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EFFECTS OF PESTICIDES ON SOIL AND WATER FAUNA AND MICROFLORA OF WETLAND RICEFIELDS

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INTRODUCTION

The increased rice production needed to meet the requirements of a fast-growing human population in the coming decades should not be at the expense of future generations. It should maintain or enhance the quality of the environment and conserve or enhance natural resources. The wetland ricefield is generally considered as a very fertile agro-ecosystem but there is no assurance that, on the long term, crop intensification will not affect its fertility.

Research on rice nutrition has shown that, at the levels of inorganic fertilizer usually applied in ricefields, most N absorbed by the plant originates from soil where it is released by the turnover of a microbial biomass which represents only a few percent of total soil N (Watanabe et al., 1988). Crop residues, rhizosphere exudates, algae and aquatic plants contribute nutrients that allow the replenishment of microbial biomass. Nutrients accumulating in algae and aquatic plants--including biologically fixed N_2-- and in the detritus layer at the soil-water interface are recycled by zooplankton and reincorporated into the soil by oligochaetes, which are therefore key components of the ricefield fertility (Roger and Kurihara 1988). An important aspect is to understand and predict how factors associated with crop intensification, especially agrochemical use, may affect the soil microbial biomass directly through toxic effects or indirectly by decreasing the productivity of the photosynthetic aquatic biomass and inhibiting invertebrate populations responsible for nutrient recycling and translocation.

The first part of the seminar present the main conclusions of a bibliographic review of pesticides impacts on populations of microorganisms and invertebrates in soil and water of ricefields. The second part summarizes preliminary results of on-going field studies of the environmental impacts of agrochemical in wetland ricefields.

2. BIBLIOGRAPHIC REVIEW

2.1. Effects of pesticides on microbial populations: A review of more than 200 papers dealing with the effect of pesticides on ricefield microorganisms (Roger 1990) showed that many studies are short term laboratory experiments in test tubes or flasks (Table 1) that cannot be extrapolated to field conditions. A limited number of field studies and laboratory studies with soil allow to draw conclusions summarized thereafter.

Pesticide degradation is often faster in wetland soils than in dryland soils because of reducing conditions (Fig. 1). Field and laboratory short term studies conducted in the presence of soil tend to show that pesticides at normally recommended field rates and intervals are seldom deleterious to microorganisms and their activities. In 349 tests of the effect of a pesticide (often applied at a rate higher than the recommended dose) on a microbial population or activity in wetland soils or in rice rhizosphere, an inhibitory effect was recorded in about 19 % of the cases (Table 2). When tests lasted for several weeks, inhibitory effects were observed to be transitory as a recovery of populations or activities was observed after 1 to 3 weeks. Herbicides seems to have more often short term negative effects on soil microflora than insecticides. Only 8 studies on microflora were conducted in the field. Five of them reported no effect of pesticide application on populations or activities, other showed a transitory drop of populations followed by a recovery within 2-3 weeks.

A few studies indicate that repeated application of a pesticide may enhance the growth of the related specific decomposing microorganisms and cause its rapid inactivation. This was observed with gamma-BHC, diazinon, aldicarb, and nitrophenols but not with carbofuran and benthiocarb. Repeated application of pesticide may change the metabolic pattern of their decomposition. With benthiocarb, this was producing a very phytotoxic compound (Moon and Kuwatsuka, 1984).

Pesticides have three major effects on ricefield algae: (1) a selective toxicity which affects preferentially green algae and thus promote BGA growth, (2) a short term promoting effect of insecticides on microalgae, due to a temporary decrease of invertebrate that graze on algae, (3) a selective effect of insecticides on BGA flora by causing a recruitment of algal grazers which results in the dominance of strains forming mucilaginous macrocolonies resistant to grazing such as *Nostoc* spp.

2.2. Effects on invertebrate populations: Insecticides are usually the most active compounds on floodwater invertebrates. Their application usually caused a decrease in populations followed by proliferation of primary consumers -- Ostracods, Chironomid and mosquito larvae, and molluscs -- (Ishibashi and Itoh 1981, Roger and Kurihara, 1988) while populations of predators such as Odonate larvae were reduced (Takamura and Yasuno, 1986). Ostracods ability to recover rapidly after pesticide application results from their resistance to pesticides and the large number of eggs produced parthenologically (Lim and Wong 1986).

Nematodes and oligochaetes are probably the only soil invertebrates studied in wetland ricefields. Usually, the specific diversity of parasitic nematodes is lower in wetlands than in uplands but apparently this results from submersion rather than higher agrochemical use in wetlands (JC. Prot, ORSTOM, personal communication). Herbicide benthiocarb had no marked effect on the number of nematodes species and their average populations during the crop cycle (Ishibashi and Itoh, 1981).

