11 AN EXPERIMENTAL ASSESSMENT OF PESTICIDE IMPACTS ON SOIL AND WATER FAUNA AND MICROFLORA IN WETLAND RICEFIELDS OF THE PHILIPPINES

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11.1. Introduction

Research on N nutrition of rice has shown that, whatever the quantity of N-fertilizer applied in a ricefield, most N absorbed by the plant originates from soil. However, only a small fraction of total soil N is available to the plant, and most of this available-N originates from the microbial biomass in soil (Watanabe, De Datta, and Roger, 1988).

Figure 11.1 presents a conceptual scheme of the role of soil microbial biomass in wetland soil fertility and the pathways involved in its replenishment. It emphasizes that rice nutrition depends on the turnover of a small biomass of microorganisms, which represents only a few percent of total soil N. The replenishment of the microbial biomass requires nutrients, which are provided by (1) crop residues incorporated at the beginning of the crop, (2) rhizosphere exudates, and (3) the photosynthetic aquatic biomass (algae and aquatic plants). Nutrients accumulating in the photosynthetic aquatic biomass (including biologically fixed

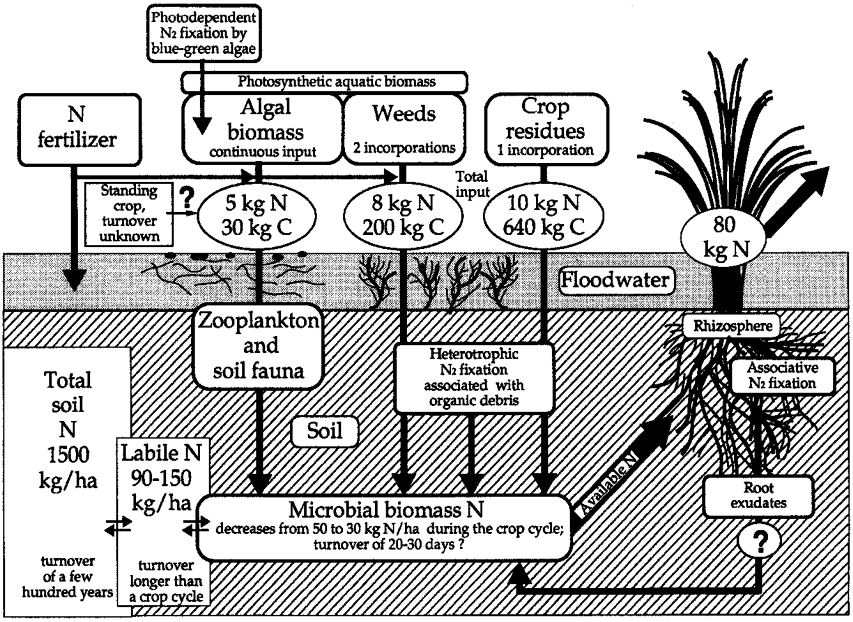

Figure 11.1. Conceptual Scheme of the Role of Soil Microbial Biomass in Wetland Soil Fertility and the Pathways Involved in Its Replenishment

N_2) are continuously recycled and reincorporated in the deeper soil layer by the zooplankton and the soil fauna, which are therefore key components of the ricefield fertility (Roger, Grant, Reddy, and Watanabe, 1987; Roger and Kurihara, 1991). Preliminary studies, under a restricted number of cultural conditions in the International Rice Research Institute (IRRI) farm, have quantified some of the inputs that allow the replenishment of the microbial biomass (Roger, Jimenez, Ardales, and Watanabe, 1989). However, a comprehensive understanding of the mechanisms involved in this aspect of N-cycling has to be developed. An important aspect is to understand and predict how crop intensification may affect the replenishment of soil microbial biomass. In particular, the overuse of agrochemicals might affect (1) the productivity of the photosynthetic aquatic biomass, (2) the populations of soil and water invertebrates responsible for nutrient recycling, and in turn (3) the microbial biomass and general soil fertility.

