the regularity of the treatment oleo-sensitive paper was placed in the field which allowed the counting of the number of droplets of the pulverized formula.
The paper was rectangular and 6 x 1 cm.
In Nioro du Rip it was directly fixed on the millet under the ears 1.5 to 1.75 m tall according to horizontal components facing upward toward the sky or vertical facing the wind. The papers 10 per

site treated were distributed every 5 m on a line passing through the centre and perpendicular to the passes of the treatment .
In Richard-Toll where the vegetation is very virgin and the plots more numerous, fixation was made every 10 m on 25 to 35 cm stakes. Only the vertical element was put in place.

After treatment, the actual dose was calculated according to the volume of pesticide spread and to the size of the site; the papers collected were carefully kept till they were taken to the laboratory where the droplets were counted

Meteorological conditions at the time of tests

The deposition of the spray in a downward direction is closely dependent on meteorological conditions in which the tests are made. The elements defining these conditions concern both the distribution of the product and physical and chemical characteristics.

The orientation and spacing of passes are fixed respectively in terms of the direction and intensity of the wind. A wind with variable intensity during treatment causes a unequal distribution of the product, some areas treated will receive overdoses whereas others will be little or not at all affected.

It is to be noted that too weak a wind demands the interruption of the treatment, whereas wind changes make modification on the placement of passes mandatory. Temperature, relative humidity and light cause volatilisation and photolysis. The first occurs in a hot and dry atmosphere. Part of the pesticide goes into the atmosphere and does not therefore reach the targeted area.The second causes the transformation of the pesticide into other metabolites which can stay inactive and in some cases are more toxic than the base product.

Rain is also a meteorological factor which affects the efficiency of the treatment. The product which has not yet attached itself to the target (vegetation, soil etc...) may be washed away. Thus in any test close observation of these four metereological elements is necessary.

At Nioro du Rip, we noted generally a lack of wind in early morning or if any, it is: 3 to 10 km/h only. The temperatures were moderate because of regular rainfalls: 25-27 °C in the morning and 33 °C in the afternoon.

At Richard Toll, there was also lack of wind very early in the morning, but the winds remain generally strong once they start : 3 to 15 km / h. Temperatures too were important 25 to 27°C at 7 h; they can rapidly go up to 37 °C before midday. Afternoons were much warmer and dryer, intensified by total absence of rain during the tests.
Table 8.2 contains the metereological data at the time of the tests.

SAMPLING

If there is a determining stage in control and research analysis of residues, it is without question sampling. It determines the coherence of the results obtained after analysis and thus is of high importance for the interpretation of the latter. Sampling has been defined (Kratochvil and Peack,1989) as the picking up of a certain quantity of elements the analysis of which will reveal the characteristics of the whole which has generated them. The quantity taken should contain all the intrinsic properties of the entity to be studied ; in other words, the sample should be representative of that entity. This concept of representativity can create difficulty with the sampling.
In fact the milieu under study can be heterogeneous in many ways:

- Heterogeneity in the distribution of the pesticide used
- Heterogeneity in the spatial distribution on / of the target

Sampling technique used

The technique used was the one recommended by FAO (1986). This method in its strategies makes it clear that the taking of the global sample is done on many points along a straight line which can cross the area on all its various properties; this line is without doubt the diagonal line in rectangular plots where treatment passes are parallel to one of the dimensions. In the case of non-rectangular areas, circular ones among others , the placement of the treatment passes which is dependent on the wind , also indicates the position of that sampling line which should cross areas obliquely. Further- more , it was decided not to include in the sampling area, the area of the site within ten or twenty metres from the limits.
This, to avoid the influence of perepheral effects which can, for various reasons, have characteristics very different from the rest of the site.

Sequence of sample taking

To establish the degradation curve of the fenitrothion, the sequence of sampling adopted, taking into account the data from the literature of the remanance of the product, is the following:

- 1 hour after treatment
- 24 hours after treatment
- 48 or 72 hours after treatment
- 1 week after treatment.

As to the diflubenzuron which has longer persistence, to these four samples a fifth was added which took place 14 days after treatment.

The sampling technique

Sampling from Nioro du Rip plots

Millet plots were characterized by a very homogeneous vegetation with plants on parallel straight lines with 0.5 to 1 meter spacing. The plants on the same line had the same distance from each other. During the tests which took place at the flowering-maturation stage of the millet, the soil was covered at more than 90 %.
The sample constituted of millet leaf and millet ear.
The sampling was made on two diagonal lines from one to two leaves per plant up to 1.5 to 2 kilogrammes on the whole plot. As to the millet ears which were heavier, the samplings were made on the same line but at larger intervals farther (the distance varying from 5 to 10 metres).

Sampling from Richard-Toll plots

The plots in Richard-Toll are dominated by *Boscia senegalensis*. This is a low shrub (height varying between 1.5 to 2.5 metres) with a dense set of branches with a lot of leaves, giving it the form of a globe. The leaves received the product on one side only determined by the direction of the wind during the treatment. All trees in the sprayed plots were sampled. Leaves were taken from the exposed side of the trees. The quantity of leaves taken from the trees of a plot depends on their

number and varies proportionnally between 1.5 and 2 kilogrammes.

Sampling of temporary ponds

Temporary ponds are sites that are low areas which collect rain and ground water. They are more or less circular, varying between 0.3 and 1 ha. Each one is rarely 1 meter deep at its center. The sampling was made on two diagonals crossing obliquely the lines of passes in 5 to 9 points according to the size of the pond. The samples are taken from the surface of the water and were strongly stirred. Two litres were taken per pond.

Preparation of the sample

The preparation of the sample, consist of two important stages: the reduction of the initial amount of the homogenized sample to a more manageable or reasonable one to the transportation and conditionning of the sample. Neither of these two phases should affect the representativity of the final sample in relation to the plot (leaves or ears) or to the pond (water).

Sample with fenitrothion

Vegetation

The millet leaves and the leaves of *Boscia senegalensis*, as well as the millet ears were cut up into small pieces with scissors, in a wooden box. The contents of the box are mixed in order to homogenize it : 500 grammes of leaves (or ears) were weighed and wrapped in aluminum foil . The sample thus obtained was kept in a cooler with chunks of ice and taken to the laboratory. Conservation before the analysis was at 18 °C in a freezer.

The water

The water taken from ponds were initially kept in a bucket where it was well shaken with a stick. 0.5 liter of that water was then put into a glass flask.
A few drops of diluted chlorhydric acid were added to the contents of the flask to lower the initial pH to 2.0-2.5. (In less acid or basic milieu fenitrothion is likely to degrade). The flask was then closed and wrapped in aluminum foil. It was kept in a cooler for transportation and placed at + 4°C in the freezer of the laboratory until analysis.

Samples with diflubenzuron

The leaves

The same cutting and homogenizing was done, but the 500 gramme-quantity was put in an envelope where it was dried in air for several days in the laboratory (diflubenzuron is rather sensitive to humidity). The sample thus obtained was kept in a cupboard until analysis.

The water

The sample of pond water was prepared in the same manner as for fenitrothion. In this case, however, more water was stored (1 l per pond).

ANALYSIS OF THE SAMPLES

The following activities were carried out:

1. Analysis of fenitrothion residues at the LOCUSTOX laboratory at Dakar.

2. Extraction of the diflubenzuron samples, which we analyzed at the residue laboratory of GTZ in Darmstadt/Germany.

Materials and methods of dosage

Fenitrothion

Fenitrothion, which is an organophosphored was analyzed by chromatography in gazeous phase (GCP). The equipment used was Delsi 200 equipped with a photometric flame detector. The column was stainless, two (2) metres long, filled with a fixed phase consisting of chromasorb (WAW HMDS 80-100 mesh 5% OV 17). At 250 °C temperature of the oven and 25 ml / mn flow of gas vector (U nitrogen), fenitrothion has a retention time of about three and half minutes (3.5 mn).
The extraction and concentration of the product from the vegetation were done according to the process proposed by Sumitomo (1981). The sample is defrosted by exposition to the environmental temperature of the laboratory and 50 g are taken and put in the recipient of a waring blendor. 50 ml of water and 25 ml of methanol are added to the contents before the grinding. To the ground product thus obtained are put 100 ml of acetonitril; then it is again vigorously shaken for harmonization.
After 5 mn of decantation, the contents of the recipient are vacum filtred on a bed of 20 g of hyflosupercel. The rest of the cake was again ground in 80 ml of the ternary mixture of methanol acetonitril (2 : 1 : 4) then filtered. To the combined filtered product which appeared only dark green (strong presence of chlorophyll) were added 30 mg of sodium chloride then 150 ml of chloroform in a separating funnel. After 5 mn of shaking and 3 mn of decantation, the heavy phase was extracted and aqueous phase was again washed with 100 other ml of chloroformic. The two organic extracts put together in a 500 ml round bottom flask were dry evaporated. The flask was washed with acetone and the residues of evaporation were finally collected in 25 ml of this solvent to be read at chromatography.
The extraction of the fenitrothion in water ponds was also done according to Sumitomo's analysis protocol (1981). Twenty (20) ml of the sample were taken then mixed with 5ml of a chlorhydric acid solution 1N in a decanter flask. To this, were added 30 ml of dichloromethan and 20 ml of 5% sodium chloride solution. After shaking and separation of the two phases, the heavy phase was withdrawn then filtered directly on sodium sulfate anhydre. The aqueous phase was washed with 30 other ml of dichloromethane which was collected on the same bed of sulfate. The filtered product was dry evaporated with a revolving evaporation and the residues were dissolved in 10 ml of acetone. It has to be noted that the other two modes of operation used here allow each one purification phase, which we went without, because of the use of a flame photometric detector.

Diflubenzuron

Diflubenzuron is detected by high performance liquid chromatography (HPLC) in UV at 254 nm wave-length. The zorbax colum was a C8 (7-8 microns) 25 cm long and 4.6 mm inner diameter. With an elution rate of acetonitril water mixture 55 - 45, the average retention time of diflubenzuron is 3mn. The purified extracts of diflubenzuron from millet plant or pond water prepared on the basis of protocol provided by Duphar (1988a, 1988b) which entails two phases : the extraction phase and the purification phase.

Extraction phase

From the vegetation, the dried sample was ground in an IKA grinder and a sample of about 12 g was done directly into a cartridge of a Soxhlet extractor. The extraction by draining was done by a backflow of 30 to 40 mn using acetonitril : water 85; 15 of 200 ml.
After cooling, the extract was filtered through glass wool and a test intake of 25 to 50 ml (according to the level of contamination of the sample) was evaporated to 3 ml and then transferred to decanter flask previously containing 100 ml of water and 25 ml of 10 % Nacl solution. The evaporation flask

was then rinced with 50 ml of hexan which was transferred to the flask. After shaking and separation of the phases, the aqueous part was washed twice consecutively with 50 other ml of hexane. To the organic phases (hexane) collected were added 5 to 10 ml of absolute ethanol for dry evaporation. The residues of the flask are dissolved again in 30 ml of acetonitril and washed twice in the decanter flask with 50 ml of hexan. The acetonitril solution was dry-evaporated and the solute was taken in 3 ml of dichlorometane (solution A).

The purification phase

In a low pressure column with 20 cm (11.7 g) of florysil previously washed with 100 ml gas ether, solution A was combined with 25 ml of the same solvant then washed successively with :

45 ml petroleum benzin
3 x 10 ml of mixture 1 (1: 9 acetone-petroleum benzin)
10 ml of mixture 2 (1: 4 acetone-petroleum benzin)

before being diluted with 50 ml of mixture 2. The eluted product thus obtained was evaporated and the residues were collected with successively 2 ml of dioxane and 18 ml of 1: 1 acetonitril water mixture used for rincing the flask.

For the pond water it was suggested to use an octadecyl C18 column (J.T Baker Brand) for extraction and purification. The 3 cm^3 column was washed successively with 5 ml of acetonitrite, of methanol and water, then with half a liter of sample processed by a Baker extraction system. This operation of the fixation of the product on the column can last from 30 mn to 1 h according to the state of turbidity of the water. After fixation, the column was washed with 35 ml of a 30 % acetonitril in water, then the diflubenzuron was diluted with 2 ml of acetonitril. To the eluted product was added 5 ml of ethanol 100 % and evaporation was done . The residues were collected in 1 ml of 10-45-45 dioxane, acetonitril, water mixture to be read with HPLC.

RESULTS

The treatment

Table 8.2 presents the result of the droplet count and the doses actually applied. This study with oleo-sensitive paper is not possible with diflubenzuron whose formulation produces patches invisible to the naked eye. The examination of the figures in table 8.3 or of the histogrammes in figure 1 shows the irregular deposition of the product: the typical gaps based on ten elements are identical to the average size. At Nioro du Rip, these typical gaps represented in relation to the average between 39 % on plot 11 and 108 % on plot 04 in vertical component confirmed the tendency by giving more significant figures ranging from 45 % on plot 11 to 122 % on plot 01 to 04.

At Richard-Toll the same observation was made despite the large difference between the two sites. The relationship gap / everage type was superior to 27 % in all cases but remained moderate on plots treated to a double dose (< 79 %). On the plots treated to a simple dose, it was twice as high as 100 % (141 % on plots 13 and 111 % on plot 20).

Table 8.2 : Results of the droplet counts.

	Plot	Dose applied g a.i/ha	Density of droplets /cm²	
			vertical	horizontal
NIORO DU RIP	1	485	115(101)	91(111)
	4	455	34(37)	108(131)
	9	410	84(54)	45(36)
	11	430	104(41)	33(15)
RICHARD-TOLL	18	905	232(115)	not measured
	13	405	17(24)	not measured
	15	990	268(210)	not measured
	8	845	267(96)	not measured
	4	990	551(151)	not measured
	20	410	36(40)	not measured
	1	470	146(70)	not measured
	9	405	106(77)	not measured

Figures in parentheses are typical gaps. The density of drops is an average counted on 6 X1 cm cards

The densities of deposition expressed in number of drops per cm² are very variable from one plot to the other. In simple dose they are not generally more than 100 and fall down to 17 on plot 11 in Richard-Toll. The passing from simple dose to double dose goes with a noticeable increase in deposition varying from 230 to 550. The examination of this deposition in relation to wind speed showed clearly that on Nioro du Rip plots when the wind speed increases, the density on vertical component become more significant than the density on the horizontal component. The same observation on the plots treated to a double dose in Richard-Toll, showed an increase of that deposition on the vertical component in relation to the wind. One will notice that on the Richard-Toll simple dose plots, that relation was not the same.

In conclusion, we see that the positioning of the oleo-sensitive paper during the treatment is interesting for research in the residues of pesticides. It allows the evaluation of the regularity of the

product in its distribution on the sites treated. In the case of the millet where the paper is at the same height as the sampled leaves, the results should be a good indicator of the initial deposition of the product on the matrix. In Richard-Toll the difference in height between the Boscia from which the sampling was made and the stakes carrying the oleo-sensitive papers does not allow to make such a parrallel.

Quality of the analysis

Fenitrothion

Fenitrothion is an insecticide with physical and chemical characteristics favourarable to analysis by chromatography in gazeous phase: a molecular weight inferior to 400 (277 only) and a tension of moderate vapor (18 mPa at 20 °C) give it a good behavior at the thermal conditions of the elution column. Furthermore, the use of a detector selective for sulfur and phosphorus (FPD, TID, NPD, AFID) while increasing the sensitivity of the detection, often makes extraction and purification less difficult, and also reduces sources of error.

Detection Limit

When readings are made by drawing the chromatogrammes of a standard which have been subjected to successive dilutions, one reaches a threshold under which nothing can be distinguished: that is the detection limit of the equipment for the product concerned.
In the case of the material used in these tests, the volume of injection being 5 µl habitually, the smallest concentration read with coherence may be up to 8.10^{3} mg a.i./l. But to reduce sources of error to a minimum mainly for the samples whose few elements of impurities create interference with the product at those low concentrations, we have deemed it necessary to consider it at 10^{-2} mg a.i/l. This value can very easily be repeated and gives a peak of at least 80 % in relation to the amplitudes of background noises which may occur.

Linearity

The linearity of the response obtained is mainly a function of the range of set sensitivity of the FPD detector which , in the analysis of residues is habitually set at 10^{-11} and 10^{-12} mV/A, 10^{-9} and 10^{-10} values are more amenable to formulation analysis. Its quality decreases with these figures while keeping an acceptable tolerance zone.
The 10^{-11} mV/A sensitivity which is very good for the analysis of concentration solutions between 0.5 and 20 ppm. offers appropriate linearity: chromatogramme free of background noise, calibration with 5 points most of the time offers a linear regression with a correlation coefficient R^2 superior to 0.96. The zero forcing of this regression creates little variation of the coefficient (around 4 % , see table below).

At the lowest concentrations (0.01-0.1) the linearity is slightly disrupted by the influence of a background noise that can be estimated to be lower than 5 % on the scale. By dividing this space into 2 parts or 3, one can get better correlations. Thus, the usual values of R^2 from 3 or 4 measuring points are higher than 0.9.

Control sample
Control sampling had been carried out on all the plots before they were treated. Their analysis did not show any residue.

Storage effects
As regards the control samples, samples taken before the treatment and containing a certain quantity of fenitrothion, with the same packaging as the samples, the results obtained are shown in the table 4.

Table 8.4. Fenitrothion control samples on millet leaves

Nature	Size	Spike (mg a.i)	Found (mg a.i)	Recovery %
1 leaf	200 g	1.00	96	96.0
2 leaf	200 g	.75	.73	97.0
3 leaf	200 g	.50	.47	94.0
4 leaf	200 g	.25	27	108.0
1 ear	200 g	1.00	91	91.0
2 cob	200 g	.50	48	96.0

Table 8.5. Fenitrothion control samples in pond waters

Water	Spike (mg a.i)	Quantité (l)	Calculated value (PPM)	Analysed value (PPM)
Distilled	0.1	1	0,1	0.11
Clear	0.1	1	0.1	0.09
Turbid	0.1	1	0.1	0.08

The control samples charged with 10 and 1 ppb have not been analysed

It has been demonstrated that the product kept frozen resists degradation well.

Recovery

Tests have also been carried out on the rate of recovery of the methods used. 4 blank leaf samples and 2 blank ear samples were spiked with a 0.1 g/l solution of fenitrothion prepared with a highly pure analytical standard. After a 24 hour storage, those samples were analysed in accordance with the analysis norms used. The results obtained show that after the analysis, 90% of the product is found again in vegetation. The table seems to show that the result obtained remains better with the samples with a weaker charge.

The recovery rate also seems to follow the simplicity of the method. In the case of the water which is analysed without the grinding or filtering stage, we found a recovery rate higher than 96 % (tables 4 & 5).

Table 8. 6 : Recovery rate of the extraction method: fenitrothion on vegetation.