2.3. Pesticide use and invertebrate biodiversity: Traditional ricefields, some of which have been cultivated for several hundred years, might be considered as climax communities. Modern technologies --which utilize fertilizer-responsive varieties, fertilizers, and pesticides -- have tremendously increased yields and production but have, indeed, caused profound modifications to traditional rice-growing environments. In general, a disturbance to a stabilized ecosystem reduces the number of species while provoking "blooms" of certain others. Those effects have been observed in ricefields (Roger and Kurihara, 1988). However, the quantitative knowledge on the long term effects of crop intensification on species diversity is extremely scarce. The only reference on the species abundance in traditional ricefields is a study conducted in 1975 by Heckman (1979) in Thailand where 599 species were recorded in one field within one year (Table 3a). Few records of aquatic invertebrates can be compared with Heckman's record of 183 species (Table 3b). In a 2 year study in Malaysian ricefields where pesticides were applied, Lim (1980) recorded 39 taxa of aquatic invertebrates. Single sampling by Takahashi et al. (1982) in 4 Californian ricefields recorded 10 to 21 taxa. In 18 sites in the Philippines and India, the highest number of aquatic invertebrates taxa recorded by single sampling at one site was 26; the lowest, 2 (Roger et al., 1987). Similarly, records of numbers of arthropod species in Japanese ricefields estimated in 1954-55 by net sweeping (Kobayashi et al., 1973) seem to indicate a higher biodiversity than in recent data collected by Heong in 5 fields in the Philippines using a suction method (Table 3c). All the above data were obtained by different methods of sampling and the time frame of the sampling were different. Therefore, the marked decrease of the values recorded since 1975 might probably be taken as a rough indication of a decrease in total number of species after crop intensification, but, in fact, there are no unquestionable data to demonstrate the generally accepted concept that crop intensification and pesticide use has decreased biodiversity in ricefields (Roger et al. in press).

3. EXPERIMENTS

We have initiated an extensive field survey and two field experiments, conducted over several cropping seasons, where the effects of pesticides is assessed by monitoring various components of the ecosystem with standardized methods.

3.1. Populations studied and methodology

Soil microbial biomass is estimated by the chloroform fumigation-incubation method, as the difference in N mineralized after 4 weeks of incubation in anaerobiosis between an untreated sample and a sample in which most of the microflora was killed by a treatments with chloroform. The difference in mineralized N (flush N) is an index of the microbial biomass. To obtain an exact estimate of the microbial biomass, the flush N has to be multiplied by a correction factor which depends on the nature of the major components of the microflora. This correction factor (>1) has not yet been determined for wetland soils. Therefore, the method is only semi-quantitative. But it is faster, more reliable, and less tedious than the classical microbiological methods of direct counts or indirect counts by plating or inoculation of soil dilutions in selective media. N_2-fixing blue-green algae (BGA) are enumerated from composite soil samples comprising 10 core subsamples of the top 0.5 centimeter of fresh soil, by using the plating method. BGA were chosen to assess possible impacts of pesticides on the photosynthetic aquatic biomass because of (1) their recognized role in maintaining the N fertility of rice soils, and (2) the existence of an important set of data on BGA in ricefields that could allow comparisons (Roger & Kulasooriya, 1980; Roger et al, 1987). The effect of pesticides on the photosynthetic activity in floodwater is estimated by measurements of dissolved O_2 between 1 and 2 pm.

Zooplankton is studied by enumerating populations of Ostracods, Copepods, Cladocerans, and Chironomid and mosquito larvae in core samples comprising the floodwater and the surface soil.

Aquatic oligochaetes were chosen to characterize possible impacts on soil fauna because a previous study and a literature survey (Roger and Kurihara, 1988) showed that they are a major component of the submerged soil fauna and might be an index of soil biological activity. Tubificids were shown to affect weed growth, soil physical, chemical, and microbiological properties, and the nutritional status of floodwater and its flora and fauna. Their major effect is to stimulates organic matter decomposition and to allow the transfer of organic matter, NH_4^+, Fe^{+2}, PO_4^{-2}, and soil bacteria to the water. Aquatic oligochaetes are enumerated from soil cores 42 mm in diameter that are collected along a transect, and processed separately.

3.1. Farmer field survey in Laguna: For two consecutive seasons, available N, microbial biomass, N_2-fixing blue-green algae, and aquatic oligochaetes were quantified in 32 farms of the Laguna area where the Social Sciences Division of IRRI had recorded agrochemical use and yields for several years. Sampling was performed at the beginning of the crop cycle before pesticide application and at the end of the crop cycle as far as possible from the last pesticide application but before the soil dried up. The rationale of this sampling schedule was to study biological variable when sort term effects of pesticide application were not expected to occur and to try to identify the long term effects of pesticide by correlating biological variables with data on pesticide use in the various farms. The study was limited by the absence of farms with no pesticide use that could serve as a control and because 21 different pesticide were used in 32 farms.