This paper summarizes data on pesticide impacts originating from controlled experiments and farmer's fields surveys conducted in the Philippines to assess the effects of agrochemicals on microflora and invertebrates in wetland rice environments. The first set of experiments studied the combined effects of pesticides and fertilizer in an experimental design of 64 plots in the IRRI farm. The second set of experiments studied the combined effects of pesticide application and fish introduction in an experimental design of twelve plots established at the Central Luzon State University (CLSU) of the Philippines. Field surveys were conducted in thirty-two farms of the Laguna region, where significant levels of agrochemical (average: 1.5 kg active ingredient (ai) of pesticides and 95 kg N,P,K fertilizer/ha/crop) are used and in thirty farms of the Lucban region, where fertilizer use was known to be minimal and pesticide applications absent.

11.2. Material and Methods

11.2.1. Selected Indicators of Pesticide Impacts in Ricefields

With regard to the methodological problems resulting from soil heterogeneity and the large number of replicated samples needed to obtain significant data in soil biology, the number of variables to be quantified to assess pesticide impacts had to be limited. We selected (1) soil microbial biomass in the upper and lower soil layer, (2) dissolved oxygen concentration in floodwater and N_2-fixing cyanobacteria populations, (3) aquatic oligochaete populations, and (4) in one of the experiments, major aquatic invertebrates, and molluscs.

The choice of an estimation of total soil microbial biomass to characterize possible effects on microflora was primarily due to our interest in using a method that is faster and less tedious than the classical microbiological methods of direct counts, or indirect counts by plating or inoculating dilutions of soil in selective media. Methods for estimating microbial biomass have been developed for upland soils (Jenkinson and Ladd, 1981), but their application in wetland soils encounters limitations. We used a method derived from the fumigation-incubation technique of Inubushi and Wada (1988), which is only semiquantitative but is faster and less tedious than direct or indirect counts. In addition, the measurement in the nonfumigated control also provided estimates of soil available-N and soil bulk density.

Pesticide effects on the photosynthetic aquatic biomass were assessed from measurements of dissolved oxygen concentration in floodwater at the time of maximum photosynthetic activity, because this value is correlated with the daily primary production in floodwater (Roger, Jimenez, and Santiago-Ardales, 1991). N_2-fixing cyanobacteria were chosen as the representative component of the photosynthetic aquatic biomass to be enumerated because of (1) their recognized role in maintaining the N-fertility of traditional ricefields and (2) the existence of a large dataset on cyanobacteria in ricefields, which allowed comparisons (Roger, Santiago-Ardales, Reddy, and Watanabe, 1987b).

The choice of aquatic oligochaete to characterize possible effects on soil and water microfauna resulted from the observation that they are a major component of soil fauna in submerged soils and might be an index of soil biological activity. Roger and Kurihara (1991) reported that aquatic oligochaetes 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 stimulate organic matter decomposition and to allow the transfer of organic matter, NH_4^+, Fe^{+2}, PO_4^{-2}, and soil bacteria to the water. This increases the activity and the biomass of bacteria, and aquatic biota, and results in a feedback effect on the aquatic oligochaete population.

11.2.2. Experimental Designs

11.2.2.1. Biological Impacts of Agrochemicals Under Controlled Conditions. An experimental design of 65 plots (16 sq m each, 5 replicates) was used to study the combined effects of N-fertilizer, the insecticide carbofuran (see Table 11.1 for information on the nature of ai in commercial formulations and their nomenclature), and the herbicide butachlor, on floodwater and soil biology. We used one unplanted unfertilized control and twelve selected combinations of

- Five N treatments: no N, 55 and 110 kg N/ha broadcast split, 55 kg N/ha deep-placed, and *Azolla* incorporated before transplanting; and
- Four levels of pesticides: one application of carbofuran at 0.1 kg ai/ha, 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 two and five applications of carbofuran also received one application of 0.375 kg ai/ha of Butachlor.