Nature	Quantity	Spike mg a.i	Found mg a.i	Recovery (%)
1 leaf	100g	1.00	92	92,0
2 leaf	100 g	1.00	93	93.0
3 leaf	100 g	.50	47	94.0
4 leaf	100 g	.50	53	106.0
1 cob	100 g	1.00	89	89.0
2 cob	100 g	.50	45	90.0

Table 8.7 : Recovery rate of the extraction method. Fenitrothion in pond water

Water	Spike mg a.i	Quantity (l)	Calculated	Analysed	Recovery (%)
Clear	0.1	1	0.1	0.096	96
Clear	0.1	1	0.1	0.099	99
Turbid	0.1	1	0.1	0098	98
Turbid	01	1	1	0.97	97

Diflubenzuron

The diflubenzuron is a compound with a high molecular weight (310.69) and a vapour presure that makes it pratically non volatile (0.033 mpa at 50°C). The way it decomposes during during distillation does not make it easy to analyse by gas chromatography but its characteristics give it a good behaviour in case of High Performance Liquid Chromatography (HPLC). The use of the Zorbak Column eluted with the binary mixture acetonitril/water presents a good separation of the peaks between diflubenzuron and the interference products.

Detection limit

A 20 µl solution injection with a Diode Array Detector at 254 nm results in a detection limit of 0,3 mg/l or 6 mg of diflubenzuron.

Linearity

The linearity in the concentration scale 0.5 - 20 ppm is very high and has correlation coefficients almost equal to the Unit. That kind of device equipped with automatic injection also offers a good opportunity of sample indication repetition. The recovery of the extraction method of diflubenzuron on vegetation carried out on 2 tests gives the following results.

Table 8.8 : Recovery rate of the extration method : diflubenzuron/millet vegetation.

Test	Nature	Size	Spike	Found (mg a.i)	Recovery %
1	Leaf	100	50	42.3	84.6
2	Leaf	100	50	46.8	93.6
3	Cobs	100	50	44.7	89.4
4	Cobs	100	50	47.1	94.2

The improvement of the results in the tests 2 and 4 is obtained with lower elution flow in the purification column : about 1 drop per second.

Recovery

The same study of the recovery rate has been carried out as regards diflubenzyron in pond water without any treatment. The control samples were charged from a 1 g/l standard solution of diflubenzuron in acetone - Some drops of 1N hydrochloric acid 1 N solution were added to the charged samples in order to reajust the pH at 2 - 2,5. Then they were stored for 24 hours at 4 ° C in a refrigerator. The results of the analyses are shown in the table 8.9.

Table 8.9 : Recovery rate of the extraction method : diflubenzuron/pond water

Test	Size (l of water)	Charge (mg a.i)	Found (mg a.i)	Recovery %
1	0,5	5,00	4.84	96.8
2	0.5	5.00	4.59	91.8

Here too, one must notice the simplicity of the extraction and purification method used (Baker), which goes almost without any loss of product. That simplicity undoubtedly caused the good recovery rate obtained.

Storage effects

In order to check the effect of preserving the products before their analyses, control samples of vegetation were taken from untreated plots and charged with given quantities of active substance. Then the indicators were put in the same storing and transportation conditions as the samples. The results of the analyses are given in table 8.10.

Table 8.10 : Diflubenzuron indicator on vegetation

Nature	Size of sample (g)	Charge (mg a.i)	Found (mg a.i)	Recovery %
Millet leaf	114,2	50.00	56,06	112.1
Millet leaf	269.2	50.00	43.78	87.6
Millet cob	129.7	50;	52.07	104.1
Boscia leaf	2.10,4	50.00	50.84	101.7

For the water, controls have been prepared with clear water, unclear water and distilled water in order to observe the influence of their states on the behaviour of the diflubenzuron in preservation. Three concentrations were targeted in order to simulate the real diflubenzuron contents of the treated ponds : 0.2 mg/l, 2 mg/l. and 20 mg/l

The proportioning carried out on the 0,2 mg/l concentration samples did not give any results because their content was under the detection limit of the device. The 2 mg/l concentrations gave results that were equal to zero or non integrated, while the ones obtained with the 20 mg/l concentration showed a better behaviour of the diflubenzuron in conservation, even if they also showed some disparities.

Table 8.11 : Fate of diflubenzuron in pond waters.

Concentration	Nature	Found mg/l	Recovery %
2 mg/l	Unclear	Non intregrated peak	-
	Clear	Undetected peak	-
	Distilled	Non integrated peak	-
2 mg/l	Unclear	33.32	166
	Clear	17.51	87.55
	Didstilled	23.51	117.55

Residues found in the samples

Qualitative aspects of the Chromatograms.

The chromatograms shown by the extracts from millet and *Boscia senegalensis* samples treated with fenitrothion have yielded two distinct peaks. The first representing the solvent is an important peak taking up 100 % of the scale and appearing within the first 30 seconds following the injection. The second one will regularly appear within 3.5 mn and its importance is geared to the level of pollution of the sample-between the two, the basic line is taken down to the lowest degree of the scale. In some cases (first hour samples) that second peak can arrive at the top of the scale. A dilution has always made it possible to bring the reading to acceptable proportions.

The reading of the extracts from pond waters required a detection sensitivity increase going from the 10^{-11} range to the 10^{-12} mV/A. That change that cannot affect the retention time of the fenitrohion, will influence the generation of residual peaks. However, it is important to say that those peaks are far from hampering the fenitrothion peak that is looked for, as almost all of them are formed around the solvent peak at the beginning of the chromatogram. One must also notice that this sensitivity increase generates a background noise when the peak of the product is about to appear. Measurement tests have shown, however, that their amplitude remains weak in general, as compared with the amplitude of the peak of the product (below 10 %).
As regards the diflubenzuron, the use of the UV detection at the 254 mm wave length makes it possible to find many residual peaks.

The readings on the (millet or *Boscia senegalensis*) leaves and on ears , sample in which the deposit is usually important, do not present any problem : the peak which is usually important as compared with the other peaks appears between 3.1 an 3.2 mn while the latter ones are often eluted before the first 2 minutes.

For the low concentrations close to the detection limit (0.3 mg/l), the roles work the other way round : the residual peaks take up 100 % of the scale while the peak of the product, despite being visible, keeps a proportion below 5 %. It is remarkable however, that there are no other peaks around the product peak, which would interfere with it.

The test results

The first remark to be made is that all the results on vegetation are quantified in kg of fresh leaves or ears. A study to the water content of the various moulds targeted here, carried out by steam-room drying (105 ° C for 24 hours) shows the following results: table 8.12.

Table 8.12 : Water content of the vegetation samples

State	Nature	Average (%)	Number of measure units	Standard gap
Fresh	Millet leaf	75.17	15	3.16
	Millet cob	62.54	11	5.35
	Boscia leaf	47.45	12	4.43
Dried *	Millet leaf	9.92	6	0.14
	Millet cob	8.99	3	0.69
	Boscia leaf	9.96	4	0.03

* The dry state of the moulds was obtained naturally : holes had been made on the sample wrapping for air circulation and the sample itself was spread for several days on the laboratory bench.Only the diflubenzuron treated samples were submitted to such a drying system.

The results on vegetation of diflubenzuron were corrected by multiplying the result obtained from the test sample analysed by the factor (1 - t_i)/(1-t_f) where :

t_i = water content of the fresh sample (see the first 3 values in the table).

t_f = the water content of the dry sample (the last 3 values in the table).

which respectively result in the following correction values :

$d_1 = 275.6\ 10^{-3}$ for the millet leaves

$d_2 = 411.6\ 10^{-3}$ for the millet cobs

$d_3 = 583.6\ 10^{-3}$ for the *Boscia senegalensis*

Table 8.13 : Fenitrothion analysis results.

On millet leaf

Hours after the treatment	mg of active ingredients/kg of wet millet leaf			
	Plot 01 467	Plot 04 454	Plot 09 408	Plot 11 428
1	67.45	56.92	71.73	53.14
24	8.56	9.942	13.42	25.11
48	0.51	1.99	1.856	.55
168	.01	0.035	.005	.87

On millet ear

Number of hours after treatment	mg of active ingredients/kg of millet cob		
	Plot 04 467	Plot 09 408	Plot 11 428
1	2.31	6.392	2.748
24	1.288	5.108	1.682
48	0.482	2.591	0.466
168	0.054	0.4	0.29

Table 8.13 continued:

On *Boscia senegalesensis* leaf

Number of hours after treatment	mg of fenitrothion/kg Boscia senegalesis leaf					
	Plot 08 844	Plot 09 450	Plot 13 442	Plot 20 453	Plot 18 906	Plot 966
1	105.7	92.1	42.29	38.96	105.5	57.8
24	36.41	31.69				
72	9	10.74				
168	6.65	1.14				

In pond water

Number of hours after treatment	mg of active ingredients/litre of water				
	Koudole 356	Wimbody 490	Gandiang 575	Daladiam 500	Mbambah 601
1	0.034	0.027	0.031	0.163	0.145
24	0.018	0.021	0.022	0.015	0.005
48	0.01	0.0025	0.013	ND	0.004
144	0.006	ND	0.004	ND	0.003

Plot code: plot number followed by real dose (fields). Ponds: see chapter 2.

Fenitrothion

On millet vegetation, the values obtained one hour after treatment range from 55 to 71 mg of fenitrothion/kg of wet leaf. Their conformity reflects the regularity of the treated place (millet plot) and the regularity of the device used (micro ULVA). On millet ear, the values obtained one hour after treatment are weak compared with the deposit on the leaves.

At Richard Toll where we carried out treatments with 2 different doses, we got two types of values. For the single dose (450 g a.i./ha) we got 2 highly comparable values : 42.29 ppm on the plot 13 and 38.96 ppm on the plot 20.

The values 92.10 obtained on the plot 09 may be due to a higher wind speed (3.75 m/s) whereas on the other plots that wind speed was below 2.5 m/s.

On the sites treated with the double dose (900 g a.i/ha) we got values close to 100 mg a.i / kg : 105 mg a.i/ kg on the plots 08 and 18 and 98 mg a.i / kg on the plot 15.

Although the rule of the proportionality seems to be implemented by the shift from the single to the double dose, one can notice certain discrepancies in the relationship between the dose and the initial deposit (figure 8.3 & 8.4)

The pond waters show low residues which are related their volume compared to the volume of pesticide spread ; the first 3 values obtained on the samples taken 1 hour after the treatment are close to each other, just ranging from 27 to 34 ppb(µg of fenitrothion/litre of pond water).

Table 8.14 : Diflubenzuron residues

On millet leaf

Hours after treatment	mg of active ingredients/kg of wet leaf			
	Plot 08 51	Plot 1054	Plot 0361	Plot 0556
1	38.59	33.33	9.15	26.74
24	23.79	30.83	Not taken	Not taken
48	16.2	12.18	Not taken	Not taken
168	9.36	8.84	Not taken	Not taken
336	8.33	.09	Not taken	Not taken

On millet ear

Number of hours after treatment	mg of active ingredients/kg of wet millet ear
	Plot 08 51
1	2.31
24	2.09
48	0.86
168	1.83
336	1.45

On *Boscia senegalensis* leaf :

Number of hours after treatment	Plot 02 118	Plot 07 120	Plot 12 116	Plot 160	Plot 16 114	Plot 17 49
1	67.83	24.54	6.93	6.20	57.23	25.54
24	6.7					
168	3.26					
336	18.0					

Pond waters

Number of hours after treatment	Concentration in mg/l of product in water				
	Fana Ava 76	Konaro 60	Degbourey 80	Peligne 102	War 105
1	0.005	0.007	0.004	0.018	0.18
24	Not detected	Not detected	Not detected	Not detected	Not detected
72	Not detected	Not detected	Not detected	Not detected	Not detected
168	Not detected	Not detected	Not detected	Not detected	Not detected
336	Not detected	Not detected	Not detected	Not detected	Not detected

Diflubenzuron

The data presented in table 8.14 on diflubenzuron lead almost to the same conclusions as the ones made on fenitrothion. This product, treated with a dose at least seven times lower than the one used for fenitrothion (60 g a.i/ha only) gives in I hour after the treatment a deposit that does not reach 40 ppm on millet leaf. That is how 26.74 ppm on plot 05 ; 33 ppm on plot 10 and 38.59 on plot 08 are obtained.The 9 ppm value obtained on the plot 03 treated with a bigger real dose is difficult to explain. As far as the ears are concerned, only one plot was subject to sample taking. The 2.31 value that was found once again shows the small deposit on that sample material. It has a small display surface and an apparent volumetric mass higher than the one of the millet leaf. On the Richard Toll sites, the 4 plots treated with the double dose make it possible, upon the first sampling, to note two diiferent values between 67.83 ppm on plot 02 and 57 ppm on plot 16. A low value of 6.93 ppm is to be noticed on plot 12 and, between the two, a middle value of 24 ppm on plot 7.

The single dose studied on the Richard Toll site shows 2 quite similar values: 6.20 and 9.93 ppm on plots 14 and 17. At the water sites, the low values are still confirmed at less than 10 ppm in

were treated with doses higher than 100 g a.s/ha (the standard one being 60) gave 18 ppb concentration

There again, the differences between the characteristics of the ponds (depth, pH ...) may well be the cause of the lack of coherence in the relationships between the nominal dose and the deposit (figure 8.4)

Degradation

Table 8.15 shows the behaviour of the studied products in the environment. In all the cases the fenitrothion on leaves is subject to a level lowering beyond 50 % within 24 hours.

In water and ears, the low contamination level is compensated by a slower disappearance. The degradation of the remaining half of the product usually takes a long time after the first 24 hours.

Table 8.15 : Half-life periods

Active ingredients	Material	Plot	R^2	$T_{1/2}$	T1	N.O.
Fenitrothion	Millet leaf	01	92	14	25	4
		04	96	17		4
		09	99	12		4
		11	99	18		4
	Millet ear	04	98	35	2.65	4
		09	99	31		4
		11	74	40		4
	Pond Water	Koudote	83	64	.025	4
	Boscia senegalensis	Gandiang	98	48	.03	4
		Wimbody	84	14	.39	3
		MBanbath	*38	46	.024	4
		Daladiam	1	7		2
	Millet leaf	06	77	45	60	4
		06*	90	20	57	4
		09	98	27	73	4
		09*	89	19		4
Diflubenzuron		06	75	173	25	5
		10	39	131	26	5
		06*	86	146	25	5
		10*	95	73		5
	Millet Cob	06	97	538	224	4
		06*	96	616	156	4
	B.Senegalensis	02	59	51	27	3
		02*	88	39	16	3

R^2 = Correlation coefficient - $t_{1/2}$: half life period - T : theoretical initial deposit.
N.O. = Number ofobservations used in the linear regression.

* = Correlation done on the hypothesis of a degradation of the 2nd order while the others follow the hypothesis of a 1st order correlation.

For the benzoylurea compound, the data from the millet leaf study reveal that the disappearance of the product is slower than of fenitrothion : half of the degradation occurs only by the end of the second day after the treatment. That persistence was definitely revealed on the millet ears where samples taken after two weeks still reveal a presence rate of the product higher than sixty two (62) %. However, the data obtained from water analysis show another aspect of the behaviour of diflubenzuron in those sites: the product was detected only on the first sample; the other samples (24 hours, 72 hours, 1 week, 2 weeks after treatment) gave results that were below the detection limits of the device, that is to say concentrations theoretically below 0.6 ppb.

Half-life periods

The mathematical processing of the results allows us to estimate the half-life period of the pesticides ($t_{1/2}$) which represents quite a good index of their persistence. It also makes it possible for us to assess by extrapolation the initial deposit rate obtained by the means that were used.

In so doing, one must adapt a mathematical model capable of keeping track of that degradation. The research of that model is done by confronting the experimental results with the results of the formal analysis : the method gives an equation of speed and integrates it using experimental results. The nature of the data (figures 8.5 & 8.6) leads us to the postulation for a simple reaction of first order degradation. The results are shown in the table 8.15.

Fenitrothion

The kinetics of the zero order was eliminated for lack of linearity between the concentrations of the various samples and the dates when they had been taken. This led to very low correlation coefficients (below 0.5) during the application of the linear regression.

The kinetics of an order higher than or equal to 2, the calculations of which do not appear in the charts, unlike the kinetics of the zero order, led to linear regressions whith a constant that makes it possible to determine the initial pesticide deposit being highly negative instead of being a positive number smaller than 1. But the correlation coefficient remains one of the best. For those various reasons, the hypotheses of kinetics of those orders were also rejected.
The hypothesis of the kinetics of the first order was confirmed in most of the cases. The linear regressions calculated on the neperian logarithm of the pesticide content of the sample as a function of time, led to correlation coefficients very close to 1. The characteristics of the regression (slope of the line Log(content) = f(time) and its intercept) are all coherent. The periods or half life obtained from those regressions based on the first order hypothesis vary with the studied materials. In so doing, they range on average from fifteen (15) hours on millet leaf to forty five (45) hours in pondwater and, in betweén: thirty five (35) hours on millet ear and thirty six (36) hours on *Boscia senegalensis*.

By observing the material, one can notice more or less important variations between the values of those periods. We think that they are due on the one hand to the statistical errors about the period estimation and on the other hand to the climatic aggressions to which each plot was differently subject. This remark is even more obvious if we consider the ponds in which the various factors such as the turbidity, the pH, the water level variations and the abundance of microorganisms, can vary in significant proportions.
By observing the initial theoretical rates of deposit one can notice a downward correction except in the case of the millet ears where they are slightly above the results founds, regarding the samples taken 1 hour after treatment.

Diflubenzuron

The same comparative study was done with diflubenzuron. Despite the restricted number of data, the results appear to be more heterogenous than in the case of fenitrothion. That is why the regression done on the diflubenzuron analysis results on millet leaf present a range of consideration choices about the order of the degradation reaction. In fact the correlation coefficients obtained increase from 0.86 for the order 2, to 0.97 for the order 4 for plot 8. However, the coherence of the values of the integration constant stops at the order 2 for plot 8. The periods respectively obtained for plots 8 and 10 are 146 and 73 hours (results obtained with the order 2) which widely expresses the importance of the persistence of the diflubenzuron compared to fenitrothion in the same material. On millet ear only one case studied presented a doubtful point (the one taken 48 hours after treatment) the regressions done on the 5 points show very bad correlations (R^2 below 0.004) whereas the truncation of that doubtful point presents a series of regressions with a better correlation coefficient. That correlation coefficient as function of the hypothetical order of the degradation shows a maximum between the orders 1 and 2. The halflife periods are of the same importance and increase as the order goes up. The periods obtained with the most probable whole orders are : 616 hours (for the 2nd order) and 538 hours (for the 1st order) those ever increasing values confirm the

capacity of this pesticide to resist degradation better on ears than on leaves (mille or Boscia) or on pond water.
The same heterogenous behaviour of the degradation movement of diflubenzuron can be noticed on *Boscia senegalensis* leaves.
By eleminating the doubtful point (the 4th one), one can get fairly good correlations for the hypotheses of the orders 2 and 3. The periods obtained are respectively 39 hours in the hypothesis of a second order and 23 hours in the one of a third order.

DISCUSSION

Fenitrothion

In millet, the figures obtained for each material vary a little when we shift from one plot to another.

Table 8.16 : Parallelism between periods on leaves and on ears.