In a first approach to relate biological variables with pesticide use, we calculated the linear correlations between the 4 biological variables determined at various sampling times and the quantities of active ingredient of insecticide, herbicide, molluscicide, and the sum of herbicide and molluscicides applied during the dry and the wet season. Among the 160 coefficients of correlation calculated, only two were significant. They indicated an inhibitory effect of herbicides + molluscicides and a positive effect of insecticides on BGA at the end of the dry season. The level of the correlation ($p = 0.05$) indicated a weak relationship. A second approach was to study the level of significance of the difference between average biological values in farms where selected pesticides were or were not used. The t test of Pearson applied on normalized data was utilized. Only pesticide used or not used by more than 5 farmers were tested. None of the tests indicated a significant difference.

3.2. Upper MN experiment: An experimental design of 65 plots (16 m^2 each, 5 reps.) was used to study the combined effects of N fertilizer and pesticides -- carbofuran and butachlor-- on major populations of aquatic and soil invertebrates, and on N_2-fixing BGA. We used a fallow control and 12 selected combinations of five N treatments (no N, 55 and 110 kg N ha^{-1} broadcast split, 55 kg N ha^{-1} deep placed, and Azolla incorporated before transplanting) and four levels of pesticides (one application of carbofuran at 0.1 kg a.i. ha^{-1}, two applications of 0.3 kg each, three applications of 0.5 kg each, and five applications of 0.5 kg each. The three treatments with 2 and 5 applications of carbofuran also received an application of 0.375 kg a.i. ha^{-1} of butachlor).

The dynamic of invertebrate populations followed a similar pattern in most plots (Fig. 2) with a peak of Chironomid and mosquito larvae at 12 DT and a peak of Ostracods at 40 DT. Copepods established early in the crop cycle and increased in number during the second half of the crop cycle. Cladocerans started to multiply only during the last third of the crop cycle. Populations of Ostracods, and Chironomid and mosquito larvae were much more abundant in the plots receiving the largest quantity of agrochemicals (Fig. 3) than in fallow plots.

There was a marked effect of N fertilizer on algivorous aquatic arthropods (Ostracods, and Chironomid and mosquito larvae), but no effect of pesticides (Fig 4). Neither N fertilizer nor pesticides significantly affected Copepods and Cladocerans which developed late in the crop cycle (Table 4). In the contrary, pesticides were found to inhibit the development of aquatic oligochaete populations.

A very clear negative correlation between BGA growth and the level of fertilizer applied was observed (Fig. 5). Deep-placement markedly decreased the inhibitory effect of N fertilizer on BGA growth. Some stimulating effect of pesticide application was observed on BGA abundance (Fig. 6) and on photosynthetic activity in the floodwater.

This experiment, conducted at rates of agrochemicals currently used in farmer fields show a clear stimulatory effect of N fertilizer on algivorous aquatic arthropods associated with an increased floodwater primary productivity at the beginning of the crop. Pesticides had no marked effect on these organisms when considered at the crop cycle level. Among tested invertebrates, only aquatic oligochaetes exhibited a significant response to pesticide application which inhibited population development.

Nitrogen broadcasting inhibited N_2-fixing BGA and favored eukaryotic algae growth. But agrochemical use did not reduce primary production in floodwater.

3.3. Rice-fish experiment at CLSU: The project study the economics and some of the biological effects of fish stocking and pesticide use in wetland rice and to quantify major components of the agroecosystem to develop a static model of N cycling in fields with and without fish using the Ecopath II simulation program (Christensen and Pauli, 1990). The first experiment was conducted in 12 experimental plots with four replicates and two treatments (with and without fish x with and without pesticide) at CLSU during the wet season of 1990. Fertilizer was applied in all plots at 5 and 30DT as urea and ammonium phosphate at 106 kg N ha-1 and 20 kg P ha^{-1}. Pesticide treated plots received 16.7 kg ha^{-1} Furadan 3G (granular insecticide with 3% a.i. carbofuran) at 1 DT, 5 kg ha^{-1} Machete 5G (granular herbicide with 5% a.i. Butachlor) at 3 DT, and Telustan (molluscicide with 60 % a.i. triphenyltin hydroxyde) applied as a solution (6 tablespoons [!!!] per liter per 1,200 m^2) at 1DT.*Tilapia nilotica* fingerlings (initial weight of 7.22 g $animal^{-1}$) were stocked at 11 DT, at a density of 12,000 ha^{-1} .

Results (Table 5) show that pesticide application caused a statistically significant increase of the photosynthetic activity in floodwater and reduced populations of aquatic oligochaetes. There was no significant effect on surface soil nitrogen, microbial biomass, available N, populations of N_2-fixing BGA, and fish and rice yields.

4. CONCLUSIONS

The literature on the effects of pesticides on microorganisms from ricefields is abundant but dominated by laboratory experiments whose results cannot be extrapolated to field conditions. Short term laboratory experiments with soil and a very limited number of field experiments lasting less than a crop cycle tend to show that pesticides at normally recommended field rates and intervals are seldom deleterious to the beneficial organisms and their activities. When effects are observed they are usually transitory as a recovery of populations or activities was observed after

1 to 3 weeks. Many of these data were obtained under conditions which might exaggerate the effects of pesticides.