Factor levels were selected to represent a range of situations either prevailing or likely to occur in farmer's fields of the tropical area. Levels of broadcast N-fertilizer were representative of average (55 kg N/ha) and high (110 kg N/ha) level of application by rice farmers. The deep-placement of 55 kg N as urea supergranules is recommended by agricultural research centers to save 30 to 50 percent N-fertilizer. *Azolla* is a green manure that has been traditionally used in China and Vietnam. Level and frequencies of pesticide application were established from a survey in thirty-two farms of the Laguna area where farmers applied between 0.5 and 2.5 kg ai/ha crop cycle (Roger et al., 1990).

To optimize the utilization of available manpower and land facilities, we retained the twelve treatments most likely to be found in farmer fields and one unplanted control among the forty possible combinations of the five N levels,

Table 11.1. Pesticides

Nature of Active Ingredient in Commercial Formulations

Insecticides	*Herbicides*	*Molluscicides*
Azocord: Monocrotophos + Cypermethrin	2,4-D: Phenoxy	Aquatin: Fentin chloride
Azodrin: Monocrotophos	Machete: Butachlor	Brestan: Fentin acetate
Brodan: Chlorpyrifos + BMPC	Solnet: Pretilachlor	Telustan: Fentin hydroxyde
Decis: Deltamethrin		
Diagran: Diazinon		
Endox: Endosulfan		
Folidol: Methyl parathion		
Furadan: Carbofuran		
Hytox: Isoprocarb		
Lorsban: Chlorpyrifos		
Thiocarb: Thiobencarb		
Thiodan: Endosulfan		
Trebon: Ethofenprox		
Sevin: Carbaryl		
Super Gran: Carbofuran		
Vindex: BPMC + Phentoate		

Nomenclature of Active Ingredients

2–4 D	(2,4-dichlorophenoxy) acetic acid
BMPC	2-sec-butlyphenyl methylcarbamate
Butachlor	N-butoxymethyl-2-chloro-2',6'diethylacetanilide
Carbaryl	1-naphtyl methylcarbamate. Carbofuran: 2.3-dihydro 2,2-dimethyl-7-benzofuranyl methyl carbamate
Chlorpyrifos	O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate
Cypermethrin	(RS)-alpha-cyano-3-phenoxybenzyl (1RS,3RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate); (RS)-alpha-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-1, 1-dimethylcyclopropanecarboxylate
Deltamethrin	(S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2.2-dibromovinyl)-2.2-dimethylcyclo propanecarboxylate; (S)-alpha-cyano-3-phenoxybenzyl (1R)cis-3-(2.2-dibromovinyl)-2.2-dimethyl cyclopropanecarboxylate
Diazinon	O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate
Endosulfan	C,C'-(1,4,5,6,7,7-hexachloro-8,9,10-trinorbon-5-en-2,3-ylene) (dimethyl sulphite) 6,7,8,9,10,10 -hexachloro -1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxa thiepin 3-oxide
Fentin	triphenyltin
Isoprocarb	o-cumenyl methylcarbamate; 2-isopropylphenyl methylcarbamate
Monocrotophos	dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate; 3-(dimethoxy phosphinyloxy)-N-methylisocrotonamide
Parathion	O,O-diethyl O-(4-nitrophenyl) phosphorothioate
Pretilachlor	2-chloro-2',6'-diethyl-N-(2-propoxyethyl)acetanilide
Thiobencarb	S-4-chlorobenzyldiethyl(thiocarbamate)

four pesticide levels, and two rice levels (planted—unplanted). In particular, treatments combining a high level of pesticides with a low level of fertilizers or organic manuring were not retained, as they are very unlikely to be used by farmers.

Measurements performed at regular intervals during the crop cycle included dissolved oxygen in floodwater, N_2-fixing cyanobacteria, aquatic oligochaetes, and major invertebrates in floodwater: ostracods, copepods, cladocerans, chironomid and mosquito larvae, and aquatic molluscs.