Plots	Period/leaf (hours)	Period/ear (hours)
09	12	31
01	14	35
11	18	-

If the plots are arranged according to the increasing order of the periods on millet leaf, the same grading persists when ears are considered. In other words, the degradation speeds seem to depend only on the site treated or, more precisely on the weather conditions that the compound is subject to, during the display period. The table 16 illustrates well the grading that was done.
Table 8.17 gives the weather conditions on the plots during and after the treatment. Plot 09 treated at 15 o'clock in a moderate (27° C) and humid (88 %) weather and having had a strong rainfall (18 mm) before the second sample was taken presented the fastest degradation of the pesticide. As the treatment conditions were almost identical to the ones regarding the plot 11, it may well be assumed that the rain water had a significant effect on that quick disappearance.
The plots 04 and 11 which presented longer half-lives had more favorable weather and a longer period before rain.
The comparative survery on the sites of Nioro and Richard Toll also reveals that fenitrothion treated in a dry area (hot and dry weather) disappears less quickly than in a rainy area.
The observations concerning ponds show more or less different half-lifes. One pond (the Mbambah one) showed bad correlation. It is difficult to interprete these differences.
The characteristics of the ponds treated with fenitrothion during the sampling process include the water level variation, the pH and the temperature prevailing during each sampling. By comparing that with the result of the analyses one can see, however, that the pond presenting the highest of degradation of fenitrothion has the lowest pH at the most constant level.

Table 8.17 : Weather conditions at the plots during the teste.

Plot 01 treated at 17 o'clock, 487 g ai/ha 34° C and 71% humidity				Plot 04 treated at 12 o'clock, 45 g ai/ha 32° C and 65% humidity			
Date	Rainfall	Total before sampling	Sample	Date	Rainfall	Total before sampling	Sample
09/12/91	0	0	1	09/12/91	0	0	1
09/13/92	1.3	1.3	2	09/13/93	1.3	1.3	2
09/14/92	0	1.3	3	09/14/91	0	1.3	3
09/19/91	14	15.3	4	09/19/92	14	15.3	4

Plot 09 treated at 15 o'clock, 406 g a.i/ha 27° C and 88% humidity				Plot 11 treated at 10 o'clock, 428 g a.i/ha 27° C and 85% humidity			
Date	Rainfall	Total before sampling	Sample	Date	Rainfall	Total before sampling	Sample
09/11/91	18	0	1	09/12/91	0	0	1
09/12/92	0	18	2	09/13/93	1.3	1.3	2
09/13/92	1.3	19.3	3	09/14/91	0	1.3	3
09/16/91	0	19.3	4	09/19/92	14	15.3	4

As a conclusion, fenitrothion on vegetation appears sensitive to rain water by two possible ways of disappearance.

The disappearence of the product by washing is very likely to occur when the rain falls during the first hours following the treatment, or if the compounds used are not systemic like the fenitrothion. That phenomenon remains all the more important as the rains are abundant, for example in the case of plot 9. The degradation by hydrolysis can also have significant effects.

The comparaison between the plots 04 and 11 in Nioro du Rip on the one hand, and the plots 08 and 09 in Richard Toll on the other hand seems to reveal that the temperature prevailing during the treatment seems to have significant effects on the disappearance of the products in those sahelian areas

Diflubenzuron

The survey on diflubenzuron is hampered by the insufficient number of cases treated. For instance on leaves, only 3 sites were completely treated : 2 in Nioro du Kip and 1 in Richard Toll.

Table 8.18 : Weather conditions prevailing during the surveys in the plots.

Plot 08 treated at 9 o'clock, 51 g a.i/ha 30° C and 93% humidity				Plot 10 treated at 11:20-12:35 o'clock at 54 g a.i/ha 34° C and 60% humidity			
Date	Rainfall	Total before sample taking	Sample	Date	Rainfall	Total before sample taking	Sample
09/13/91	1.3	0	1	09/13/91	1.3	0	1
09/14/92	0	1.3	2	09/14/93	0	1.3	2
09/15/92	0	1.3	3	09/15/91	0	1.3	3
09/20/91	16	1.3+14(19)=15	4	09/20/92	16	1.3+14(18)=15	4
09/27/92	0.1	15.3+27.1(26)+1=58	5	09/27/92	0.1	15.3+27.1(26)+1=58	5

In Nioro du Rip there is a significant difference between the two periods found in the results from the plots 08 and 10 (Table 8.15). Again, the product treated under milder weather conditions lives longer. In fact plot 10, treated at 12 o'clock under a 33% relative humidity decrease and 4° C temperature increase as against the weather conditions prevailing during the treatment of the plot (Table 8.18) presents shorter periods.

The slight rainfall before the second sample taking does not seen to have much influence on the first time degradation and, unlike the fenitrothion case, the effect of that rain is not obvious on the freshness of the treatment. Furthermore, the 58 mm rain that eventually fell on the product during the sampling period do not seem to have speeded up the desappearance phenomenon of the product. One can logically infer from those observations that the diflubenzuron on vegetation is not very affected by rainwater. Moreover, this conclusion is confirmed by IRPTC (1991) which, while dealing with the diflubenzuron remnants on cotton, confirms its persistence during the wet season.

For Richard-Toll, the observation of the results of the plot 2 only do not make it easy to draw any conclusions. But, despite the absence of weather data, we noted that that area is generally sunny, hot and dry with strong winds, which can contribute in speeding up the degradation of the product : At least that is what the result reveals, with a shorter period, compared to the values found in the Nioro du Rip site (only 51 or 39 hours depending on weather we consider the hypothesis of the first or the second order). The absence of rain in that Richard-Toll area highlights the effect of the high temperatures and the weak humidities and the wind (volatilisation or photochemical decomposition). This remark also agrees with the conclusion of IRPTC (1991).

The result from the millet test match badly too. However we can see (table 8.6) that the product, here again, resists rain water. In fact,despite 14mm of rainfall on the 6th day and 27.1mm on the 13th day after treatment, more than 62% of the product was remaining at the end of the 2nd week. Here again,we find the same capacity of the millet ear to keep the compound longer than the leaves do.

The results of the work done on pondwater leave many questions unanswered. In fact,unlike what we found on vegetation,the figures revealed by the analyses show a clear instability of the diflubenzuron in pondwater. No trace of residues has been detected 24hours after the treatment. Any attempt to explain this phenomenon necessarily leads us to considering the characteristics of the waters treated. We notice, while observing the chart including the so called characteristics of the waters (see annex) that except the pond of Kouare, all the other ponds have a pH over 7, which proves that those sites are basic.Furthermore, by observing that very annex,one should note the relative importance of the temperatures noted during the sampling. They are different depending on which pond is considered and can change from 28 - 29°c in the morning, to 35 - 37 in the afternoon. If this physical and chemical aspect of the waters cannot provide any explanation on the quick disappearance of the diflubenzuron one can still refer to many authors of publications according to whom diflubenzuron can only be well kept in water if the pH and temperatures are low.

Besides, most of the pondwaters in the Nioro du Rip area are turbid partly because of an important biological activity but mainly because of the daily presence of animals that come and drink. This observation on the turbid waters (see the visibilities expressed in cm of depth as regards the same ponds during the same period of 1992: table 20) provides explanations about the presence of suspended substances and sediments that,due to the highly lipohile character of difubenzuron, may mix well with it. Thus they may gradually absorb it.To sum it up, we found from the results of the surveys the characteristic of persistence of diflubenzuron on vegetation. In the 3 cases of solid materials studied, the periods that it presented were by far superior to the fenitrothion periods. the results obtained from the analyses done on the ponds show however that this persistence of diflubenzuron depends on the milieu which, in the present case causes its lifetime to be cut down to less than 24 hours. Morever, diflubenzuron looks less affected in rainy areas with moderate temperatures than in hot dry and sunny areas: the comparative study of the Nioro du Rip sites and the Richard-Toll sites shows a longer remanence of diflubenzuron in the former than in the latter cases.

COMPARISON WITH OTHER RESULTS AND STANDARDS

We can easily compare our results with some surveys already carried out in the sahelian environment. Those surveys include the studies by Everts (1990) and the U.S. agency for International Development USAID (Dynamac 1988). In the first survey, the tests were carried out in Richard Toll. The products tested were the same but the treatment devices used, the material bearing the product and the Nioro du Rip site brought about the difference. As regards the second study, the common product is fenitrothion; the treatment devices and the mould are also different.

However, we can notice that the specific data are alike: the simple nominal doses used are:
As regards fenitrothion

485g a.i./ha	Everts
500g a.i./ha	USA tests
456g a.i./ha	Actual study

The result comparison can be done at 2 levels:

Level 1 : The initial deposit

The chart comparison (figures 8.7) shows, despite the differences already expressed, a good similarity about the fenitrothion results in the 2 surveys. the numbers found are similar. However, it is to be noticed that the deposit values are less important in the results of the 1988 and 1989 surveys. That phenomenon may be due to the different means of treatment used.

Level 2 : The degradation of the products

The observation of the chart lines relative to each survey do show that fenitrothion has the same degradation pace in these sahelian environments. The non presistent characteristic of fenitrothion is shown in the 3 surveys. In most of the cases, the disappearance of half of the the product happened before the end of the first 24hours. Except the case of the 1989 surveys in which the sample taking was stopped 4 days after the treatment, the disappearance of the product at the end of the first week is almost complete. The relatively slow disappearance of fenitrothion in the dry environment of Richard Toll in 1989 also exists in the present survey.

The persistent characteritics of the diflubenzuron pesticide on leaves are also shown in the 1989 survey. despite a certain disparity of the results, the product is still notably present 14 days after the treatment: a rate over 2 ppm in all cases.

Table 8.19 shows a representation of the test results in the scale of the standard.
We notice that fenitrothion treated on vegetation also complies by the standards regarding animal fodder right on the first day. If, in addition to that, we consider the leaves of millet or Boscia as edible by human beings, 2 remarks are to be made.

In a rainy environment like Nioro du Rip, the disappearance of the product before the maximum residue limit (MRL) is underway right during the first week.

In Richard Toll,in the two cases studied,the consumption of the product by human beings was to be prohibited during the first week following the treatment.

When comparing ears and grains, we notice the low deposit of fenitrothion on mould. The MRL is not exceeded in 2 cases, in the third, the product is ready for consumption before the end of the second day.

As regards diflubenzuron we lack standards for the fodder and the grains. The admission of millet leaves and boscia to human consumption indicates compulsory deadlines longer than 14 days in the cases studied here

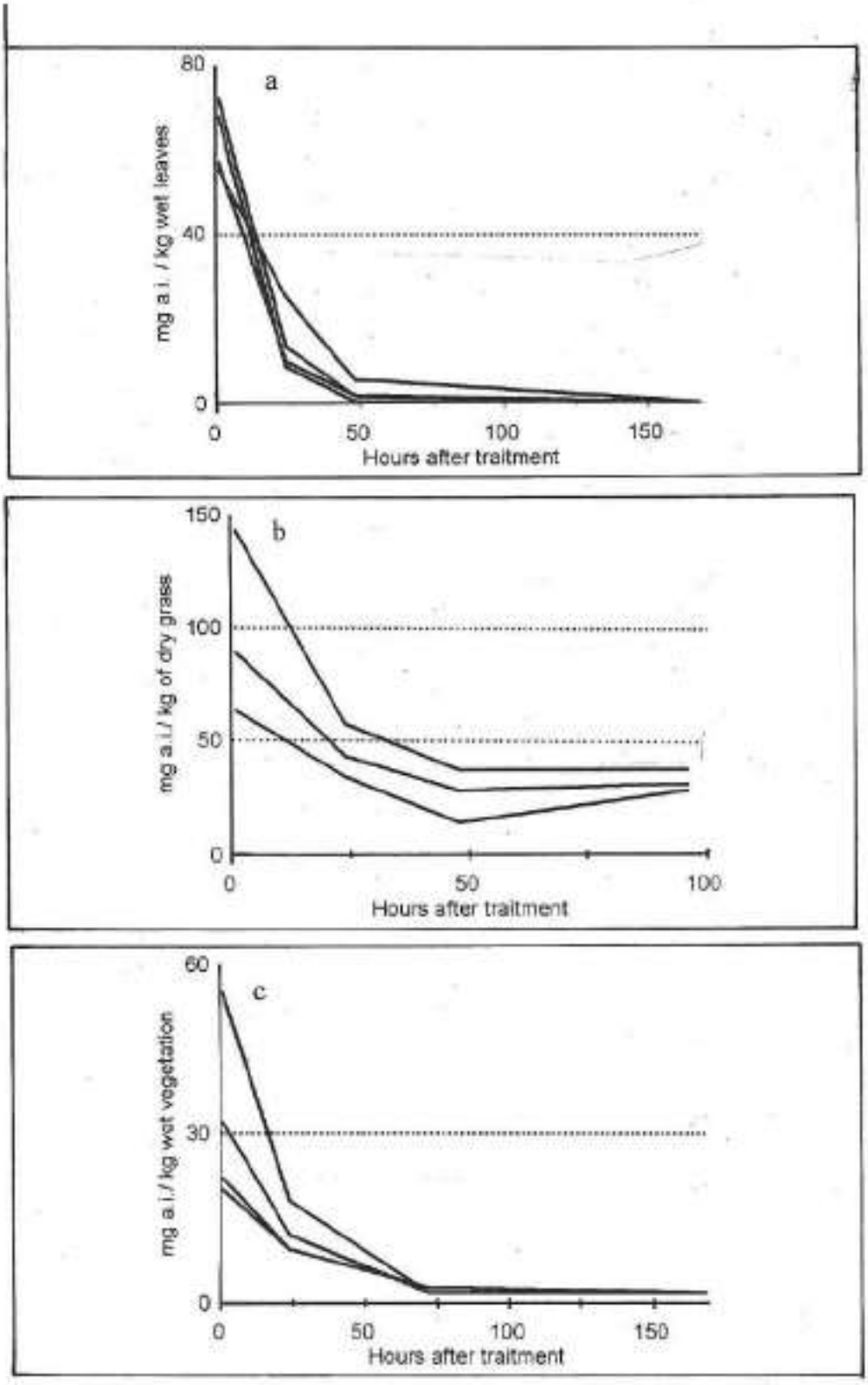


Fig. 8.1 : Disappearance curves of fenitrothion in three different studies.

REFERENCES

American Chemical Society (1990) Handbook of Chemical Estimation Methods. American Chemical Society, Washington DC, USA.

Australian Plague Locust Commission (1981) Technical Report N° 3.

Dynamac (1988) Results of the locust pesticide testing trials in Sudan. Dynamac Corporation, Rockville, MD, USA.

Duphar (1988a) Liquid chromatographic determination in tea (Analytical Development Department). Duphar, Weesp, The Netherlands.

Everts James Williams (Editor, 1990) Environmental effects of chemical locust and grasshopper control. A pilot study. FAO, Rome.

Duphar (1988b) High Performance Liquid Chromatographic Determination of diflubenzuron in water. Duphar, Weesp, The Netherlands.

F.A.O. (1986) Pesticide Residue Trials to provide data for the registration of pesticides and establishment of Maximum Residue Limit. FAO, Rome, Italy.

F.A.O. (1991) Controlling Desert Locusts. FAO, Rome, Italy.

Federal minister of environment (1989) Environmental effects of fenitrothion used in forestry. Minister of the Environment, Darmouth, Canada.

(IRPTC) 1991 : International Registor of Potentially Toxic Chemicals, United Nations Environment Programme, Genève, Suisse.

Kratochvil & Peack (1989) The analytical methods for pesticides and plant growth regulators.

NRI N° 28 A guide to the establishment and maintenance of pesticide laboratories in developing countries. National Resources Institute, Chatham Maritime, UK.

Sumitomo (1981) Sumitomo Technical Manual. Method for residue analysis (pp 23 - 27). Osaka, Japan.

Sumitomo (1988) Residue analytical method for fenitrothion and fenitrothion in water. Laboratory of Biochemistry and Toxicology, Takarazuka Research Center, Sumitomo Chemical Co. (Ltd), Japan.

Van der Valk (1991) The effect of fenitrothion and diflubenzuron on natural enemies of millet pests in Senegal. Projet FAO-Locustox, FAO, Dakar.

ANNEX 8.1 : Fenitrothion: pond caracteristics during the sampling period.

Pond	Dates	pH	Depth	Temperatures	hours
	17.09.91	6.05	0.4	27	8H 30
	18.09.91				8H 20
Koudole	20.09.91	6.25	0.4	26	8H 30
	23.09.91	6.23	0.4	27	9H 20
	17.09.91	6.75	0.39	35	13H 30
	18.09.91				13H 30
Wimbodi	20.09.91	6.59	0.34	27	9H45
	23.09.91	8.23	8.23	33	12H 20
	17.09.91	6.73	0.5	25.7	8H 55
	18.09.91				13H 20
Bamba 1	20.09.91	6.71	0.36	29	11H 30
	24.09.91	6.71	0.39	31	10H 45
	17.09.91	6.94	0.28	32.5	13H 05
	18.09.91				16H 50
Gandian	20.09.91	7.23	0.37	31	13H 15
	24.09.91	8.22	0.48	35	11H 00
	17.09.91	6.4	0.2	29	11H 15
	18.09.91				9H 45
Daladiam	20.09.91	6.4	0.17	26	8H 30
	23.09.91	6.2	0.17	29	9H 20

ANNEX 8.2 : Diflubenzuron: Pond caracteristics during the sampling period.

Pond	Dates	pH	Depth	Temperatures	hours
	15.09.91	7.18	0.43	35	9H 05
	16.09.91				8H 45
	19.091	6.49	0.4	26	8H 55
Sadiowar	23.09.91	7.03	0.39	29	9H 50
	30.09.91	6.86	0.4	30	10H 20
	15.09.91	7.12	0.56	31	16H 00
	16.09.91				16H 35
	19.09.91	7	0.55	28	17H 45
Debreya	23.09.91	7.3	0.7	35	16H 00
	30.09.91	6.81	0.6	35	17H 00
	15.09.91	6.6	0.45	30	12H 40
	16.09.91				13H 12
	19.09.91	7.26	0.39	33	14H 00
Fana-awa	24.09.91	7.14	0.39	31	11H 20
	01.10.91	7.32	0.3	31	11H 00
	15.09.91	6.84	0.5	26.5	8H 48
	16.09.91				15H 50
	19.09.91	6.95	0.5	31	12H 20
Kouaré	24.09.91	6.76	0.45	29	9H 10
	01.10.91	6.6	0.5	28	9H 00
	15.09.91		0.74	37	12H 45
	16.09.91	7.2			10H 00
	19.09.91	8.12	0.77	31	10H 50
Palagne	23.09.91	8.33	0.65	33	13H 00
	30.09.91	7.81	0.6	34	13H 15

CHAPTER 9

Deposition and degradation of fenitrothion and diflubenzuron on vegetation and in the soil in senegal & residue survey in the millet stores in rural areas (1992 Campaign).

SUMMARY

Millet parcels at the flowering or maturation stage at Nicro du Rip (Centre of Senegal) and semi-arid pasture sites at Richard-Toll (North of Senegal) have been separately treated with fenitrothion, diflubenzuron, bendiocarb and chlorpyrifos. Deposition and fate of these pesticides was studied.

To this end, the vegetation and soil of the parcels at the flowering time and the grass of the pasture sites were sampled after treatments.

Millet ear was sampled on millet parcels during a week before their being harvested. After harvest, the part of threshed millet is stored in bags and the other part of millet ears is stored in a loft. Samples were taken to follow the residues during the storage.

A micro-ULVA treatment with the recommended doses (450 g a.i./ha for fenitrothion, 60 g a.i./ha for diflubenzuron, 100 g a.i./ha for the bendiocarb and 240 g a.i./ha for chlorpyrifos) resulted in average initial depositions of: 40 mg a.i./kg of wet millet leaf for fenitrothion, 50 mg a.i./kg of wet leaf for diflubenzuron. Half-life periods are 6 hours for fenitrothion and 400 hours for diflubenzuron. On the soil, the average initial depositions for these two products are recpectively 0.95 and 3.94 mg a.i./kg of wet soil and the average half-life periods are: 30 and 50 hours.