No field studies were conducted over several crop cycles and information on the long term effects of pesticide use on wetland soil microflora and fauna is lacking. However it has been shown that, when applied repetitively, pesticides could lead to the disappearance or depression of components of the microbial community, thus leading to a new equilibrium and changes in the pattern of their microbial decomposition. Studies of the impacts of pesticides on microflora and microbial activities in flooded rice soils, hitherto restricted to short term experiments, must be performed under more realistic field conditions and on long term basis.

Our experiments show that N fertilizer affect more floodwater biology than pesticides do. Agrochemical use did not decrease primary production in floodwater but, by favoring algivorous aquatic arthropods, it also favored nutrients recycling. On the other hand, the clear negative effect of pesticides on aquatic oligochaetes indicates that pesticide use might reduce the translocation into deeper soil of recycled nutrients accumulating at the soil/water interface, and thus reduce their availability to rice plant. However data on microbial biomass do not show any significant effect of pesticide use at the level of a crop cycle.

As compared with aquatic arthropods tested, N_2-fixing blue-green algae and soil microbial biomass, aquatic oligochaetes seems to be the most sensitive indicator of pesticide use in wetland soils.

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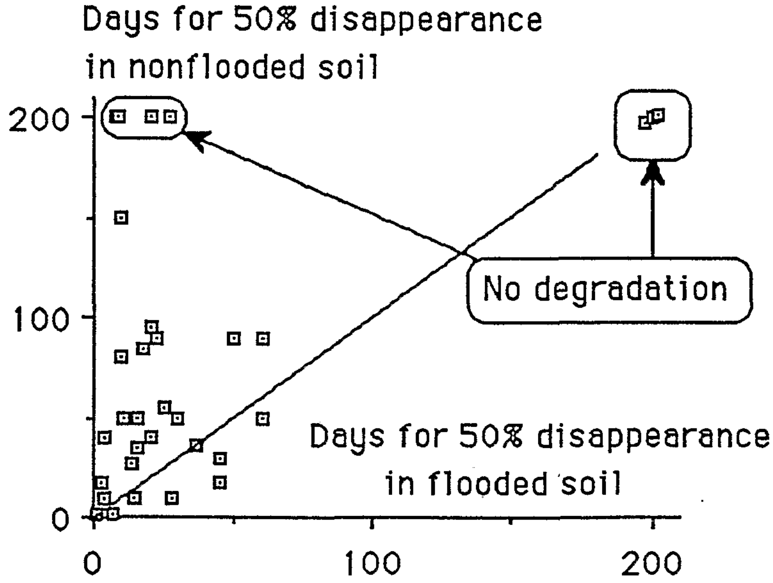
Table 1. Methods used to study the microbiological effects of pesticides in wetland soil.

Type of experimental design	Type of study Bacteriological	 Algological
Flask/tube cultures of microorganisms	6	102
Soil in test tube or beakers	52	-
Pot experiments	13	6
Microcosm experiments	10	-
Field studies on degradation and residues	12	-
Field studies on populations and activities	8	12
Total	101	120

Table 2. Summary of a bibliographic study on the effects of pesticides on microflora and microbial activities in wetland ricefields (values are nº of reports).

POPULATION/ ACTIVITY	Inhibition	no effect	enhancement
Counts of specific groups	**26**	**87**	**31**
Total bacteria in soil	4	13	3
N_2-fixing bacteria	1	15	6
Enzymatic activities	**4**	**72**	**5**
Microbiological activities	**34**	**40**	**42**
Nitrification	14	6	2
Denitrification	4	16	0
N_2-fixation (soil)	1	3	28
N_2-fixation in rhizosphere	13	10	9
TOTAL	**64** (19%)	**199** (58%)	**77** (23%)

Fig. 1. Comparison of the half live time of pesticides in wetland and dryland soils*



* Drawn from data by Sethunathan and Siddaramappa (1978)

Table 3. Summary of quantitative records of species/taxa in wetland ricefields

a. Number of species recorded by Heckman in 1975 in a one-year study of a single field in northeastern Thailand (6 samplings)

Sarcodina	31	Cyanophyta	11
Ciliata	83	Algae	166
Rotifers	50	Pteridopyta	3
Platyhelminthes	7	Monocotyledonae	25
Nematoda	7	Dicotyledonae	10
Annelida	11	Pisces	18
Mollusca	12	Amphibia/Reptilia	10
Arthropoda	146	TOTAL	599

b. Number of species/taxa of aquatic invertebrates, excluding protozoa, recorded by different authors

- Heckman (1979) (species), one traditional field, one year study (Thailand)..............183
- Lim (1980) (taxa), two-year study of pesticide application (Malaysia)..........................39
- Takahashi et al. (1982) (taxa) 4 fields, single samplings, (California)....................10 - 21
- IRRI 1985 and Roger *et al.* 1985 (species) single samplings in 18 fields with pesticide applied (Philippines and India)..2 - 26

c. Records of arthropod species in ricefields over one crop cycle

- Kobayashi et al (1973) : study in 1954-55 of several fields by net sweeping (area of Shikoku, Japan)...450
- Heong et al. (unpublished): study in 1989 of 5 ricefields by suction (Philippines): ....Fields considered separately: 146, 125, 116, 92, 87
  Five fields combined..240

Table 4. Effect of N fertilizer and carbofuran on aquatic invertebrates and N_2-fixing BGA populations [a]. Upper MN experiment, IRRI DS 1990.