11.2.2.2. Rice-Fish Culture Experiments. We studied during two rice-growing seasons some of the biological effects of fish stocking and pesticide use in twelve experimental plots corresponding to four replicates and two treatments (with and without fish x with and without pesticide). The plots were part of two larger experimental designs established at the Central Luzon State University (CLSU, Philippines) for a CLSU, International Center for Living Aquatic Resources Management (ICLARM), IRRI and Institut Francais de Recherche Scientifique Pour le Development en Cooperation (ORSTOM) collaborative study of the effects of various managements for rice-fish culture.

Fertilizer was applied in all plots at five (2/3) and thirty days after transplanting (DAT) (1/3) as urea and ammonium phosphate at 106 kg N/ha and 20 kg P/ha. Pesticide-treated plots received 16.7 kg/ha Furadan 3G (granular insecticide with 3 percent ai carbofuran) at 1 DAT, 5 kg/ha Machete 5G (granular herbicide with 5 percent ai Butachlor) at 3 DAT, and Telustan (molluscicide with 60 percent ai triphenyltin hydroxide) applied as a solution (6 tablespoons per liter per 1,200 sq m)[1] at 1 DAT. Tilapia fingerlings (average initial weight about 7 g) were stocked at 11 DAT at 12,000/ha.

Measurements performed at four regular intervals during the crop cycle included dissolved oxygen in floodwater, N_2-fixing cyanobacteria and aquatic oligochaete populations, and microbial biomass, bulk density, available-N, total N in the surface soil layer (0–2 cm) and the deeper soil layer (2–10 cm) of the soil. Fish yield and rice yield were determined at the end of the crop.

11.2.2.3. Surveys in Farmers' Fields. For two consecutive cropping seasons (wet season [WS]; dry season [DS]), available-N, microbial biomass, N_2-fixing cyanobacteria, and aquatic oligochaetes were quantified in thirty-two farms of the Laguna area and thirty farms of the Lucban, Quezon area (Philippines) where the Social Sciences Division of IRRI had recorded agrochemical use and yields for several years. No pesticide other than rodenticides were used in the farms of the Quezon area. In Laguna area, a wide range of pesticides in terms of formulation (21) and quantity (0.5 to 2.5 kg ai/ha/cropping season) was applied.

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 soil dried up. The rationale of this sampling schedule was to study biological variable when short-term effects of pesticide application were not expected to occur and to try to identify the long-term effects of pesticides by correlating biological variables with data on pesticide use in the various farms.

Measurements performed included (1) major physicochemical properties of the soils—that is, pH, C, available P, active iron in the plough layer (0–10 cm); and bulk density, available-N, total N in the surface soil layer (0–2 cm) and the deeper soil layer (2–10 cm), (2) microbial biomass estimated as flush-N in the surface soil layer and the deeper soil layer, and (3) populations of N_2-fixing cyanobacteria and aquatic oligochaetes.

11.2.3. Agroecological Data. Soils were analyzed by the Analytical Services Laboratories of IRRI according to standardized methods, using composite samples of at least five cores, 42 mm in diameter, collected from each site at the beginning of two cropping seasons. In the farmers' fields survey, data on yield and agrochemicals (nature, date of application, quantity applied, and cost) were collected by IRRI's Social Sciences Division.

11.2.4. Soil Microbial Biomass. Soil microbial biomass was estimated as the difference in N mineralized after four weeks of incubation in anaerobiosis between a soil sample in which most of the microflora was killed by a treatment with chloroform and an untreated control. This value (flush-N) is an index of the microbial biomass (Jenkinson and Ladd, 1981). The measurement in the nonfumigated control also provides an estimate of soil available-N.