At Richard Toll, the treatment of pastures has given the following average initial depositions on grass *(Tribulus terrestris)* : 82.0 mg a.i./kg of wet grass for fenitrothion, 40.5 mg a.i./kg of wet grass for diflubenzuron, 37 mg a.i./kg for bendiocarb. The half-life periods are markedly different from those found at Nioro du Rip and are of 25 hours for fenitrothion, 63 hours for diflubenzuron, and 18 hours for the bendiocarb. On the soil, the average initial depositions and the half-life periods are respectively: 0.19 mg a.i./kg, 62 hours for fenitrothion; 3.25 mg a.i./kg, 223 hours for diflubenzuron; and 0.028 mg a.i./kg. 114 hours for bendiocarb.

The millet parcels at Nioro du Rip, treated with the recommended doses in the framework of the residues survey in farm stocks, have given initial depositions of 0.44 mg of fenitrothion/kg of wet ear, 0.20 mg of chlorpyrifos/kg of wet ear, and 0.18 mg of bendiocarb/kg of wet ear. After harvest, the products stored in the lofts and in bags are very little contaminated and respond all to the standards of consumption.

INTRODUCTION

As for the '91 campaign, the '92 campaign was carried out in the rainy season going from the month of July to that of October by the same pluridisciplinary team (Chapter 8).

The studies described in this chapter aim at:

1) The continuation of the 1991 research activities for a confirmation of results on the initial deposition of pesticides on certain matrixes, and of studies on the degradation of pesticides in the environment. In 1991, diflubenzuron has been studied on a less important number of samples than fenitrothion. The present study will allow to make up this deficit by increasing the number of studied cases by an intense sampling strategy as well in the sites of Nioro du Rip as in those of Richard-Toll.

2) The extension of research activities to other fields and to other pesticides by profiting from experiences acquired both in field and laboratory works :

That is how the study on millet vegetation in four parcels for fenitrothion and diflubenzuron has been taken up again for the Nioro du Rip area where samplings have been done on millet leaves and on the soil. The sampling on millet ears has not been possible due to the fact that the treatment date has been advanced to the last week of August, a period when the millet is only at the start of its flowering stage.

The same study including fenitrothion and diflubenzuron, but also bendiocarb has been carried out at Richard Toll. A change in the choice of geographic sites and in the meteorologic conditions of the pre-treatment period failed the sampling of the *Boscia senegalensis* which has been replaced by an herbaceous species : the *Tribulus terrestris*.

Still for these three compounds, soil samples have been taken on the Nioro du Rip and Richard Toll parcels with the same periodicity as for the vegetation.

The study includes a treatment with pesticides on the millet parcels of Nioro du Rip and on the pastures of Richard-Toll. This stage is followed by sampling over a period of one to two weeks according to the remanence of the product. The prepared samples are stored untill they reach the laboratory where they will be subject to the residue analysis. The results of analyses are used to describe the behaviour of these pesticides in a sahelian environment.

In addition to that, this campaign has been marked by a residue survey in the farm millet stores of Nioro du Rip. Inspired by the 1991 results on more or less long half-life periods of degradation of fenitrothion on millet ears, we thought it necessary to search for the residues of the pesticides generally used in Senegal and in the Sahelian sub-region in case of locust invasion: fenitrothion, chlorpyrifos, and bendiocarb were the targets.

In this study, we are doing a simulation by separately treating with three products three parcels to assess the degree of exposition to residues of populations who consume the millet. The doses used are the recommended ones for each pesticide. Four first millet ear samples are taken from each field during the first week to determine the initial deposition and follow the degradation on the ground. Harvesting is done seven days after treatment. According to local practices, the harvested ears are dried (during 3 days) before being put in the loft or beaten in mortars for the gathering of grains. Half of the harvest in ears is then put in the loft and the other half is stored in bags in the store. A sampling is done on these two parts differently stored for 5 months so as to be subject to laboratory anlysis of residues.

During this campaign meteorologic conditions during the testings and during the sampling period were monitored carefully : temperature, daily pluviometry, relative humidity.

The soil temperatures have also been taken during the entire sampling period. Soil drying in an incubator was done for the determination of their water content.

MATERIAL AND METHODS

General elements of the treatment

Products applied

In addition to the products used in the '91 campaign (fenitrothion and diflubenzuron), two other products are concerned by the treatments of the present campaign: bendiocarb and chlorpyrifos.

Bendiocarb

Bendiocarb is an insecticide belonging to the family of the carbamates. The IUPAC name is: 2.2 dimethyl-1.3 benzodioxol-4-yl methyl carbamate ($C_{11}H_{13}NO_4$).

The pure compound is a solid crystalline of white colour with 380° C as melting point. Not very soluble in water (0.04g/l at 25° C), it remains very soluble (up to 200/l) in ordinary organic solvents : acetone, chloroform, dichloromethane, dioxane. A low vapor pressure 0.667 10-6 kPa (those of the water and the acetone at practically the same temperature are respectively of 3.2 and 30.47 kPa) makes that it practically doesn't vaporize at the use temperature. Its physical chemical properties give it good remanence qualities. The product is slightly systemic and penetrates by contact and by ingestion and causes an inhibition of cholinesterase. A very wide action spectrum and a moderate toxicity among mammals, puts bendiocarb in the category of pesticides largely used in agriculture, horticulture and forestry but also in public health programmes to struggle against agricultural pests and desease vectors.

In this present study, bendiocarb used in the formulation FICAM 200 g a.i./l ULV was subject to tests on parcels at Richard-Toll where grass samples (*Tribulus terrestris*) and soil samples have been taken. At Nioro du Rip it has been used for of pesticide residues survey in farm stores.

Ethyl chlorpyrifos

Like most of the organophosphates, chlorpyrifos is an insecticide which acts by inhibition of cholinesterase. Its chemical name (IUPAC) is O,O-diethyl-0,3,5,6-trichloro-2-pyridinyl phosphathioate ($C_9H_{11}C_{13}NO_3PS$). The pure product is a white grained crystalline, with a melting point between 42 and 43.5° C. Its odor reminds of that of the mercaptans. Not very soluble in water, it has a big affinity with solvents like acetone (6.5 kg/kg), benzene (7.9 kg/kg), chloroform (6.3 kg/kg), methanol (450g/kg) at 25°C. Chlorpyrifos is a little more volatile than bendiocarb with a vapor pressure of 2.5 10^{-4} kPa at 25° C. Chlorpyrifos has a persistence that varies a lot according to the environment: its decomposition by hydrolysis in alcaline waters is quick but keeps a notable persistence in soils going from 60 to 120 days. Its wide action spectrum covering the totality of insects including locusts at the larva and adult stages implies it in the treatments of market gardening and cereal growing areas before and after sowing, in the treatment of stocks and dwelling buildings, in the treatment of soils, polluted waters, wood, concrete etc...
Chlorpyrifos is used in this study in the DURSBAN 450 g a.i./l formulation.

Technique of treatment

At Nioro du Rip, the Micro-ULVA has been used for the treatment of parcels which has allowed to conduct comparisons with the data of the preceding year (Chapter 8).

Table 9.1 : Comparison of treatment data at Nioro du Rip

Traitement parameters	Fenitrothion (SUMITHION)		Diflubenzuron (DIMILIN)	
	1991	1992	1991	1992
Sprayer used	Micro ULVA	Micro ULVA	Micro ULVA	Micro ULVA
Formulation concentration (g/l)	500	500	60	60
Nominale dose (g/ha)	450	450	60	60
Flow (ml/mn)	50	46-50	50	50
Spacing of passes (m)	11	10	11	11
Moving speed (m/mn)	50	55	30	46
Disk frequency (t/mn)	6000-6400	6000-6400	6000-6400	6000-6400
Size of droplets	80-100	80-100	80-100	80-100
Height of spray (m)	1.5-2.0	1.5-2.0	1.5-2.0	1.5-2.0

The a drift treatment technique has been applied. The size of all plots was equal (< 2 ha). The treatment parameters did not or marginally change. In table 9.1 the compared data of '91 and '92 campaigns for the treatment of parcels are given.

For products used for the research of residues in farm stores, used treatment data are given in Table 9.2.

Table 9.2 : Residues in farm stocks : technical data treatment.

Produits & formulation	Fénitrothion SUMITHION	Bendiocarb FICAM	Chlorpyrifos ethyl DURSBAN
Sprayer used	Micro ULVA	Micro ULVA	Micro ULVA
Formulation concentration (g/l)	500	40	240
Nominal dose (g/ha)	450	100	240
Flow (ml/mn)	55	50	35
Spacing of passes (m)	10	10	10
Moving speed (m/mn)	-	-	-
Disk frequency (t/mn)	8100-8500	8100-8500	8100-8500
Size of droplets	80-100	-80-100	80-100
Height of spayer (m)	1.5-2.0	1.5-2.0	1.5-2.0

At Richard Toll, the equipment and the choice of sites were different. The choice of sites which was dictated by the biological research (Chapter 4) (Richard Toll South-East in 1991, Richard Toll South-West in 1992) as well as the more reduced parcel sizes depending on the mobility of studied populations (400m x 400m parcels in 1991 for the study of flying insects, 125m x 125m parcels in 1992 for the study of termites and ants). This reduction in the size of the parcels has involved more adequate and more economic use of the MicroULVA instead of the ULVA-Mast. Table 9.2 draws up the technical data relating to both campaigns in the Richard Toll zone.

Table 9.3 : Comparison of treatment data at Richard Toll

Traitement parameters	Fenitrothion (SUMITHION)		Diflubenzuron (DIMILIN)		BENDIOCARB (FICAM)
	1991	1992	1991	1992	
Spayer used	ULVAMAST	Micro ULVA	ULVAMAST	Micro ULVA	Micro ULVA
Formulation concentration (g/l)	500	500	60	60	200
Nominal dose (g/ha)	450	900	60	120	200
Flow (ml/mn)	410	46-50	450-460	50	50
Spacing of passes (m)	40	10	40	10	10
Moving speed (m/mn)	50	55	30	46	
Disk Fréquency (t/mn)		6000-6400		6000-6400	6500
Size of droplets	80-100	80-100		80-100	
Height of spray (m)	2.5	1.8-2.0	2.50	1.8-2.0	1.8-2.0

Positioning of oil-sensitive paper

Positioning of oil-sensitive paper has been done during the treatment of sites to allow us to give account of the regularity of the deposit. This paper is white, 6 cm x 1 cm. It is put in position before the treatment on the perpendicular median to treatment passes at the rate of 10 per parcel. Positioning covers two components : a vertical component which turns face to the wind therefore in the product's attack direction, and a horizontal component facing the sky. Both components vertical and horizontal describe the distribution of the product at the level of the parcel.

At the Nioro du Rip sites where the treatment was carried out on millet, the paper was fixed on the ear at a variable height between 1.5 and 1.75 meters.

At Richard Roll, the paper was fixed on iron stakes. Depending of the size of the vegetation of the area the height was to 0.25-0.35 meter.

At the end of the treatment, the oil-sensitive paper is recovered and stored carefully until it gets to the laboratory where the counting of droplets is done by microscope.

SAMPLING

Sampling technique

Samples were taken from a diagonal track, recommended by the FAO (FAO 1986).

Sampling frequency

The sampling frequency depends both on the nature of the study and on that of the product searched for. The following frequency of sampling has been adopted for fenitrothion for the determination of the initial deposit rate and for the establishment of the pesticides degradation curves :

- 1hour after treatment
- 24 hours after treatment
- 48 to 72 hours after treatment
- 1 week after treatment.

For diflubenzuron and bendiocarb, the study of which revealed a longer persistence, a fifth sample was taken 14 days after treatment.

For the residue research programme in farm stores, there are two stages for the three studied pesticides:

Stage 1 : after the treatment, sampling is carried out on the parcel during a week at the same frequency indicated for fenitrothion.

Stage 2 : one week after treatment, harvesting is done, and the millet is stored (in grains or in ears). Sampling is done in the bags or on the lot at the frequency of one per month.

Hour and date of each sample are mentionned on each sample and the real duration that the sample has spent on the ground is calculated for the determination of half-life periods of the studied insecticides.

Sampling Method

There are four types of samples in this study and each of them necesitated a specific technique.

a) *Vegetation sampling*

Vegetation samples concern millet leaves at Nioro du Rip and the *Tribulus terrestris* at Richard Toll. Both are homogeneously distributed at their site. At Nioro du Rip, the millet plants are equidistant from one another and provide a vegetable cover of the soil variable from one parcel to another between 50 and 90 % while at Richard Toll, apart from the shoals which present a thick nature of herbaceous vegetation presence, the other spaces are regularly covered between 20 to 80 % by *Tribulus terrestris*. The sampling is done along a diagonal, at the rate of one or two leaves per plant.

On the Richard Toll grasslands, the grass was cut by scissors every 1 to 1.5 m on the diagonal. The final size of the sampling as well for the millet leaves as for the grass varied from 1 to 2 kg according to the size of the parcel.

b) *Soil sampling*

Soil samples are simultaneously taken with the vegetation in the same parcels. The soil is taken down to a depth of 4 cm with a cylindrical drill with a 3.9 cm diameter and a penetrating part of 25 cm. A sliding plate along the body of the drill sets the depth of the sampling. The sample is taken every 2 to 3 meters on the diagonal of the parcel. Humidity due to rain facilitates the capture. The final size of the sampling goes from 2 to 3 kg of soil according to the size of the parcel.

c) *Millet sampling*

Millet ear were sampled before harvesting at the parcels the same way as the leaves. The collected

quantity varies between 1 to 2 kg.

At the level of the cylinder-shaped loft which has a diameter of 1 m and a height of 1 m, and which is locally made with tree or small shrub branches, the sample is taken on three spots at a rate of 5 points per spot : 1 at the centre and 4 others at the corners of a concentric square at the base of the loft, and are located at 20 cm from the edge.

The three sampling spots are chosen in such a way as to avoid the edge effects. They are taken at 20,50 and 80 cm from the bottom of the loft.

Two ears are taken per spot and their temperature is measured by a contact thermometer.

d) *Millet grains sampling*

Millet grains are separated from the ears by threshing. It is stored in bags and kept in the farm store. The grain samples are taken using a drain pipe long enough to reach deep in the bag. The instrument allows to take a sample right at 5 points of the diagonal.

Preparation of the samples

The sample preparation includes two important stages: the reduction of the inital size of the sample to a more reasonable quantity for the transportation and conditionning. Both of these two phases must not affect the representativity of the sample.

a) *Sample treated with fenitrothion, bendiocarb and chlorpyrifos*

Millet leaves at Nioro du Rip and the *Tribulus terrestris* at Richard Toll, as well as the millet ears are split up in little pieces using scissors and then well mixed up. 500 grams are weighed and packed in aluminium foil. The thus obtained sample is kept in an icebox with ice blocks where it will be transported to the laboratory. The preservation before analysis is at -18°C in a freezer.

The grains are well mixed and a quantity of 500 g is wrapped in aluminium foil and the whole is closed in a plastic jar. After putting it in an icebox for the transportation, the sample is stored under the same conditions as the millet ears.

b) *Samples treated with diflubenzuron*

Samples of millet leaves and *T. terrestris* were homogenized and split in the same way. 500 g was air dried in the laboratory.

Preparation of controls: Preservation method testing

A control sample is prepared by spiking a clean sample with a known quantity of product. The spiking solution is prepared in acetone with the formulation at 300-2000 mg a.i./l. Acetone is known for its high volatility and its capacity to disolve pesticides. The volume of solution corresponding to the deposit is taken with high-precision micro pipette and poured drop by drop on the surface of the spread control sample. After a few minutes of evaporation of the acetone, the control is carefully mixed, wrapped and stored under the same conditions as the corresponding samples till the moment of analyses.

It will be noticed that the preservation duration of the samples and of the controls that go with them, vary between 3 to 6 months : the first ones with fenitrothion were analyzed in December 1992, and the last ones with bendiocarb have been anlyzed in February 1993.

Preparation of controls : Recovery rate

The sample preparation for the determination of the recovery rate of the analysis methods is done in the same way apart from that the spiking solution is an analytical standard of purity next to 100% and the storing duration exceeds seldomly 24 hours (Annex 4 : preparation of controls).

ANALYSIS OF RESIDUES

Materials and methods of extraction, purification and reading of samples and controls

Fenitrothion and Chlorpyrifos

These two organophosphorous compounds were analyzed by gas chromatography. The device used is a Delsi 200 equipped with : a Flame Photometry Detector (FPD) selective for sulphurated or phosphorous compounds and a filled glass column 2m long, 0.64mm (1/4") of inner diameter and 2mm of outer diameter. The inert is a chromosorb WHP HMDS consisting of particles with a 80-100 mesh. The liquid phase is a gel OV 101 impregnated at 5% on the support.

The extraction and concentration of the compound on the millet leaves were carried out according to the protocol proposed by the manufacturer (Sumitomo 1981).

a) For the millet ears and grains, the extraction of pesticides in the cereals is carried out using an adapted of the Steinwandter method used by the GTZ/Darmstadt.

   1) The grains are first ground in an IKA Grinder. Then a test quantity of 25 g is moistened with water in a WARRING Blender. 200 ml of acetone and 30 g of sodium chloride are added and mixed up for about a minute. 150 ml of dichloromethane is re-added before proceeding to a second more vigorous and longer mixing. After a 5 minute pause, the mixture is filtered on anhydrous sodium sulfate bed (Na_2SO_4) (4 to 6 spoonfuls). The progressive and complete recovery of the mixing solvent is done by addition of a small quantity of NaCl .

   2) The thus obtained filtrate is decanted in a 500 ml glass balloon and evaporated in the Rotavapor up to 5 ml.

   3) The 5 ml are transfered in a 10 ml gauged vial and the balloon is rinsed with acetone so as to bring back the whole to the gauge line.

b) From the soil, fenitrothion is extracted according to a method copied from the residue analysis laboratory of GTZ/Darmstadt/Germany :

   1) 50 g of moistened soil with 15 ml of distilled water (DW) are carried with 400 ml of a 1:1 hexane mixture: aceton in a Shott Duran flask and shaken for 3 hours at the mechanical shaker.

   2) The flask content is filtered in a balloon on folded paper and the bottle is rinsed with 50 ml of the mixture. At the end of the filtration, the paper walls are washed with aceton jets using a Pasteur pipette.

   3) The balloon content is then evaporated up to 60-80 ml and decanted in a decanting vial with 600 ml of DW and 10 ml of a solution saturated with NaCl for washing. Recovery of the organic phase will be followed by washing of the aqueous phase with 35 ml of hexane. The collection of the organic phases in a same decanting vial of 250 ml allows to have them undergo two new washings with 100 ml of DW in presence of 5 ml of the NaCl solution.

   4) A last filtration on a Na_2SO_4 bed and then an evaporation of the solvent finish the operation followed by a resumption of the sample in 5 ml of hexane using a gauged vial.

Diflubenzuron

Diflubenzuron is detected in High Performance Liquid Chromatography (HPLC) by UV at the wavelength of 254 nm. The Zorbax column is a C8 (7-8 microns) 25 cm long and with an inner diameter of 4.6 mm. The elution solvent is a mixture of dioxane : acetonitril : water (10 : 45 : 45). At 1.5 ml/mn flow, the average retention time of diflubenzuron is about 2mn50.