Organisms	Rice	N fertilizer		Carbofuran		
	Fallow vs Planted No	Deep Placement	Increasing Level	at 110 N 4 levels	at 55 N 2 levels	with Azolla 2 levels
Ostracods	-	0	+++	0	0	-
Copepods	0	0	0	0	0	0
Cladocerans	-	+	0	-	0	-
Chironomid larvae	-	---	+++	0	0	0
Mosquito larvae	+	---	+++	0	0	0
Aquatic oligochaetes	nd	nd	++[b]	---	nd	nd
Snails	nd	nd	0	nd	0	nd
N_2-fixing BGA	---	+++	---	+++	+	0
Dissolved O_2 (0-20 d)	---	---	+++	0	0	0

[a] 0: no effect; + or - not very marked, possibly incidental, positive or negative effect; +++ or — clear positive or negative effect; nd no data. [b] data from previous year

Table 5. Effects of pesticide treatment in a rice-fish experiment. CLSU/IRRI WS 1990.

Variable	no pesticide	pesticide	p
Dissolved O_2 (av.two first weeks) (ppm)	**8.2**	**16.0**	**0.01**
Dissolved O_2 (av. whole crop) (ppm)	**6.8**	**8.6**	**0.01**
Av. bulk density of surface soil (g cm^3)	0.70	0.71	0.73
Av. change in surface soil N%	**0.015**	**0.022**	**0.12**
Av. available N (0-10 cm)(kg ha^{-1})	18.3	16.3	0.16
Av.extract. N after fumigation. (0-10 cm) (kg ha^{-1})	64.2	62.3	0.62
Av. flush N (0-10 cm) (kg ha^{-1})	45.9	46.0	0.97
Av. nº N^2-fixing BGA (CFU cm^{-2})	6.7 10^4	5.4 10^4	0.35
Av. nº oligochaetes (nº m^{-2})	**1760**	**183**	**0.01**
Fish yield (kg ha^{-1})	199	179	0.36
Rice yield (t ha^{-1})s	4.4	4.8	0.21

Av. = average value over the crop (4 samplings)

Fig. 2. Average dynamics of zooplankton in Upper MN experiment (13 treatments)