The method we used was modified from that of Inubushi and Wada (1988) for wetland soils. After fumigation, chloroform was removed by evaporation for one hour in a ventilated hood, instead of using a vacuum pump. To obtain an exact estimate of the microbial biomass, flush-N should be multiplied by a correction factor that depends on the nature of the major components of the microflora. This correction factor (> 1) has not yet been determined for wetland soils. Therefore, we used noncorrected flush-N values and the method was only semiquantitative. Determinations were done in triplicate on the surface soil layer (0–2 cm) and the deeper soil layer (2–10 cm) of composite samples comprised of ten cores samples 2.7 cm in diameter. Values of available-N (control) and flush-N (difference between control and chloroform treated soil) were calculated in ppm NH_4-N on wet and dry soil basis and were extrapolated in kg N/ha on the basis of bulk density measurements.

11.2.5. Photosynthetic Aquatic Biomass

The effects of pesticides on photosynthetic activity in floodwater were estimated by measuring dissolved O_2 around 1–2 pm. In one experiment, the abundance of floodwater algae was assessed, using a visual index (Roger, Jimenez, and Santiago-Ardales, 1991).

N_2-fixing cyanobacteria were enumerated from composite soil sample comprising ten core subsamples of the top 0.5 centimeter of fresh soil. Suspension-dilutions of soil from 10–2 to 10–6 were plated in triplicate on 1 percent agarized BG-110 medium (Stanier, Kunisawa, Mandel, and Cohen-Bazire, 1971) without mineral-N. Petri dishes were incubated for three weeks at laboratory temperature (22–30^{0}C) under continuous light (800 lux) provided by cold white fluorescent lamps. Counts were performed at two consecutive dilutions under a stereoscopic microscope and were expressed as number of colony forming units (CFU) per sq cm of soil. Detailed information on the methods is available in Roger, Jimenez, and Santiago-Ardales (1991).

11.2.6. Invertebrate Populations

Zooplankton was studied by enumerating ostracods, copepods, cladocerans, and chironomid and mosquito larvae in composite samples of 5 cores (dia. 71 mm) comprising the floodwater and the surface soil. Samples were washed through stacked 1,000, 750, 500, 350, 250, and 128 μm sieves. Material retained on each mesh was backwashed into separate petri dishes and organisms enumerated.

Aquatic oligochaetes were enumerated from composite samples of 10 to 18 soil cores, 27 mm in diameter, collected along a transect in the fields or the experimental plots. The depth of each core sample was determined by the plow layer. Aquatic oligochaetes were extracted by gentle washing with water through 0.25, 1 and 2 mm sieves. Counts were performed on live specimens.

Molluscs were estimated from three surface soil (0–3 cm) samples collected by using 25 × 25 cm metallic quadrants inserted between the rice hills. Soil was washed through 1 and 2 mm sieves.

Population densities were expressed on area bases (number/sq m). Detailed information on the methods is available in Simpson, Roger, Oficial, and Grant (1993b, 1994a, 1994b).

11.2.7. Statistical Methods

Analysis of variance, correlations, and linear regressions were studied on original data for soil properties, ai of pesticides, available-N and flush-N, and on transformed data for cyanobacteria, aquatic oligochaetes, and zooplankton. Cyanobacteria are known to exhibit log-normal distributions and the counts were transformed by $y = \log 10 (x + 1)$. Aquatic oligochaete and zooplankton populations exhibited aggregative distributions, which were transformed by $y = x^{1-b/2}$ where b is the slope of the regression between logarithms of mean and variances of measurements among replicated plots (Roger, Jimenez, and Santiago-Ardales, 1991). Differences among individual treatments were identified using Least Significant Difference (LSD) multiple range tests at the 95 percent level.

11.3. Results

11.3.1. Biological Impacts of Agrochemicals Under Controlled Conditions

Results presented in this section are summarized from a series of papers (Simpson, Roger, Oficial, and Grant, 1993b, 1993c, 1994a, 1994b, 1994c), that present results of studies of the combined effects of pesticides and N-fertilizer in a design of sixty-four plots in the IRRI farm.

11.3.1.1. Impacts on Primary Production. In plots where N-fertilizer was broadcast, primary production in floodwater exhibited a marked increase (about 10 ppm) for several days after N application due to the proliferation of unicellular eukaryotic algae. In these plots, pesticide application had no significant effect. Incidental significant differences due to pesticide application were observed at the higher pesticide level at four occasions (15, 42–49, and 55 DAT) in the zero N treatment and at three occasions in the deep-placed N treatment (15, 42, and 49 DAT) whereas measurements were performed weekly during the crop cycle (Table 11.2).