Diflubenzuron samples are extracted and purified on the basis of the protocol provided by the manufacturer Duphar (1988 a) ; a more detailed description is given in chapter 8.

Bendiocarb

Bendiocarb is analyzed by gas chromatography with the same device and the same column as for fenitrothion and chlorpyrifos. Its detection is done using an Electron Capture Detector (ECD) endowed with a Ni63 as radioactive source. At the derivatized state and under oven temperature of 250°C, the retentiont ime of bendiocarb is 8 mn.

Extraction, purification and derivatization are carried out according to the protocol proposed by Browne (1981):

a) Millet leaf

1) After weighing of the millet leaves 180 ml of dichloromethane (CH_2Cl_2) and 20 ml of methanol (CH_3-OH) is added and put in a WARRING Blender for grinding. After a 60 mn pause, the filtration takes place at the Büchner and the grinding rests are rewashed with 50 ml of a mixture of CH_2Cl_2 and CH_3-OH(1:9)

2) The combined filterings are washed at the decanting vial with 50 ml of water before passing on an anhydrous sodium sulfate bed (Na_2SO_4) for drying.

3) The concentration is done at the Rotavapor at 50°C after addition of 2 ml of iso-octane ($(CH_2CH_2)_2$-C-CH_3) to help completely chase out the CH_2Cl_2.

4) Purification of 1 ml final volume concentrate is done on SEP-PAK silica cartridges. The concentrate is disolved in 2 ml of hexane and passed on the silica cartridge. The fixation is followed by a double rinsing of the column with 2 ml of the same solvent. A second rinsing is effectuated with 10 ml of a mixture 1:9 of ethyl acetate : hexane. The elution of the bendiocarb is assured with 8 ml of the modified mixture of the same solvants in the ratios of 1:3. The remains of the elution are then evaporated till dry for the derivatization.

5) The purified extract is recovered in 1 ml of a 1% fluoro-1 dinitro-2-4 benzene solution (($F-C_6H_3-(NO_2)_2$) to which is added 10 ml of a phosphate buffer solution (pH11). The reactional environment thus prepared in the evaporation balloon is maintained to 50°C in a double boiler with agitation. The solution, clear in the beginning turns to turbid as it goes along.

6) At the end of the operation (20 mins.), the obtained solution is cooled down to room temperature (25-30°C) and extracted at the decanting vial with 20 ml of hexane. The yellowish aqueous phase is thrown away and the light phase is retained and completed to the gauge line of a 20 ml vial with hexane for the chromatographic detection.

b) Millet ear

Bendiocarb extraction in millet ears and grains begins with a dry grinding in an IKA Grinder. Soxhlet treatment which should have been used has not been carried because of the loss of solvent due to the temperature of the freezer water which is not low enough to totally condense the dichloromethane vapors. It is for this reason that the same treatment as that of the millet leaves has been applied.

c) Soil

For the soil, instead of the Soxhlet, the mechanical shaker has been used : the weighed sample is moistened with distilled water then put in a Duran flask with the solvant mixture to be vigorously agitated for 2 hours.

The controls are treated in the same way as samples but as a result of their being generally more loaded, the filtering rests obtained from the grinding are partially analyzed: only a quarter has been used for the operations.

RESULTS

Meteorology

A wind stillness in the early morning at Nioro du Rip forced us to treat between 8:30 and 10:00. When started, its direction was generally North-South which is dominant at the monsoon period of the year. The speed was oscillating between 1 and 4 meters/second. (Annex 1).

The ambient temperature taken in the shade was characrteristic of the rainy season: it varied between the morning and the mid-afternoon on an average between 25°C and 33°C. In the same period, the soil temperature taken at sunshine between 0 and 4 cm of depth to give account of the thermal exposure of pesticides in this environment varied from 25°C to 38°C (Annex 9.2).

The pluviometry in this Nioro du Rip sector at the moment of the testings remained regular (2 precipitations on an average every 3 days) and abundant (25 to 31 mm). (Annex 9.2).

At Richard Toll, the wind started earlier in the morning which allows to do the first treatments at from 8:00 onward. It is generally of the same importance as at Nioro du Rip but adopts directions that may change from one day to another.

The temperature appeared to vary from 27 to 33°C between seven and ten o'clock. The soil temperature taken in the same conditions as at Nioro du Rip showed a quick rise of 1.8°C/hour between 7 and 12-13 hours maxima going up to 45°C : (Annex 9.2).

It did not rain during study period.

Qualitative aspect of the treatment

The qualitative aspect of the treatment is described by statistical analysis of the distribution of the spray droplets. Table 9.4 gives results of the droplet counting on oil-sensitive paper, the treatment dosed of the sites and the wind speeds. Figures 9.1 and 9.2 show the graphic representations based on these data.

Table 9.4 : Doses and density of deposit and wind speed

Fenitrothion/Nioro du Rip				
Parcel	Applied dose g a.i./ha	Density of drops (/cm²)		Wind speed
		Vertical	Horizontal	(m/s)
01	465	72(57)	27(53)	1.25-2.0
04	490	97(97)	117(105)	1.7-2.5
06	445	59(71)	37(50)	1.75-1.75
10	415	59(34)	28(23)	1.25-1.75
Fenitrothion / Richard Toll				
Parcel	Applied dose g a.i./ha	Density of drops (/cm²)		Wind speed
		Vertical	Horizontal	(m/s)
01	1030	110(74)	43(25)	1.4-1.8
04	710	33(18)	36(10)	2.75-2.75
08	800	20(15)	48(47)	1.75-2.0
11	790	83(62)	49(30)	1.26-1.65
Bendiocarb/Richard Toll				
Parcel	Applied dose g a.i./ha	Density of drops (/cm2)		Wind speed
		Vertical	Horizontal	(m/s)
02	169	24(22)	18(15)	1.5-2.25
05	194	17(16)	26(13)	1.75-2.25
10	167	na	na	1.25-1.95
14	153	86(65)	26(9)	1.5-3.5

The numbers between parentheses are the standard deviations. The density of drops is an average counted on 10 cards.
na : no values available.

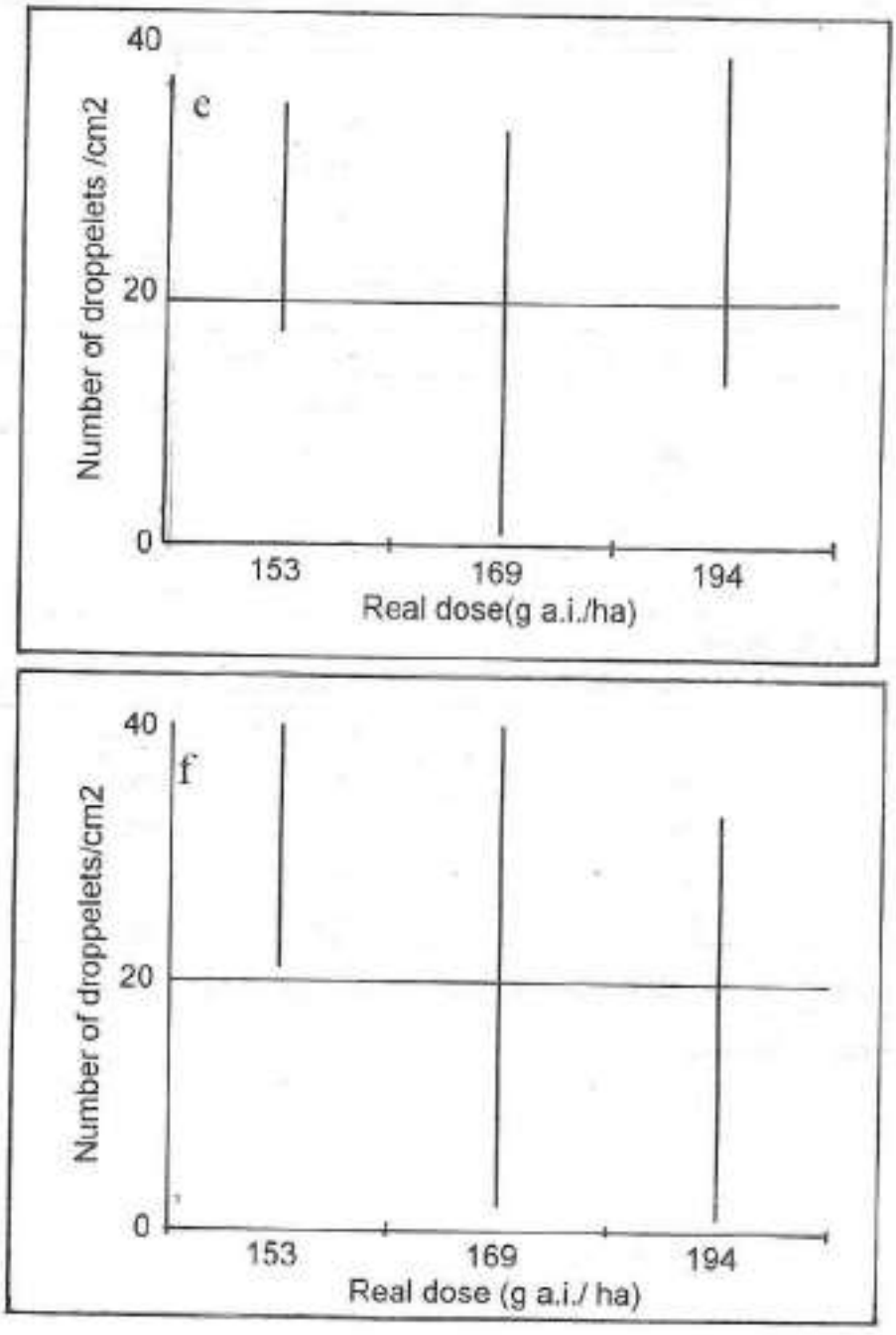
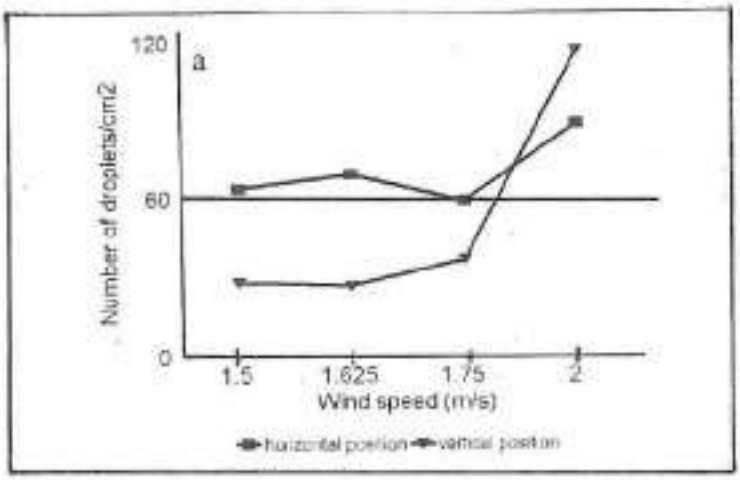
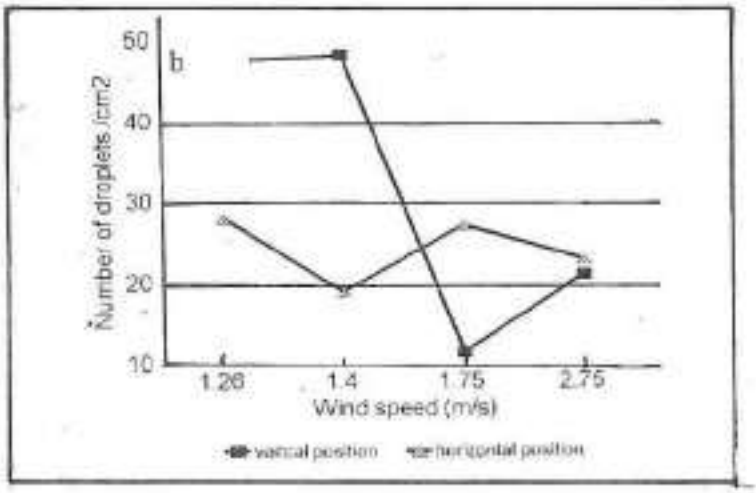


Figure 9.1 bis : Distribution of bendiocarb droplets on oil-sensitive paper. The small square and the vertical line respectively designate the deposition density average and the corresponding standard deviation.
e) Richard Toll, Horizontal component
f) Richard Toll, Vertical component





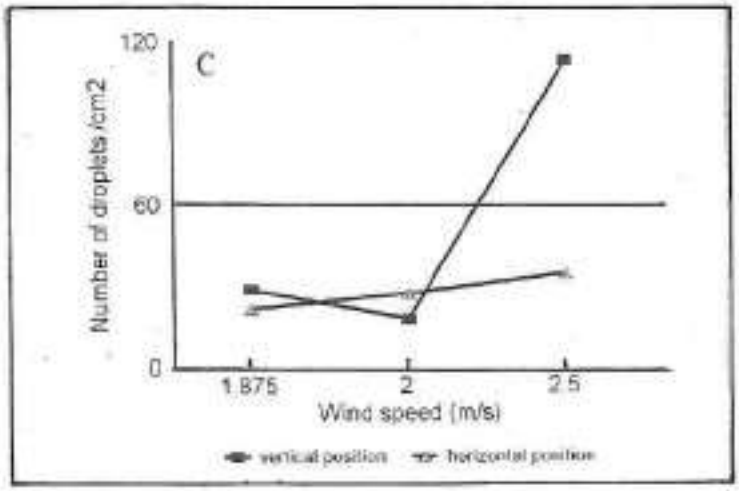


Figure 9.2 : Distribution of droplets and average speed of wind during treatment.
a) Fenitrothion on millet at Nioro du Rip
b) Fenitrothion on grass at Richard Toll
c) Bendiocarb on grass at Richard Toll

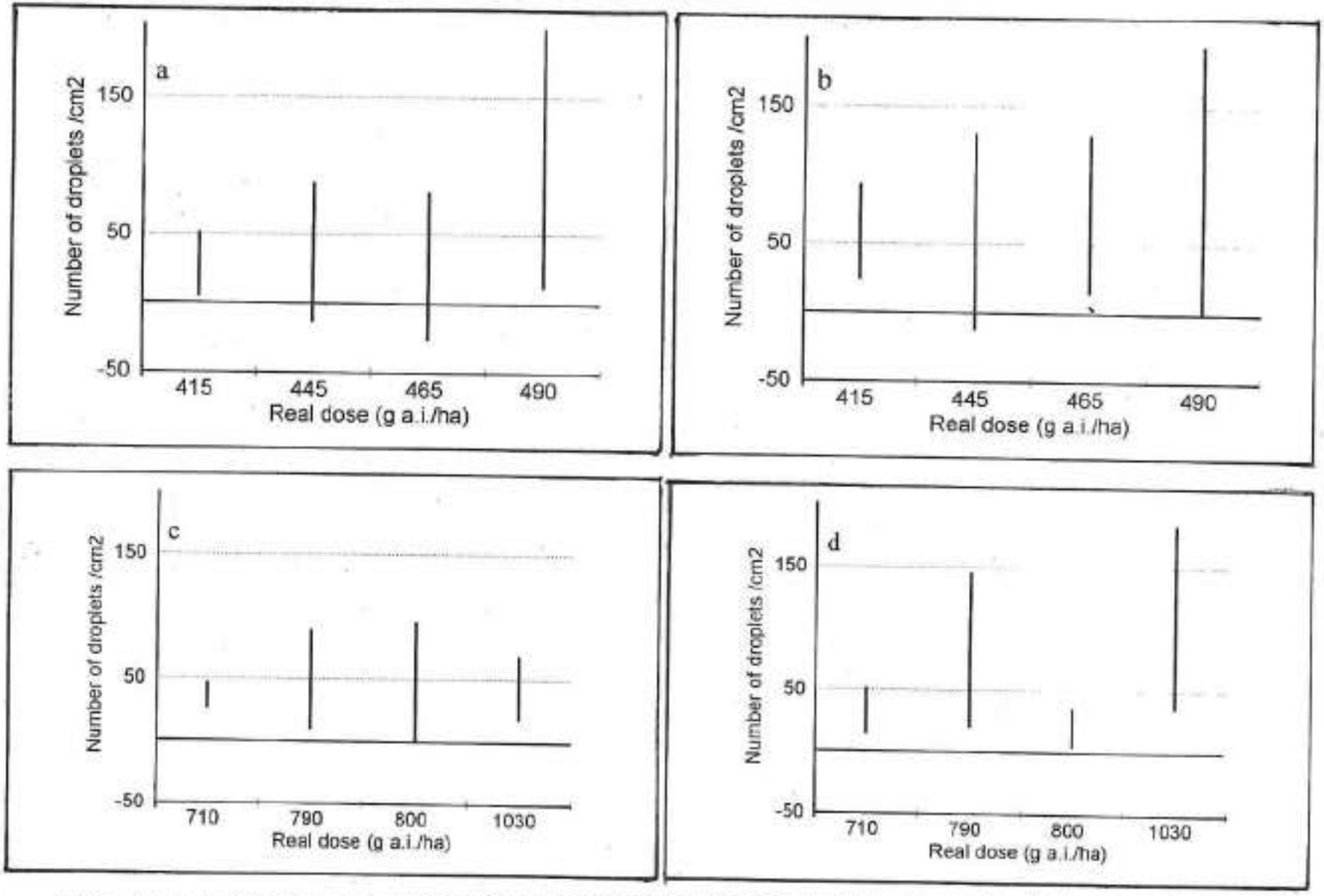


Figure 9.1 : Distribution of fenitrothion droplets on oil-sensitive paper . The small square indicates the value on the axis of the deposition density average, the vertical line designates the corresponding standard deviation.

a) Nioro du Rip, horizontal component b) Nioro du Rip, vertical component

c) Richard Toll, horizontal component d) Richard Toll, vertical component

Deposit, discussion of the results

The results in table 9.4 show that the droplet deposition varies from one parcel to another and sometimes independently from the applied dose.

The standards deviations show an unequal distribution of the product on the treated site. They are in some cases of the same range as the averages and largely exceed them in some cases. This irregularity may depend on the treated environment.

Especially, the deposition in the millet, an environment cluttered by the presence of high plants, shows standard deviations largely superior to the average: 196 % on the horizontal component of parcel 01, 120 % and 135 % on both components of parcel 08. The treatment on the Richard Toll sites, show standard deviations in the order of 25 to 100 % and reveal an open environment. Figure 1 shows that the deposition on the vertical component of the paper is generally higher than on the horizontal which is characteristic for a drift spray .

The graphic representation of the droplet density according to the wind speed (Figure 9.2) generates also another aspect of the treatment quality. (450 g a.i./ha fenitrothion, and 200 g a.i./ha for bendiocarb). The curves show in the case of fenitrothion at Nioro du Rip and in that of bendiocarb at Richard Toll, that the vertical component is related to the wind speed. The same tendency on the horizontal component is observed. As for fenitrothion on grass at Richard Toll, however, the curves seem to show an opposite effect.

In conclusion, the interest of laying the paper is in recording the regularity of the deposit on the site knowing that the deposit on the paper does not reflect exactly the deposit of the product on the vegetation.