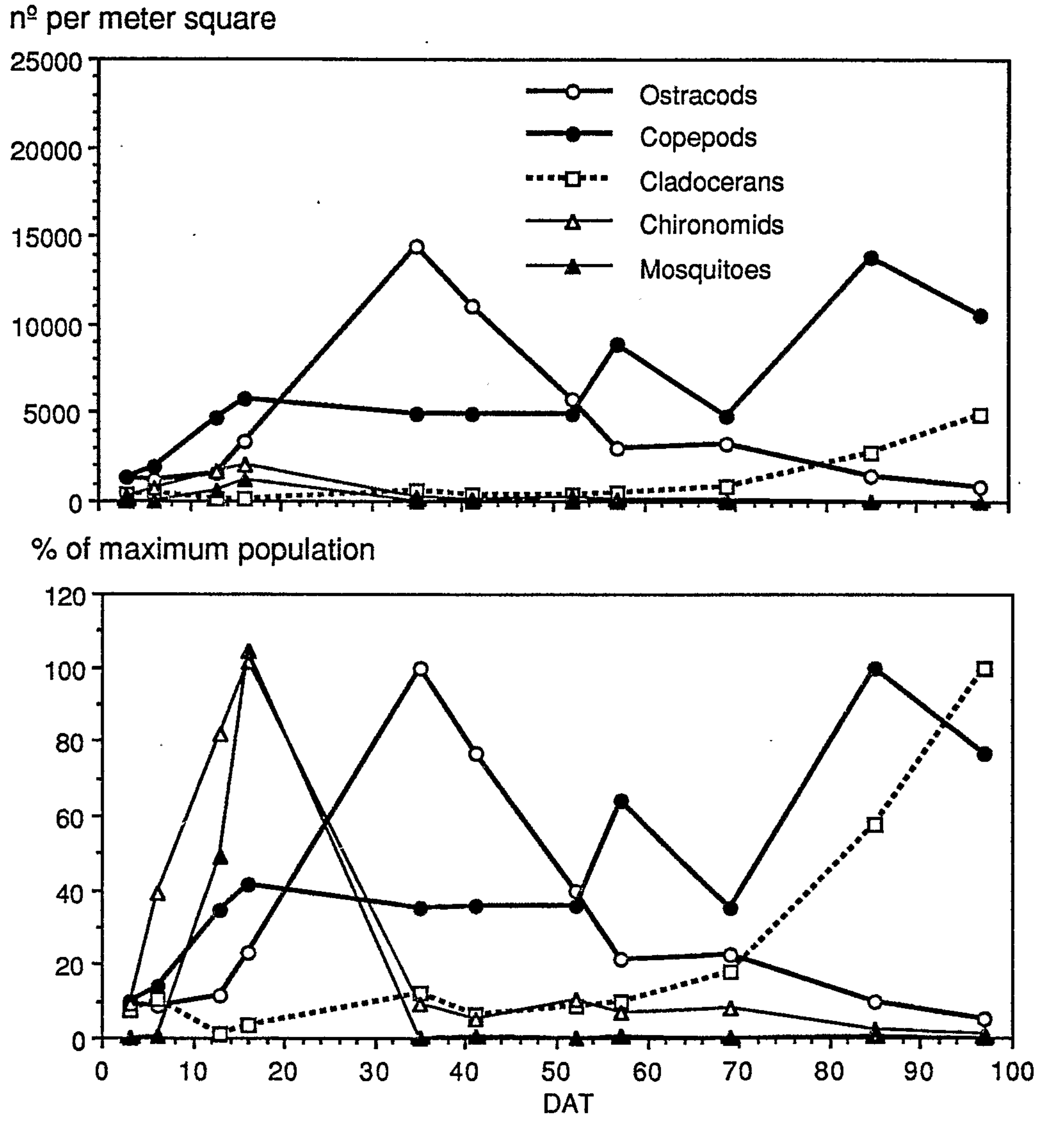


Fig. 3. Comparison of the dynamics of zooplankton in planted plots receiving a high input of agrochemicals (110 kg N + 2.8 kg a.i. pesticide ha^{-1}) and in fallow plots.