11.3.1.2. Impacts on N_2-Fixing Cyanobacteria. Cyanobacteria proliferated in zero N control and fallow plots. A very clear negative correlation between their growth and the quantity of N-fertilizer broadcast was observed. Deep-placement markedly decreased the inhibitory effect of N-fertilizer on cyanobacteria growth (Table 11.2).

On the other hand, pesticide effects were not striking. There was no consistent evidence of significant relationships between cyanobacteria blooms and pesticide regimes across fertilizer treatments or in the four 110 kg N/ha treatments. Some stimulating effect of pesticide application was observed on cyanobacteria abundance when N-fertilizer was not applied or deep-placed. In the absence of N, cyanobacteria blooms developed more rapidly in the 0.3 kg ai/ha × 2 than in the lower input treatment, but abundance maxima were similar. When N was deep-placed, population development was faster and maximum cover was increased in the higher pesticide treatment. Despite the consistency of these differences, they were mostly not statistically significant.

Table 11.2. Selected Biological Effects of Rice, Urea, and Pesticides in Experimental Ricefields[a]

Organisms	Rice[b]	N-fertilizer: Deep Placement	N-fertilizer: Increasing Level	Pesticides: All N Treatments 2 Pesticide Levels	Pesticides: at 110N 4 Pesticide Levels
Ostracods	–	– – –	+ + +	– (3/11)	– (5/45)
Copepods	0	0	0	– (1/11)	– (4/45)
Cladocerans	–	+	0	– (3/11)	– (5/45)
Chironomid larvae	–	– – –	+ + +	0	0
Mosquito larvae	+	– – –	+ + +	0	0
Aquatic oligochaetes 1990	nd	nd	+ +	– – –[c]	– – – (11/12)
Aquatic oligochaetes 1991	nd	nd	+ +	+ +	+ +
Snails	nd	nd	0?	nd	0?
N_2-fixing cyanobacteria	– – –	+ + +	– – –	+ + +	+
Dissolved O_2 (0–20 d)	– – –	– – –	+ + +	+ (6/32)	0

Source: Summarized from Simpson, Roger, Oficial, and Grant (1993a, 1994a, 1994b, 1994c).

a. 0 = no effect; + + + or — = clear positive or negative effect; + or – = not very marked, possibly incidental, positive or negative effect (values in parenthesis are the number of significant differences over the total number of records); nd = no data.

b. Comparison between planted and fallow plots with no N-fertilizer applied.

c. Effect observed during DS 1990 but not in 1991 where some positive effect was observed.

11.3.1.3. Impacts on Zooplankton. The dynamics of invertebrate populations followed a similar pattern in most plots with a peak of chironomid and mosquito larvae at 12 DAT and a peak of ostracods, the most abundant organisms, at 40 DAT. 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 than in fallow plots.

There was a marked positive effect of N-fertilizer broadcasting on populations of algivorous aquatic arthropods (ostracods, and chironomid and mosquito larvae) which developed in response to blooms of readily palatable algae. Populations of dipteran larvae were suppressed by N-fertilizer deep-placement. N-fertilizer had much less effects on populations of copepods and cladocerans (Figure 11.2). When considered at the crop cycle level, aquatic invertebrate populations were not significantly affected by applications of butachlor and carbofuran, especially when N-fertilizer was broadcast in the floodwater (Figure 11.2). Few significant effects were found for ostracods, copepods, and cladocerans by statistical analysis of daily measurements, but they represented a very low percentage of the total number of measurements (Table 11.2). In particular, a few incidental significant differences in copepod densities were observed among pesticide treatment levels. All indicated an inhibitory effect of the highest level of pesticide. Populations of cladocerans, which developed late in the crop season, were significantly denser in the lowest than the highest pesticide treatment from 52 to 85 DAT. The length of time before populations started to expand increased with the quantity of pesticide applied and the time of the last application.