Qualitative aspect of the analyses

The qualitative aspect of the analyses concerns a certain number of analytical parameters likely to affect the validity of definitive results. It is the behaviour of the preservation controls which indicates the state of studied products during the preservation of samples and the recovery of controls which give information on the reliability of the analyses done on the basis of protocols.

Fenitrothion and diflubenzuron have been subject to a comparable study during the 1991 campaign (Chapter 8) on vegetation and in pond waters. In that case, verification of the control and of the recovery rate in the soils was done for fenitrothion.

Bendiocarb is subject to a complete study while chlorpyrifos is only checked on millet ears and grains.

Table 9. 5 : Controls of fenitrothion on soil : preservation mode tests.

Nature	Size	Load mg a.i.	Found mg a.i.	Recovery %
1 Soil	100g	0.75	0.69	92.0
2 Soil	100g	0.50	0.51	102.0
3 Soil	100g	0.25	0.17	68.0

Table 9.6 : Recovery rate of the extraction method : Fenitrothion in soil.

Size (g)	Load (mg a.i.)	Found (mg a.i.)	Recovery %
50	0.50	0.391	78.2
50	0.25	0.278	111.2
50	0.10	0.087	87.0

Table 9. 7 : Controls of chlorpyrifos on grains and ears : preservation mode tests.

Nature	Size	Load mg a.i.	Found mg a.i.	Recovery %
1 Seed	100g	1.00	1.170	117.0
2 Seed	100g	0.50	.474	94.8
3 Seed	100g	0.25	.232	92.8
1 Ear	100g	1.0	.931	93.1
2 Ear	100g	0.50	.410	82.0
3 Ear	100g	0.25	.222	88.8

Table 9. 8 : Recovery rate of the extraction method of chlorpyrifos on grain and ear.

Nature	Size (g)	Load mg a.i.	Found mg a.i.	Recovery %
1 Grain	50	1.00	0.937	93.7
2 Grain	50	0.50	0.412	82.4
3 Grain	50	0.25	0.181	67.0
1 Ear	50	1.00	1.09	109
2 Ear	50	0.50	0.391	78.2
3 Ear	50	0.25	0.217	86.8

Table 9.9 : Controls of bendiocarb on grass, soil, millet grain and ear : preservation mode tests.

Nature	Size	Load mg a.i.	Found mg a.i.	Recovery %
1 Soil	100g	1.00	1.210	121.0
2 Soil	100g	0.50	0.327	65.4
3 Soil	100g	0.25	0.199	79.7
1 Grass	100g	1.00	0.905	90.5
2 Grass	100g	0.50	0.420	84.0
3 Grass	100g	0.25	0.179	71.6
1 Grain	100g	1.00	0.920	92.0
2 Grain	100g	0.50	0.444	88.8
3 Grain	100g	0.25	0.192	76.8
1 Ear	100g	1.00	1.023	102.3
2 Ear	100g	0.50	0.479	95.8
3 Ear	100g	0.25	0.251	100.4

Table 9.10 : Recovery rate of the extraction methods of bendiocarb on grass, soil, millet grain and ear.

Nature	Size	Load mg a.i	Found mg a.i.	Recovery %
1 Soil	50g	0.500	0.490	98.0
2 Soil	50g	0.250	0.219	87.6
3 Soil	50g	0.100	0.157	157.0
1 Grass	50g	1.00	0.845	84.5
2 Grass	50g	0.50	0.434	86.8
3 Grass	50g	0.25	0.190	76.0
1 Grain	50g	1.00	0.795	79.5
2 Grain	50g	0.50	0.384	76.8
3 Grain	50g	0.25	0.191	76.4
1 Ear	50g	1.00	1.100	110.0
2 Ear	50g	0.50	0.467	93.4
3 Ear	50g	0.25	0.242	96.2

The results presented in tables 9.5 to 9.10 show that the storing quality in high freezing (temperature inferior to -15°) of the active matters of fenitrothion, bendiocarb and chlorpyrifos on vegetation, on millet grain and ear is fairly good : all averages on the results of controls are superior to 80 %. As well, we notice that the found recovery rates reveal the simplicity of the different extraction, purification and reading methods used. There also, the results are on average generally superior to 85 % with a small disparity in the level of bendiocarb where derivatization.

Results of analyses

The results are given in mg of active ingredient (a.i.) per kg of vegetation, ears, grain or wet soil. The water content(w) of samples has been determined several times for each matrix following the sampling period. The averages and standard deviations on (w) are calculated and arranged in Table 9.11.

Table 9.11 : Water content of sampled material.

State	Nature	(-) medium %	Nber of measures	Standard deviation(%)
Fresh	Millet leaf	76.17	16	0.23
	T. terrestris	83019	10	1.87
	Soil N. du Rip	11.37	16	2.90
	Soil R. Toll	1.64	9	0.77
	Millet grain	10.12	4	1.20
	Millet ear before drying	29.62	5	5.34
	Millet ear after drying	5.67	5	1.64
Dried*	Millet leaf	12.00	14	1.23
	T. terrestris	12.95	11	3.53

*Dried matrixes after sampling concern diflubenzuron samples. This drying is natural and is obtained by opening and aeration of leaves or grass during several days on the laboratory draining board.

Then for diflubenzuron results to be considered in mg a.i./kg of wet matrix, they are multiplied by the factor (100-(w)r)/(100-(w)i) where (w)r and (w)i respectively designate the sample's water content at the fresh state and the sample's water content at the natally dried state. This factor is on an average of :

0.271 for the Nioro du Rip millet leaves.
0.193 for the Richard Toll *T. terrestris*.

Fenitrothion results (Table 9.12) show that the values of the deposition in pesticide one hour after treatment vary between 30 and 55 mg a.i./ha (ppm) on millet leaf. At Richard Toll on grass with practically doubled doses, the range is from 62 to 95 mg a.i./kg.

Comparison with the 1991 results shows a small drop of the present values, the former figures varying between 55 and 71 mg a.i./kg on the Nioro du Rip leaves. The soil samples taken on the first 4 cm indicate initial rates (one hour after treatment) varying between 0.5 and 2 mg a.i./kg. The deposition values found at Richard Toll are lower : 0.245 and 0.134 mg a.i./kg.

Table 9.13 shows the results of diflubenzuron analyses. The values represent the average between analysis results coming from two different laboratories. The samples have been sent to them for chromatographic reading after extraction and purification at the project laboratory. We point out that the results of both laboratories keep a same size order concerning the vegetation samples, but concerning the soil, we notice some disparities which can go up to a ratio of 10. The figures on the initial deposition are between 30 and 45 mg a.i./kg. Only the value obtained at parcel 02 of Niorodu Rip exceeds this range to rise up to 80 mg a.i./kg. This disparity is again accentuated while reading the column of sample 2 which displays values superior to those of the first one. Diflubenzuron depositions found in the soil are generally more important than those found with fenitrothion.

Two depositions at 3.24 and 2.39 mg a.i./kg on the Richard Toll parcels were comparable to the depositions of 1.26 and 1.52 mg a.i./kg found on two Nioro du Rip parcels. Other depositions found at Nioro du Rip are 5.49 mg a.i./kg on parcel 02 and 8.68 mg a.i./kg on parcel 06.

Table 9.12 : Fenitrothion residues on vegetation and soil (mg a.i./kg of wet matter)

Site	Parcel	Nature	Sample1	Sample2	Sample3	Sample4
N. du Rip	01 465	Leaf	34.34	1.890	0.005	0
		Soil	1.859	0.337	0.002	0
		Hr/rain	2.67/0	27.42/31	71/101	168/254
	04 490	Leaf	53.22	2.277	0.009	0
		Soil	0.478	0.131	0.099	0.014
		Hr/rain	1.75/0	27/31	70.5/101	167.5/254
	08 445	Leaf	28.55	0.6	0.053	0
		Soil	1.044	0.244	0.013	0
		Hr/rain	1.92/0	25.58/4	72/55	168.5/191
	10 415	Leaf	44.95	0.404	0.029	0
		Soil	0.501	0.061	0.044	0.042
		Hr/rain	1.58/0	28.27/4	72.92/55	168.42/191
R. Toll	04 711	Grass	85.87	44.4	0.877	0
		Soil	0.245	0.151	0.149	0.034
		Hr/rain	1.5/0	24.5/0	73/0	169/0
	08 800	Grass	62.10	0.224	14.79	2.70
		Soil	0.134	22/0	0.124	0.213
		Hr/rain	1/0		70.5/0	167/0
	01 1030	Grass	86.89			
		Hr/rain	2/0			
	17 789	Grass	95.09			
		Hr/rain	1.5			

* The parcels are coded by a five digit number, the first two indicating their sequence number and the last three the real dose in mg a.i./ha with which they have been treated. In the line Hr/rain, the first number indicates the number of hours spent since the treatment before the sample has been taken from the site, the second number shows the pluviometric cumul in mm which the parcel receives between the treatment date and that of the sampling.

Table 9.13 : Diflubenzuron residues in mg a.i./kg of vegetation and wet soil.

Site	Parcel	Nature	Sample1	Sample2	Sample3	Sample4	Sample5
N. du rip	02 065	Leaf	60.25	83.9	33.83	26.16	18.65
		Soil	5.49	1.94	0.12	1.33	0.10
		Hr/rain	1/0	23.75	72/68	167/175	336/210
	06 062	Leaf	41.28	60.62	20.59	16.78	25.60
		Soil	8.68	2.27	0.31	1.83	2.08
		Hr/rain	1.5/0	24.7/0	71.7/68	167.3/175	335.5/210
	09 081	Leaf	31.83	24.29	16.18	0.39	17.53
		Soil	1.52	1.08	0.80	0.43	0
		Hr/rain	1/0	23.8/16	72/122	167/144	335/153
	12 061	Leaf	Lost	25.03	39.47	13.72	23.57
		Soil	1.26	0.97	0.07	0.68	0.59
		Hr/rain	2.3/0	26.3/16	75.2/122	170.144	338/153
R. Toll	08 111	Grass	47.62	42.83	20.58	6.85	3.48
		Soil	3.24	1.90	4.21	1.41	0
		Hr/rain	1.4/0	23.6/0	72.5/0	168.5/0	337/0
	16 104	Grass	34.31	78.45	49.69	16.18	6.12
		Soil	2.39	0.75	1.33	1.23	0.57
		Hr/rain	0.8/0	24/0	72.7/0	168.2/0	336.2/0

** Same comments as Table 12*

Table 9.14 : Bendiocarb residues in mg a.i./kg of vegetation and wet soil.

Site	Parcel	Nature	Sample1	Sample2	Sample3	Sample4	Sample5
R. Toll	02 169	Grass	26.90	3.33	2.14	1.650	0.049
		Soil	0.029	0.053	.008	0.012	0.007
		Hr/rain	1.5/0	25/0	71.75	168/	337/0
	05 194	Grass	51.37	7.95	0.14	0.47	
		Soil	0.025	0.005	0.005	0	
		Hr/rain	0.17/0	94.25/0	167/0	336/0	
	10 167	Grass	38.96	8.61	2.06	0.92	
		Soil	0.03	0.011	0.009	0.004	
		Hr/rain	0.5/0	94/0	167.7/0	335.3/0	

** Same comments as Table 12*

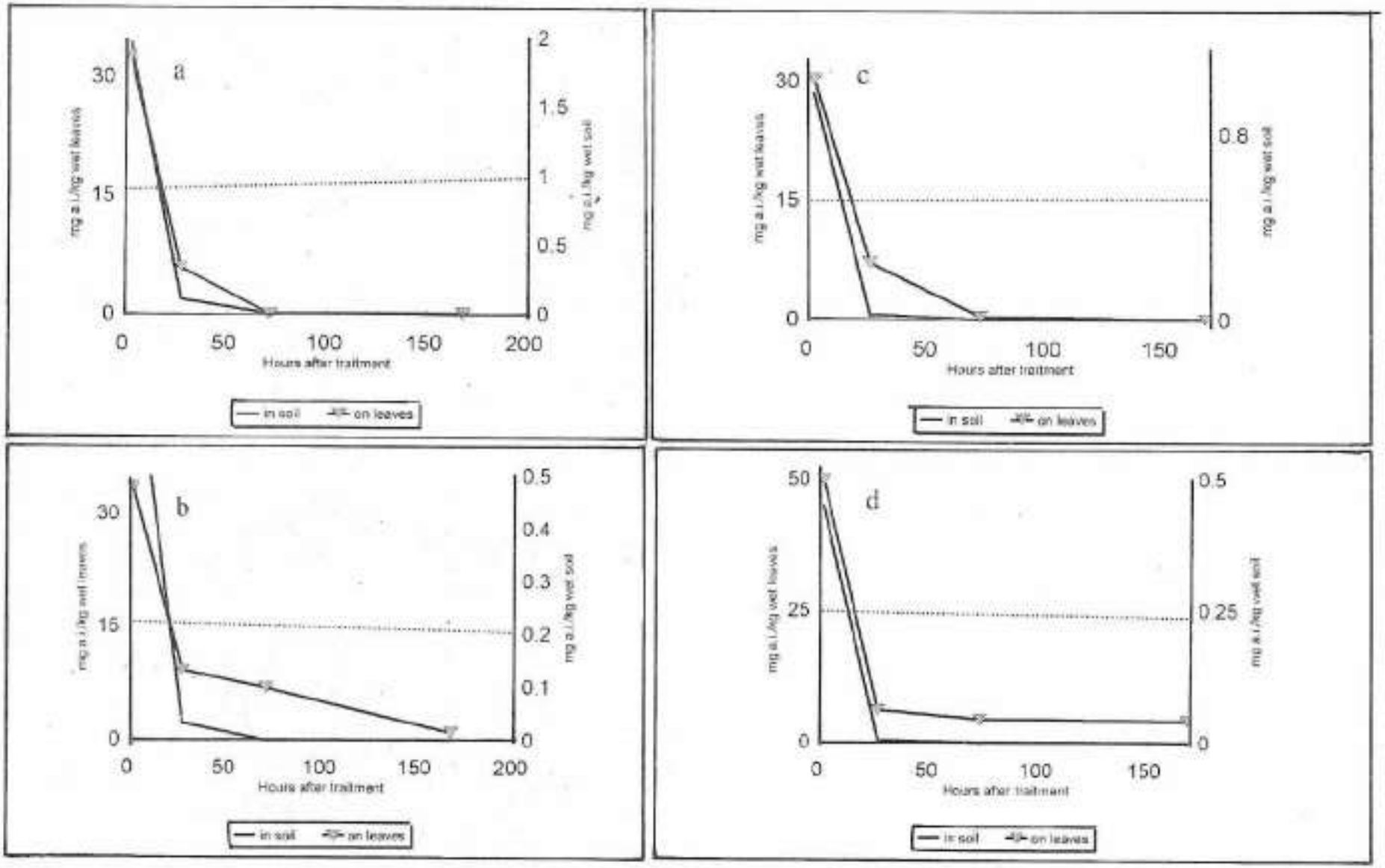
Bendiocarb has been measured on Richard Toll parcels. Table 9.14 indicates the results. The initial depositions found are low when compared to the values of fenitrothion and those of diflubenzuron, they are respectively 26.9, 39.0 and 51.4 mg a.i./kg for parcels 02, 10, and 05.

Disappearance of the compound after treatment

The curves (Figures 9.3, 9.4, 9.5) respectively show a very quick disappearance on the relatively rainy sites of Nioro du Rip. In all cases the drop of the residue down to 6% of the initial rate is observed within 24 hours following the treatment. The product continues to disappear with certainly a less pronounced slope, but ends up being no longer detectable at the end of the week after treatment. This is also observed on millet leaves and on the soil where the product disappears within the first 24 hours by a value between 73 and 88%. At Richard Toll on the hot and dry sites, even if the appearance has remained almost the same, the disappearance slopes are less steep. Fenitrothion disappeared by 59% and 49% within 24 hours in both situations. On the soil, degradation is slow on parcel 04 (13% of product subsisting one after week) and uncertain on parcel 08.

Figure 9.4 shows the behaviour of diflubenzuron on vegetation and in the soil. At first, we notice a slower disappearance than in the case of fenitrothion. Despite a pluviometry more or less identical to Nioro du Rip, no total disappearance of the product on vegetation could be observed. The degradation on millet leaves during the first 14 days is a little bit significative compared to those of fenitrothion : only of 78 % on parcel 02 and 40 % on parcel 06. In the Nioro soil, even if the disappearance speed of the porduct proves more quick, the last remark is also valid. At Richard Toll the product persists less on vegetation : it disappears from 83 % on parcel 16 to 93 % on parcel 03. On the soil of the same site, diflubenzuron disappeared at the end of two weeks after treatment by 77 % in one case and by 100 % in another.

The fate of bendiocarb is presented in Figure 9.5. At Richard Toll where it has been experimented, no total disappearance is observed, but the remaining rates at the end of the two weeks are small: between 0.1 and 2 % of the initial rate. On the soil, the product disappears quickly and is almost non-existing after 14 days.