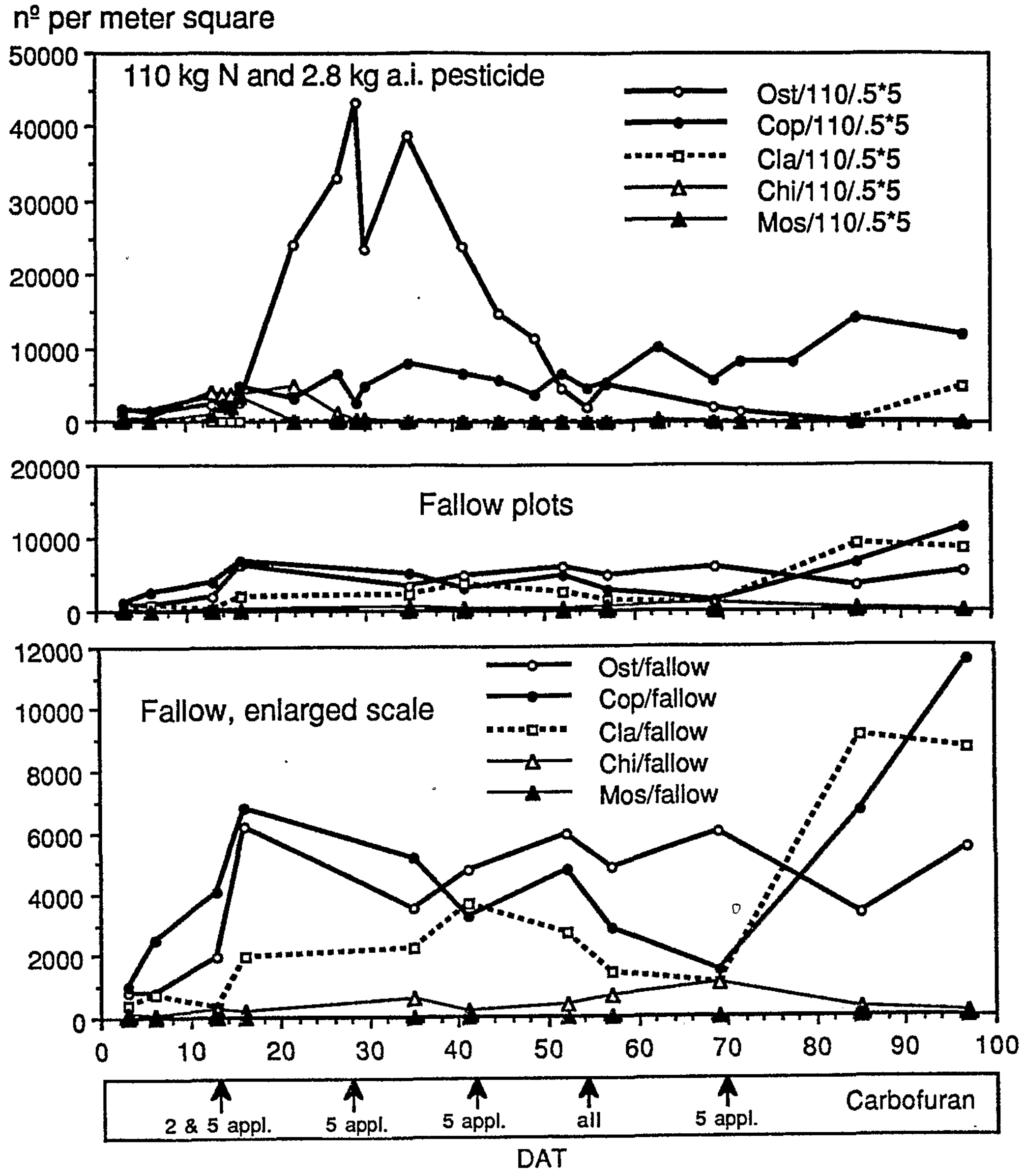
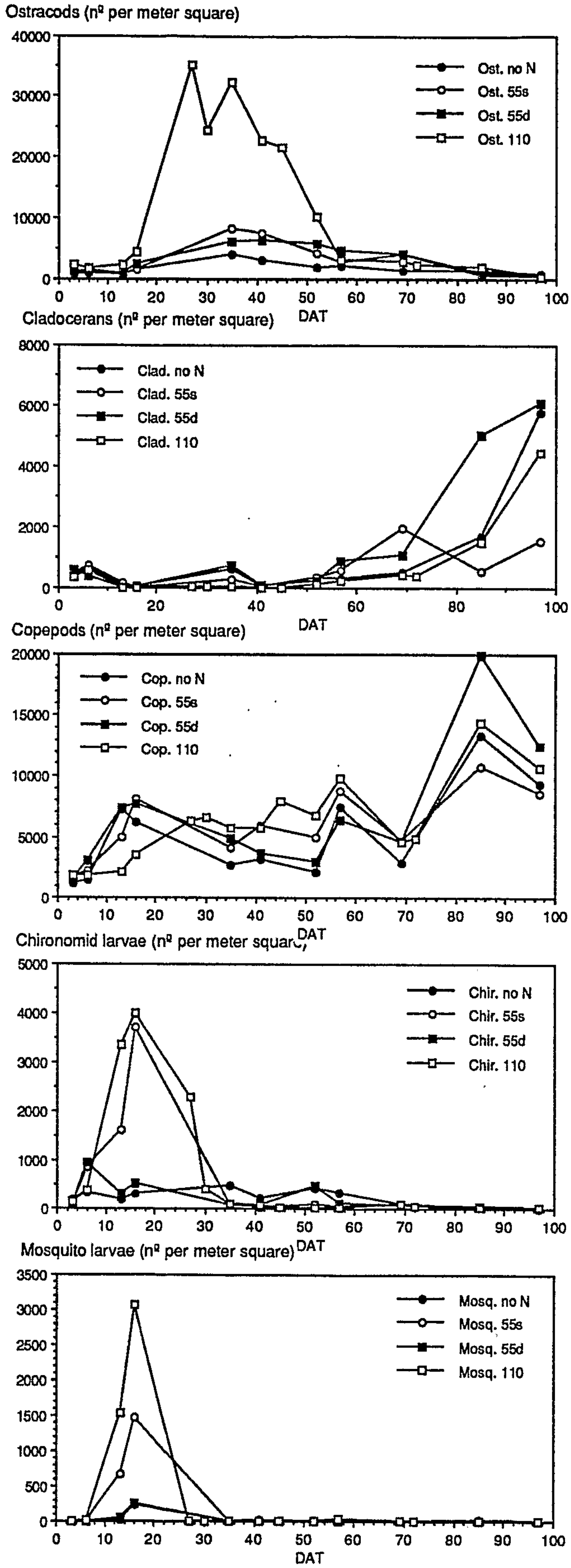


Fig. 4. (on next page) Effect of N and pesticides on the dynamics of major components of the zooplankton in Upper MN experiment, IRRI DS 1990.

Figures on the left side of the page present average values of treatments with no fertilizer, 55 kg N ha^{-1} broadcast or deep-placed, and 110 kg N ha^{-1} broadcast. Figures on the right show the effects of 4 levels of pesticide in plots receiving 110 kg N ha^{-1}.