11.3.1.4. Impacts on Aquatic Oligochaetes. Populations of aquatic oligochaetes were dominated by tubificidae. Total densities ranged from 0 to 40,000/sq m. Their dynamics were associated with the crop cycle, peak densities being achieved thirty to fifty days after transplanting. Oligochaete numbers increased in response to additions of urea.

An inhibitory effect of carbofuran on oligochaetes was observed during the first season (1990 DS) of pesticide treatments, but not during the following year (1991 DS).

Adverse effects of carbofuran in the 1990 DS (Table 11.2) were expressed as an inhibition from further development relative to treatments that did not receive pesticide and not as density reductions. It was suggested that carbofuran concentrations were insufficient to cause adult mortality but sufficient to affect immature worms, which are more susceptible, thus reducing recruitment to the adult population (Simpson, Roger, Oficial, and Grant, 1993c).

At the start of the 1991 DS, oligochaetes were more numerous in the low-pesticide than the no-pesticide treatments. After pesticides were applied 50 DAT oligochaete population densities remained significantly higher in the presence of pesticides. At 81 DAT after all pesticides and fertilizers had been applied, the interaction between pesticide and N-fertilizer became significant. Oligochaete populations were significantly denser at the high pesticide level only in the absence of mineral-N application. The apparent stimulatory effects of carbofuran in the 1991 DS could be explained by the combined effects of antecedent conditions, the oligochaete life-cycle, and adaptation. Populations in the pesticide-treated plots were depressed the previous year. If resources were underused, populations could have developed to exploit them during the wet fallow period. Oligochaete density reductions caused by carbofuran in the 1990 DS were probably not a consequence of adult mortality. Therefore, established adult populations may not decline after pesticide applications but could be prevented from further increase (Table 11.3).

11.3.1.5. Impacts on Aquatic Molluscs. The most abundant snail species recorded were *Melanoides tuberculata* and *Melanoides granifera*. Snails were more abundant in fallow than in planted plots where they declined as the crop