Figures 9.3 : Fenitrothion disappearance on millet leaf and in the soil at Nioro du Rip.
a) Parcel 01, b) Parcel 04, c) Parcel 06, d) Parcel 10

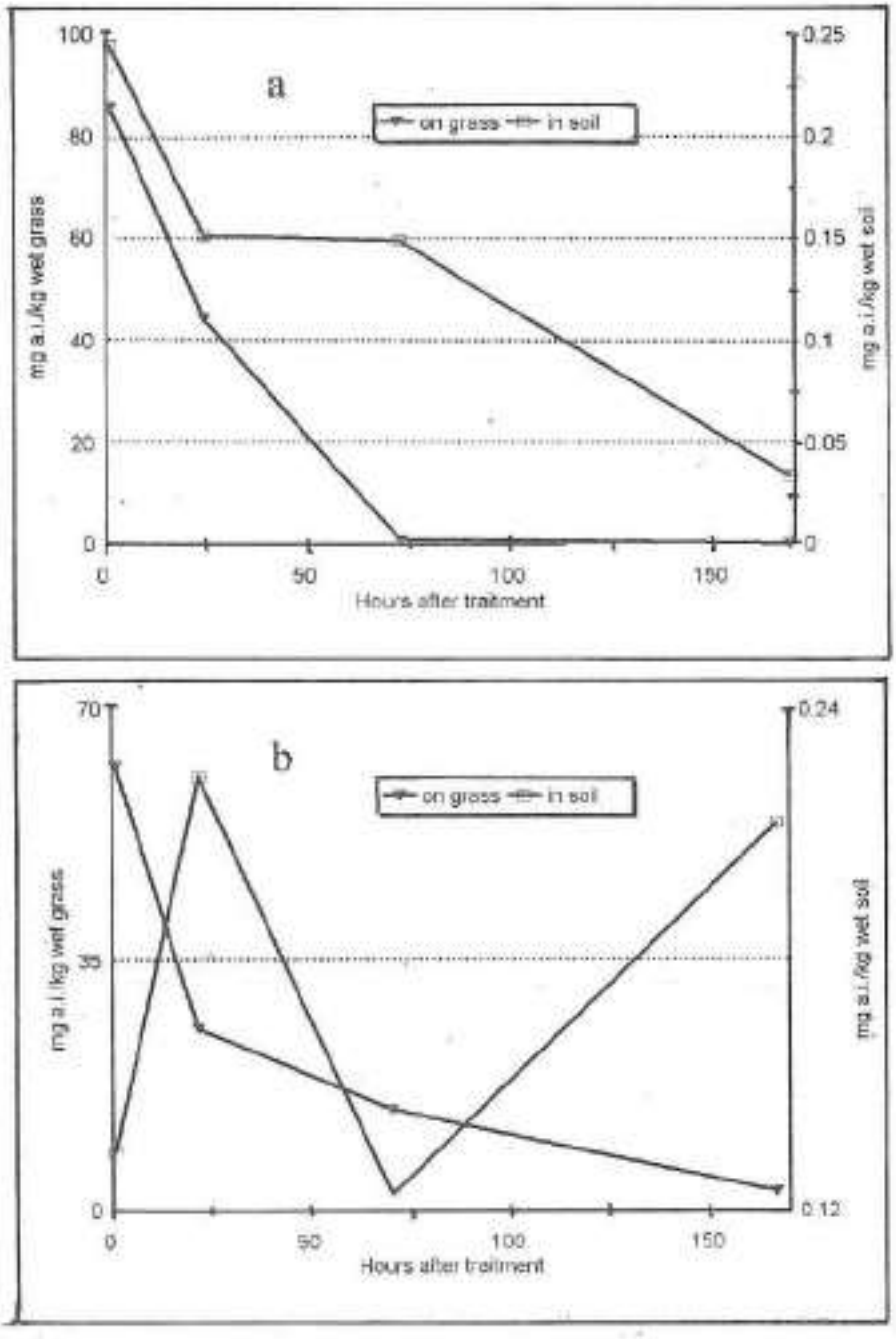


Figure 9.3. : Fenitrothion disappearance on T. terrestris and in the soil at Richard Toll.
a) Parcel 04 b) Parcel 08

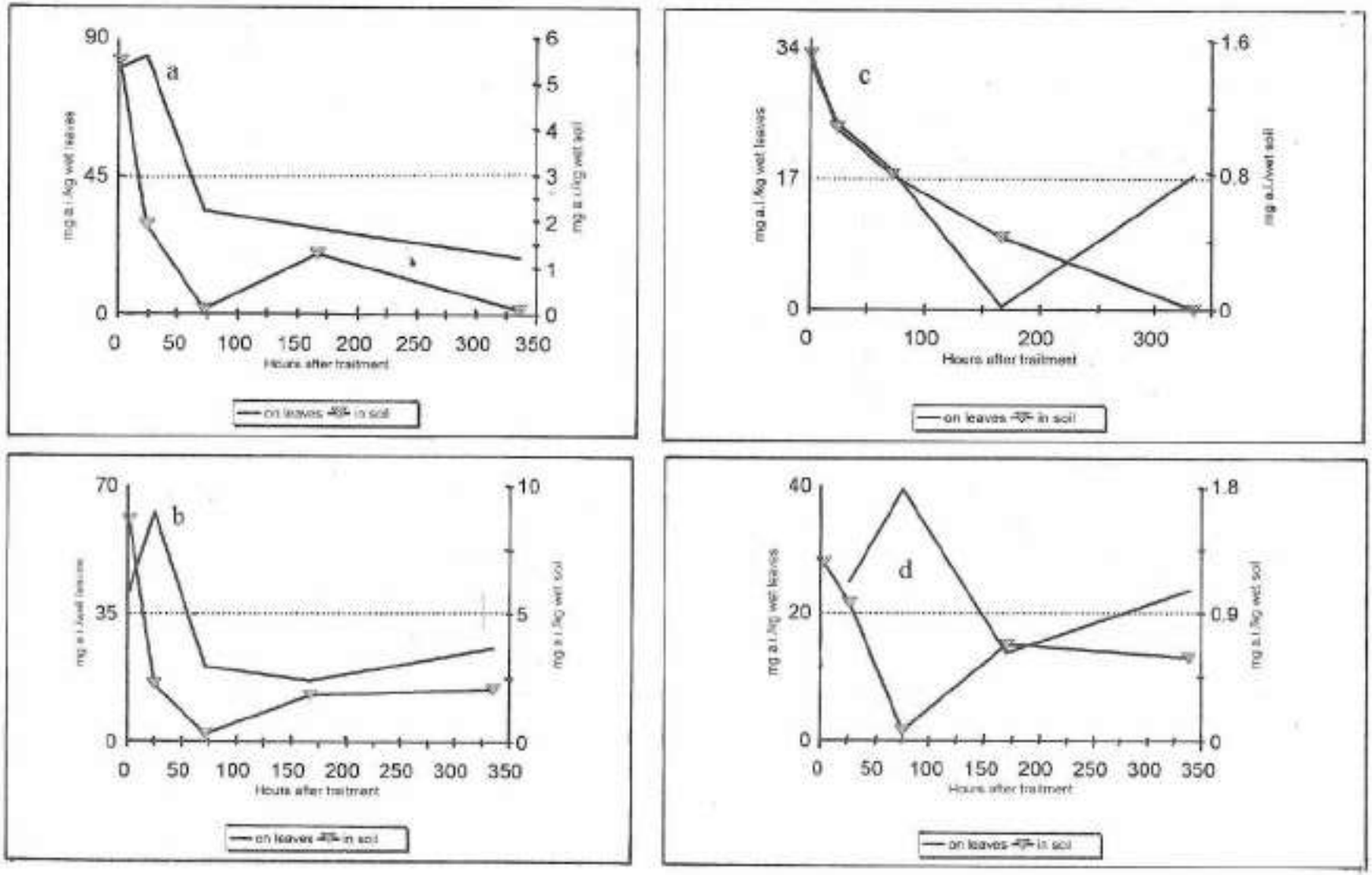
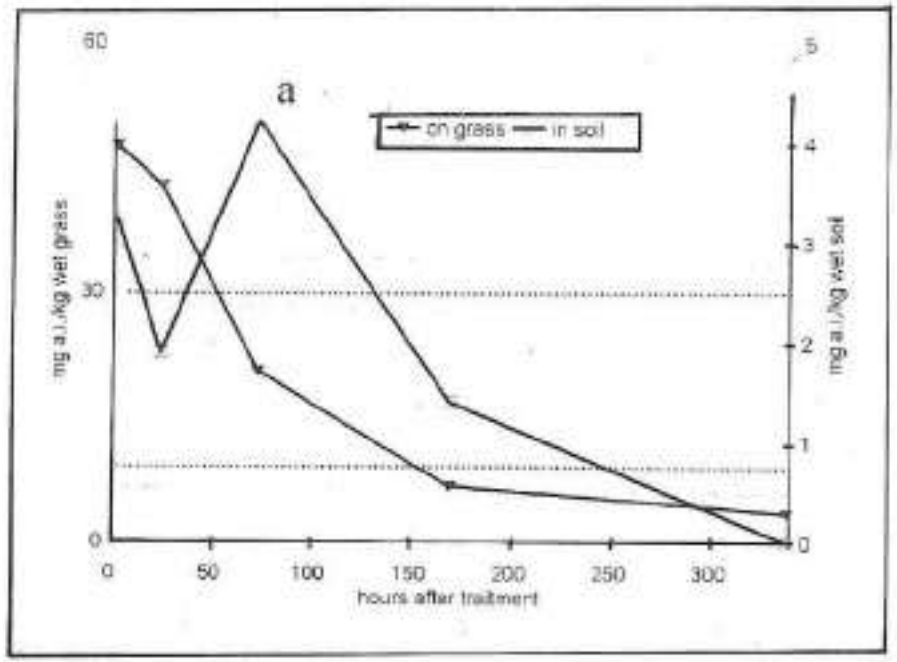
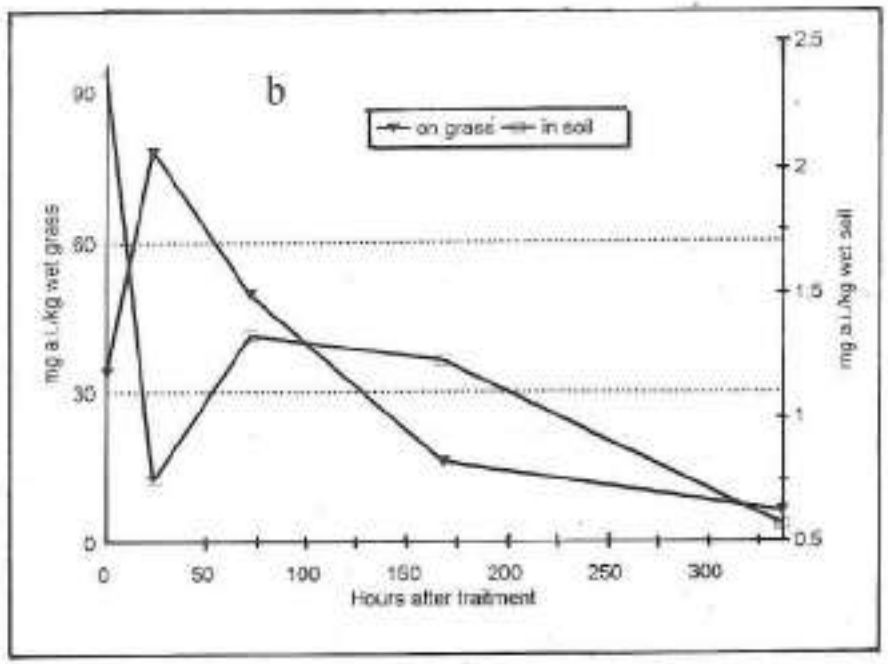
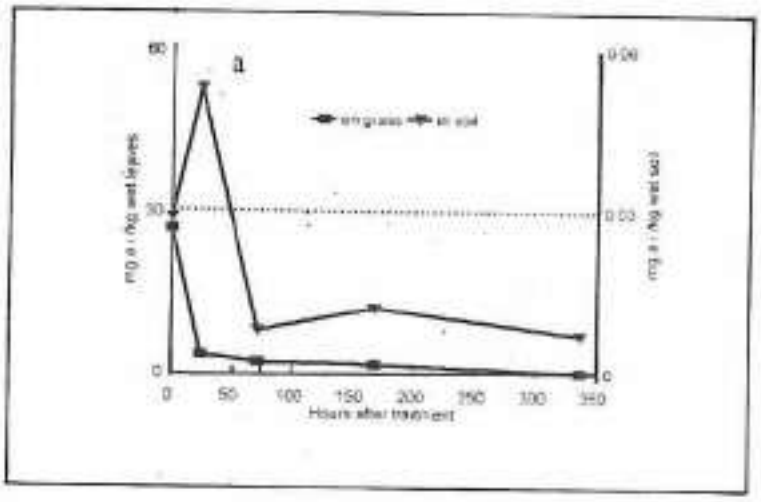


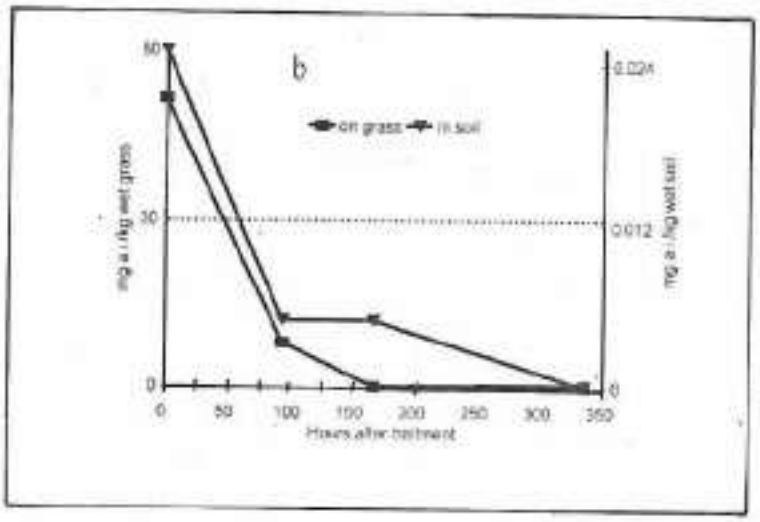
Figure 9.4 Diflubenzuron disappearance on millet leaf and in the soil at Nioro du Rip
a) Parcel 02, b) Parcel 05, c) Parcel 09, d) Parcel 12





Figures 9.4 bis : Diflubenzuron disappearance on T. terrestris and in the soil at Richard Toll.
a) Parcel 03. b) Parcel 16.





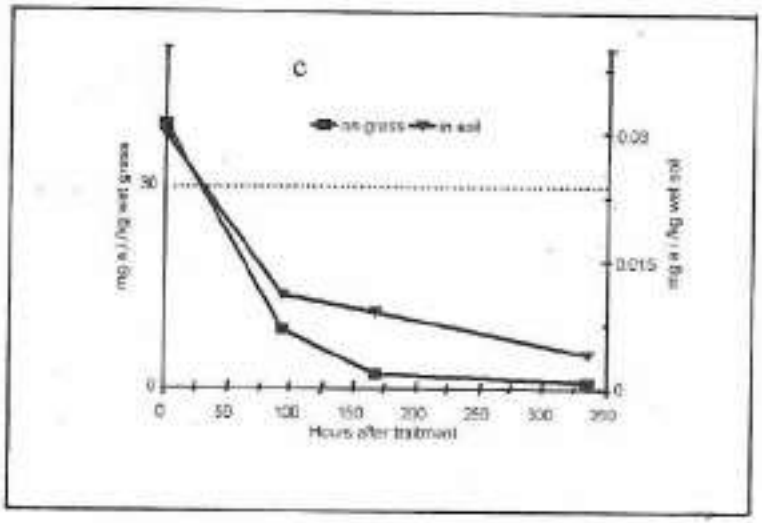


Figure 9.5 : Bendiocarb disappearance on *T. terrestris* and in the soil at Richard Toll.
a) Parcel 02, b) Parcel 05, c) Parcel 10.

Residue monitoring in farm stores : results

Table 9.15 gives the residues analysis results on the samples coming from farm stores of millet grains and ears. Figure 6 gives the corresponding disappearance curves.
Fenitrothion formed a deposit at the rate of 0.45 mg a.i./kg of wet ears. This value progressively decreases to settle at 0.25 mg a.i./kg at the end of the week before harvesting. The disappearance of the product during the drying period is represented by the difference of rates between sample 4 and sample 5 that is to say 33 %. The analysis results of samples 7 and 8 show well that the product disappearance continues its process during the stocking in the loft. On the grains compared to the ears (0.101 mg a.i./kg the day of the storing) we find lower residues. The nature of the curve (Figure 6a) shows the progressive lowering of fenitrothion content of the grains during the first 63 storing days: 80 % disappearance. Beyond this time limit, the level remains almost constant until the 140th day.

Bendiocarb deposition on millet ear is lower than that found for fenitrothion: 0.20 mg a.i./kg only. Its disappearance on the parcel also seems quicker : 90% of product is lost at the end of the first seven days following the treatment. Before the storing, during the drying phase 50 % of the product disappears going from 0.02 to 0.01 mg a.i./kg on the respective samples 4 and 5. The residue rate of ears is so low at the moment of being put in the loft that the storing effect can not be perceived. On the grains, the same remark is valid: the pesticide rate is low (0.01 mg a.i./kg).

Rates of 0.18 and 0.19 mg a.i./kg have been found between the first and second day of treatment for chlorpyrifos on millet ears. The product was very quickly degraded and disappeared by more than 95% between the first and seventh day. The values before storing are already low. On the grains, chlorpyrifos has not been found.

Half-life periods

Table 9.16 contains the results of linear declines after neperian logarithm transformation of the residue data and the time between the treatment of the parcel and the sampling. The objective is to describe the degradation by a theoretical model which allows us to estimate the half-life periods $t_{1/2}$ and the initial depositions on the concerned matrixes. The model chosen is that of the first order reaction.

The table shows the behaviour of fenitrothion described by the model: the coefficient of correlation is close to one. The $t_{1/2}$'s found on millet leaf are between 5 and 8 hours while on *T. terrestris* at Richard Toll, they are higher: 10 and 39 hours. On the soil, the $t_{1/2}$ values found at Nioro vary between 11, 15, 36 and 63 hours. A 62 hour value is found on parcel 04 of Richard Toll.

The reaction model of first order doesn't fit well with diflubenzuron disappearance at Nioro du Rip: the coefficients of correlaton are generally low on vegetation and on soil. The $t_{1/2}$'s vary between 346 and 690 hours on vegetation, on the soil they are smaller: between 97 and 247 hours. The declines are more homogenous on *T. terrestris* at Richard Toll where R^2 is superior to 0.90 and the periods obtained are smaller (42 and 85 hours only).

Table 9.15 : Pesticide residues in farm stores.

Fenitrothion (Pcl 13)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample6	Sample9
Sampling date	10/8/92	10/9/92	10/10/92	10/15/92	10/19/92	11/4/92	12/10/92	02/9/93	03/5/93
Hour	10:15	10:45	10:00	8:45	8:45	11:30	10:30	12:20	9:15
Nber of days after treatment	0	1	2	7	11	27	63	124	148
Residue in ppm (grains)					0.101	0.04	0.016	0.021	0.019
Residue in ppm (ears)	0.446	0.309	0.282	0.245	0.163	0.293	0.072	0.055	
Bendiocarb (Pcl 14)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9
Sampling date	10/9/92	10/10/92	10/12/92	10/16/92	10/20/92	11/4/92	12/10/92	02/9/93	03/5/93
Hour	11:00	10:30	10:45	8:45	9:45	12:45	11:30	11:00	11:15
Nber of days after treatment	0	1	3	7	11	26	62	123	147
Residue in ppm (grains)					0.01	0.01	0.01	0.00*	0.00
Residue in ppm (ears)	0.20	0.03	0.01	0.02	0.01	0.00	0.01	0.01	
Chlorpyrifos (Pcl 15)	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample6	Sample9
Samplin date	10/9/92	10/10/92	10/12/92	10/16/92	10/20/92	11/4/92	12/10/92	02/9/93	03/5/93
Hour	10:00	9:30	9:45	8:45	8:45	10:00	9:00	9:15	9:30
Nber of days after treatment	0	1	3	7	11	26	62	123	147
Residue in ppm (grains)					0.002	0.00	0.00	0.00	0.00
Residue in ppm (ears)	0.176	0.191	0.004	0.008	0.074	0	0.006	0.0005	

The void values indicate that the residue rate is under the detection limit of the analysis device.
Harvesting was done the seventh day after treatment. The drying took place between the 7th and the 10th day after treatment. The threshing and storage were carried out the 11th day after treatment.