.1*1 = 1 application of 0.1 kg a.i. ha^{-1} carbofuran
.3*2 = 2 applications of 0.3 kg a.i. ha^{-1} carbofuran + 1 application of 0.375 kg a.i. ha^{-1} butachlor
.3*5 = 5 applications of 0.3 kg a.i. ha^{-1} carbofuran + 1 application of 0.375 kg a.i. ha^{-1} butachlor
.5*5 = 5 applications of 0.5 kg a.i. ha^{-1} carbofuran + 1 application of 0.375 kg a.i. ha^{-1} butachlor

Fig. 4 See legend on previous page



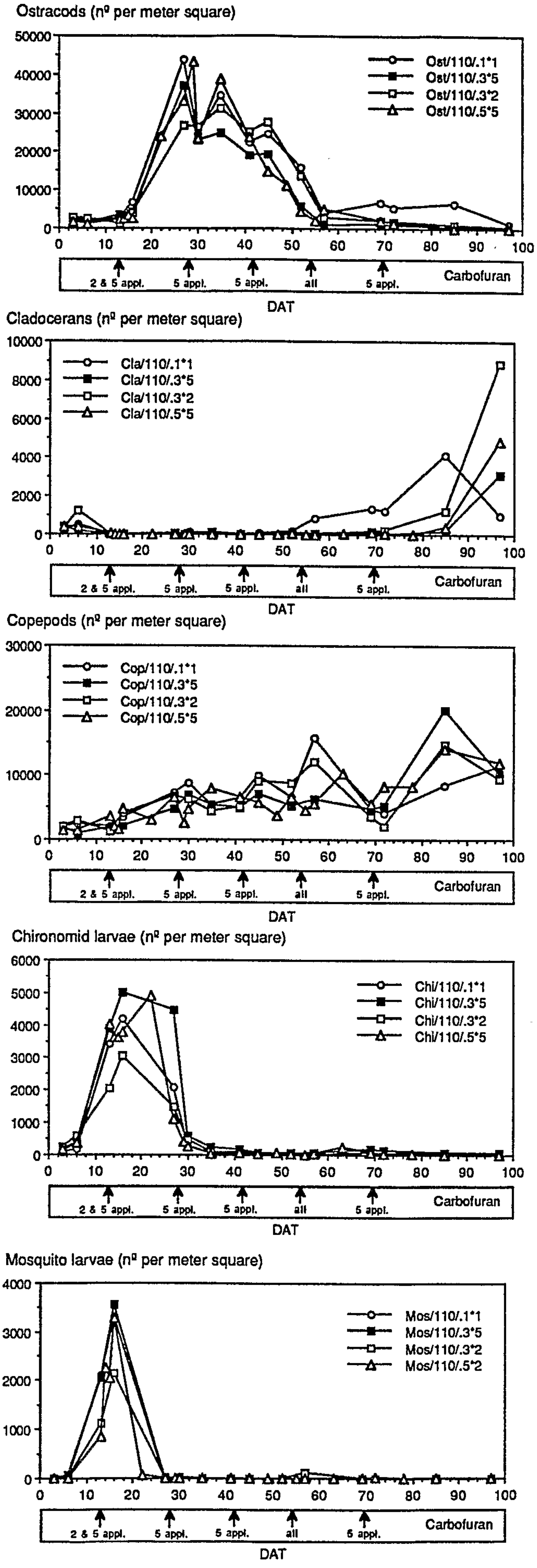


Fig. 5. Effect of N fertilizer on the dynamics of populations of N_2-fixing BGA.

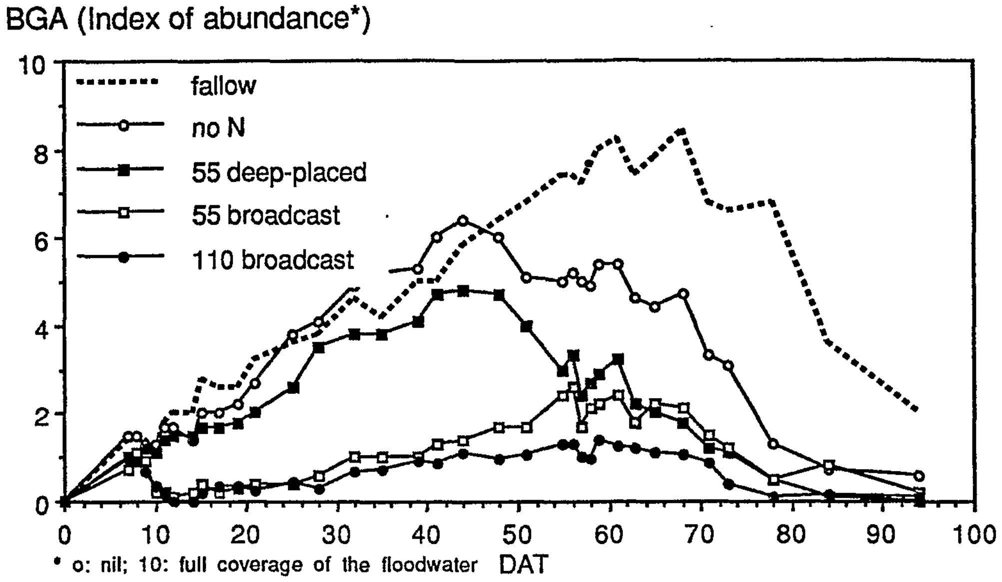


Fig 6. Effect of four levels of pesticide on the dynamics of populations of N_2-fixing BGA in plots with 110 kg N applied.