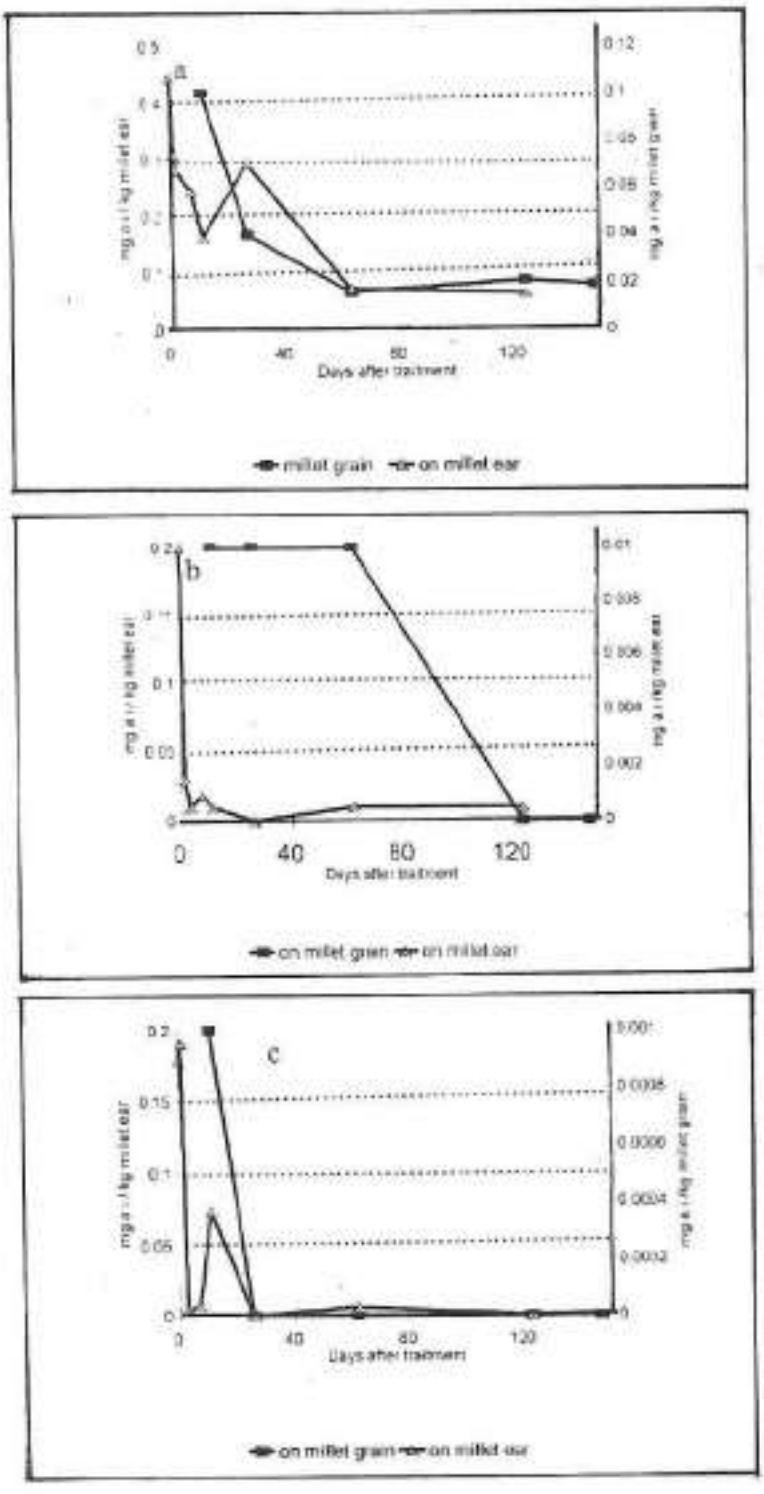


Figure 9.6 : Residues in farm stores : disappearance curves
a) fenitrothion b) bendiocarb c) chlorpyrifos

Table 9.16 : Initial deposition and calculated half lives.

		Vegetation				Soil			
a.i.	Parcel	R^2	$t_{1/2}$(h)	ti(mga/kg)	Obs.	R^2	t1/2(h)	ti(ppm)	Obs.
Fenitrothion	O1 N. Rip	0.99 -s	5	55	3	0.77 -s	15	0.69	4
	O4 N. Rip	0.99 -s	5	67.4	3	0.94 -s	36	0.35	4
	08 N. Rip	0.90 -s	6	16.0	3	0.99 -s	11	1.19	3
	10 N. Rip	0.88 -ns	7	20.0	3	0.46 -ns	63	0.18	4
	06 R.Toll	0.97 -s	39	50	4	0.13 -ns		0.15	4
	04 R. Toll	0.96 -s	10	135	3	0.93 -s	62	0.24	4
Diflubenzuron	02 N. Rip	0.39 -ns	68	20.6	5	0.62 -ns	108	3.65	3
	06 N.Rip	0.27 -s	346	37.5	5	0.35 -ns	247	4.27	4
	09 N.Rip	0.51 -ns	532	25.7	4	0.97 -s	97	1.39	4
	12 N. Rip	0.10 -ns	690	25.7	4	0.001 -ns	1982	0.5	5
	03 R.Toll	0.93 -s	42.1	66	5	0.29 -ns	190	3.14	4
	16- R.Toll	0.97 -s	85.6	84	4	0.44 -ns	257	1.54	3
Bendiocarb	02 R.Toll	0.88 -s	11.5	44	5	0.52 -ns	151	0.03	5
	05 R.Toll	0.59 -ns	19.9	48	4	0.77 -s	70	0.02	3
	10 R.Toll	0.90 -s	23.3	63	4	0.94 -s	122	0.02	4

R^2 : The square of the coefficient of correlation obtained on the linear decline of the neperian logarithm of the residue.
s = $p < 0.05$;
ns = not significant.
Obs : the number of points used to perform the decline.
t1/2 : the half-life period (theoretical) calculated from the decline results.
ti : Theoretical initial depositions (at t = 0) in mg a.i./kg estimated from the decline results.

The Table presenting bendiocarb data shows intermediary values between fenitrothion and diflubenzuron : the R2's are between 0.59 and 0.90, the t1/2's are in general superior to those of fenitrothion and vary between 11 and 23 hours.

DISCUSSION

Fenitrothion

The discussion concerns primarily the results taking into account the meteorologic data of the treated sites. During these studies, parcels have been treated in the morning with corresponding meteorologic conditions depending on whether it is at Nioro or at Richard-Toll. The parameters which differ are mainly the total rainfall between the treatment day and the sampling day, and the temperature of the environment. If we compare the periods obtained at the Nioro du Rip and at the Richard Toll sites, we come to the conclusions of 1991 Chapter 8). On millet leaf found at Nioro, where a variable rainfall between 191 and 254 mm has been recorded, the half-lifes are shorter (between 5 and 8 hours) than those obtained on *T. terrestris* at Richard Toll (10 and 39 hours). The observation on the soils leads to the same conclusion if we don't take into account the cases where the R2 coefficient is low (< 0.5): (Table 9.16). The comparison of Nioro parcels supports the assumed impact of pluviometry or humidity on the disappearance of fenitrothion: the first two parcels which have received more water than the two others, mostly on the first day, have given the lowest $t_{1/2}$ on millet leaf.

Everts (1990) found, after aerial application at the dose of 485 g a.i./ha, initial depositions varying between 60 and 145 mg a.i./kg of dry grass. These values are comparable with those of the present study if the factor due to the water content is taken into account : our results for wet grass are lower. In this same study, the half-life periods of fenitrothion, calculated on the basis of a process of first order disappearance, are between 60 and 75 hours. On the other hand, it is emphasized (EPA 1987) that fenitrothion becomes quickly degraded in non-sterilized clayey soils or in sandy soils with a half-life period inferior to a week; which is entirely in accordance with the results we have obtained and for which $t_{1/2}$ varies between 11 and 63 hours.

Diflubenzuron

The comparative study on the behaviour of diflubenzuron also reflects climatic differences of the Nioro du Rip and Richard Toll sites. Diflubenzuron shows, as in 1991, a lower tendency to disappear on vegetation at Nioro du Rip than at Richard Toll. The difference of half-life periods is remarkable (Table 9.16). On the soil, this difference is less marked even if the maximum temperatures in this area show appreciable differences : 38°C at Nioro and 45°C at Richard Toll.

A study of the degradation of diflubenzuron on grass has been carried out in Mali (Duphar 1991). The average dose used was 62.5 g/ha. The sampling was done until the 49th day after treatment. The evaluation of half-life periods, supposing a first order degradation, gives values between 140 and 270 hours, which is comparable to the results of our study. Periods relating to the disappearance of diflubenzuron in a relatively rainy environment (Everts 1990) are estimated to be of first order kinetic between 320 and 380 hours, these values are also close to those we have obtained.

Bendiocarb

Bendiocarb has only been studied in the Richard-Toll zone, which excludes e a comparative study taking meteorological data differences into account. We can simply notice that in this area, it has a less stable behaviour on vegetation than on soil: the half-life period goes from 11-23 hours on *T. terrestris* to 70-150 hours in the dry soil of Richard Toll. These results can be compared to those obtained on a series of tests of bendiocarb degradation on vegetation carried out in the United States and in Yugoslavia (Camco 1986). In the United States, during an aerial application at 180 g a.i./ha the residue analyses revealed pesticide contents of 50 g a.i./kg of grass on the samples taken imediately after treatment. On the 15th day, the rate found is only of 0.7mg/kg and the estimated half-life period is 2.4 days. In Yugoslavia, the grass samples taken 12 hours after treatment at 80-100 g a.i./ha have given rates varying between 1 and 25 mg a.i./kg.

Comparison with standards

Table 9.17 gives the results in relation to the standards. Results partly confirm the conclusions drawn from the analysis of the half-life periods.

In fact fenitrothion shows a withholding period in the different areas where it has been studied. At Nioro du Rip, on the millet leaves, it complies to the standard for forage of animals before the end of the first 24 h in two cases, in the two other cases, the compliance to this standard settles before 72 hours after treatment. At Richard Toll, on *T. terrestris* the product complies to the standard in three days on the first parcel while on the second, the withholding period exceeds seven days. A study (IRPTC 1991) based on the feeding of dairy cows with forage having a certain rate of fenitrothion concluded that individuals receiving a daily dose of 3mg a.i./kg of body weight give milk containing 0.002 mg a.i. of fenitrothion/litre. This residue is equal to the fenitrothion standard for milk (FAO & WHO 1991). Even if the risk of the consumption for the cattle does not require a long withholding period (generally inferior to 3 days), the risk presented by milk consumption obliges to extend withholding periods or to restrict grazing of the cattle in the parcels.

Comparison with the Maximum Residue Limit (MRL), which is stricter (0.05 mg a.i./kg instead of 5 mg a.i./kg for the TL) gives longer time-limits : at Nioro du Rip a unique case indicates a time-limit inferior to three days, the other three cases give withholding periods which are inferior to seven days. On the two results obtained at Richard Toll, the first indicates a withholding period inferior to seven days while on the second, the *T. terrestris* does not respond to the standard within seven days.

Comparison of results for diflubenzuron leads to a remarkable conclusion : all withholding periods found are superior to 14 days. This is due to the strictness of the standard and to the persistence of diflubenzuron. At Nioro du Rip where we have noticed a stronger persistence of the product (see half-lifes), the withholding periods are largely superior to fourteen days: the rate on the 14th day sample is superior to 75 mg a.i./kg of dry millet leaf, which largely exceeds the standard of 1 mg/kg. At Richard Toll, the rate found expressed in in the same unit is 40 times inferior the standard of 1 mg a.i./kg.

For bendiocarb, at Richard Toll on *T. terrestris*, the withholding period on the animals' forage is shorter than 7 days in two cases, in the third of the studied cases, it remains inferior to 2 weeks. Compared to the Maximum Residue Limit (MRL) which is also (0.05 mg a.i./kg) for vegetables, the three studied cases at Richard Toll give high residue rates. At the end of the 14 days, the rates found on parcels 02, 05 and 10 are respectively close to 5.5 and 105 times the standard. Which shows a remanence similar to that of diflubenzuron.

For the study done on the farmers' millet in rural storing, the comparison of the standards and the results of Table 18 show a minor contamination on the grains and the millet ears.

Table 9.17 : Standards and estimated withholding periods for the treated vegetation.

Pesticide	Dose (g a.i./kg	th***	ts***	Number of days to reach the residue limits		
				TL(forage)*	MRL vegetables**	MRL (grain)
				5 ppm****	0.05ppm	10 ppm
	465	34	144	< 3	< 3	
	445	28	119	< 1	< 7	
Fenitrothion	490	53	223	< 3	< 7	
N. du Rip	415	44	188	< 1	< 7	
Fenitrothion	710	86	511	< 3	< 7	
R. Toll	800	62	369	> 7	> 7	
Fenitrothion	740	Farm stocking		Harvested grains satisfy to the MRL		
				1 ppm	1 ppm	
	65	80	336	>>14	>>14	
Diflubenzuron	62	41	172	>>14	>>14	
N. du Rip	61	32	133	>>14	>>14	
	61			>>14	>>14	
Diflubenzuron	111	47	293	> 14	> 14	
R. Toll	104	34	204	> 14	> 14	
				10 ppm****	0.05 ppm	0.05 ppm
Bendiocarb	169	27	160	< 7	> 14	
R. Toll	167	39	232	< 14	> 14	
	194	51	306	< 7	>>14	
Bendiocarb		Farm stocking		The harvested grains satisfy to the MRL		
				5 ppm		0.25 ppm
Chlorpyrifos		Farm stocking		The harvested grains satisfy to the MRL		

* TL(forage) : tolerance level for the animal forage standard US EPA (CFR 1990)

** MRL : Maximun Residue Limit for vegetables, Codex Alimentarus Standards (FAO & WHO 1991)

*** th is the initial deposition (Tables 12, 13, 14) of pesticide on treated vegetation expressed in mg a.i./kg of wet vegetation. ts is the same deposition expressed in mg a.i./kg of dry vegetation? ts is obtained by dividing th by the corresponding coefficient (1-(-)). (-) is the water content of the matrix. (-) = 76.17% for the millet leaves, 83.19% for the Richard Toll T. terrestris, 10.12% for the millet grains, and 29.62% for the millet ears.

**** The tolerance limit (TL) for fenitrothion or bendiocarb has been calculated on the basis of the No Effect Dose (NOEC) for rat (C.R. Worthing & R.J. Hance 1991) applied to a sahelian cow of average weight (P) 300 kg, with a daily consumption (DC) of 3 kg of dry forage. The security factor (Sf) for the estimation of the acceptable daily dose (ADD) is set to 100. Tolerance limit is given by the formula TL = (NOEC x P) / (Sf x DC).

The withholding periods are found by converting TL or of the MRL into mg a.i./kg of vegetation or wet grains.

The withholding period for fenitrothion on the grains is obtained from the millet threshing, the value found is inferior to 10 times the MRL. By comparing the ears with the grains, we find that the residue rate at the treatment day is 0.63 mg a.i./kg while after three days following harvesting during which the sun-drying took place, this value has decreased to 0.17 mg a.i./kg, which is far from the MRL value of 10 mg a.i./kg.

Of chlorpyrifos, there were almost no residues found on the grains after threshing : the only detected value found on the first sample taken after threshing was low compared to the standard (at least 100 times smaller). On the ears, the same remark is valid. Only the first two samples give rates which are near the standard level, the others are far below the MRL.

For bendiocarb, the residue rates on millet grain are of the order of 1/100 mg a.i./kg on the first 3 samples and negligible on the last two. Which means that the MRL on the grains is obtained during the threshing operation. On the millet ears, only the first day sample displays a rate of 0.28 mg a.i./kg (dry). All the other values are inferior to the standard of 0.05 mg a.i./kg.

CONCLUSION

The difficulties linked to the realization of the pesticide residue monitoring in the environment, are the following. Heterogenity is strong at all levels of the study. Like the results of the oil-sensitive paper which show high irregularity in the pesticide distribution on the treated site, the residue analyses sometimes show high irregularities as well.

Fenitrothion has been studied on vegetation, on soil and on millet in maturation. The results confirm its low persistency, also found in the first year study (Chapter 8). The reduction of half-life periods on millet leaf at Nioro du Rip where the rain has been more abundant also confirm its quicker disappearance in rainy conditions. At Richard Toll, a zone characterized by higher temperatures, its disappearance is slightly slower, although the withholding periods generally are inferior to 7 days. The study on millet stores carried out in rural area shows that the millet doesn't have important residues before storing if it is treated one week before harvesting.

Diflubenzuron has been studied on the same number of parcels as fenitrothion. The results confirm that it is a remanent product and match the conclusions of the first year study: its half-life period on millet leaf at Nioro du Rip is much longer than that at Richard Toll on *T. terrestris*. The comparison with the standards in all studied cases imposes withholding periods superior to 14 days.

Chlorpyrifos, studied only in farm stores, gives values that meet the standards in a short time-limit. However, this conclusion must take into account an essential factor: the applied doses give low initial depositions (< 1 mg a.i./kg on millet ear). Whereas in reality sometimes a big difference exists between the treatments carried out here and those which are done by farmers or even the agents of the crops protection. The lack of training and of knowledge of the products among the first and the use of treatment devices that are difficult to handle among the second have result in chemical dosage far superior to the recommended dose.

REFERENCES

Browne P M (1981) Analytical method for residues of bendiocarb in water. Schering Agrochemical Limited, NC 6897/R140 C158, Essex.

Camco (1986) Decline of bendiocarb residues on grassland/ Safety to grazing animals, Cambridge Animal and Public Health Limited, Cambridge p 1.

C F R (1990) Code of Federal Regulations (40. parts 150 to 189), National Archives and Record Administration, Washington D.C.

Duphar (1988) Liquid chromatographis determination in tea, Duphar, Weesp.

Duphar (1991) Diflubenzuron in Grass from Mali, Duphar (results not yet published).

EPA (1987) Pesticide Fact Sheet : fenitrothion (N° 42). Environmental Protection Agency, Washington DC, pp. 3.

Everts J W (1990) Environmental Effects of Chemical Locust and Grashopper Control, a Pilot Study, FAO, Rome.

F.A.O. (1986) Pesticide Residue Trials to Provide Data for the Registration of Pesticides and establishment of Maximum Residue Limit, F.A.O., Rome.

F.A.O. (1991) Controlling Desert Locusts F.A.O., Rome.

F.A.O. and W.H.O. (1991) Codex Alimentarus Commission, F.A.O., Rome/ Italy.

Gadji B (1993) Deposition and Degradation of Fenitrothion and Diflubenzuron on millet vegetation and in the ponds in Sahelian Environment, F.A.O., Locustox Project Dakar.

IRPTC (1991) Intnational Register of Potentially Toxic Chemicals. QR 3217, Fenitrothion, United Nations Environmental Program, Geneva.

Steel RGD and Torrie J.H. (1980) : Principles and Procedures of Statistics (a Biometrical Approach) 2th Edition, McGraw-Hill Book Company, p. 597, New York.

Sumitomo (1981) Sumithion Technical Manual, Method for residue analysis of fenitrothion, (pp 23..27), Osaka.

Worthing C R and Hance R J (1991) The Pesticide Manual 9th Edition, British Crop Protection Council, 680 Bendiocarb (p. 54), 6120 fenitrothion (p. 369).

Further details can be obtained upon request from the author.

1. Weather conditions during treatments
2. Droplet counts on oil sensitive paper.

The LOCUSTOX Project ECLO/SEN/003/NET, GCP/SEN/041/NET has been founded in 1989, by the governments of Senegal and the Netherlands. The project is based at the Directorate for Plant Protection of the Senegalese Ministry of Agriculture and it is executed in collaboration with the Food and Agricultural Organization of the United Nations.

The project's objective 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.

In the period from 1991 to 1994 studies were carried out on following subjects :

- The risk to human health of anti-locust and anti-grasshopper campaigns.

- The side-effects on aquatic fauna and beneficial insects.

- The synthesis of research and literature data for advice to the government and FAO on selectivity and safety of chemical treatments.

The Locustox Building at km 15 Route de Rufisque - DAKAR - (Sénégal)