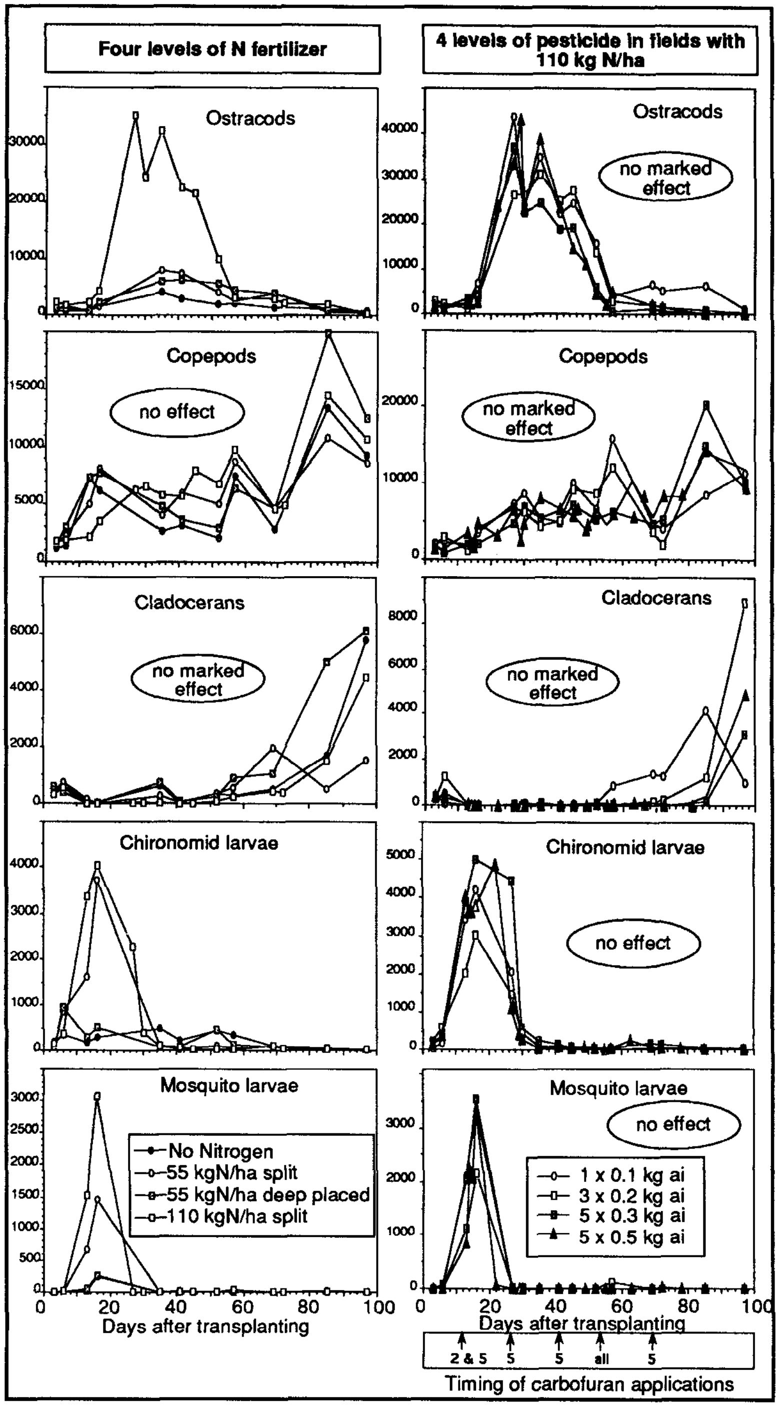
Four levels of N fertilizer
4 levels of pesticide in fields with 110 kg N/ha
Ostracods
no marked effect
Copepods
no effect
no marked effect
Cladocerans
no marked effect
no marked effect
Chironomid larvae
no effect
Mosquito larvae
no effect
No Nitrogen
55 kgN/ha split
55 kgN/ha deep placed
110 kgN/ha split
1 x 0.1 kg ai
3 x 0.2 kg ai
5 x 0.3 kg ai
5 x 0.5 kg ai
Days after transplanting
Days after transplanting
2 & 5
5
5
all
5
Timing of carbofuran applications

Table 11.3. Aquatic Oligochaete Populations (hundred/sq m) During the 1991 DS and One-Way ANOVA Analysis on Log-Transformed Data

	Days after Transplanting					
	–3	*18*	*39*	*60*	*81*	*Mean*
ANOVA (P values)	0.23	0.01	0.01	0.01	0.01	0.01
No N-fertilizer:						
No pesticide	64	36[a]	64[ab]	28[a]	30[ab]	45[a]
Low pesticide level	146	91[c]	63[ab]	58[b]	54[bc]	82[b]
High pesticide level	91	75[bc]	78[b]	84[b]	116[d]	89[b]
110 kg N/ha:						
No pesticide	66	69[bc]	97[b]	58[b]	71[bcd]	72[b]
Low pesticide level	95	68[bc]	89[b]	75[b]	79[cd]	81[b]
High pesticide level	110	123[c]	101[b]	72[b]	112[d]	104[b]

Note: Numbers followed by the same letter are not significantly different at the five percent level.
Source: After Simpson, Roger, Oficial, and Grant (1993b).

season progressed. Populations were significantly reduced by the broadcast N-fertilizer at 110 kg N/ha. Changes in snail numbers were more dramatic than changes in biomass, partly because smaller snails were more affected by treatments, and they contribute little to the biomass. Limited evidence suggested that snails were favored by carbofuran and/or butachlor applications (Table 11.4).

11.3.1.6. Conclusion. These experiments, conducted at rates of agrochemicals currently used in farmer fields, showed that, as a general trend, N-fertilizer application and the presence of rice plant had more effects on studied populations than pesticides.

Figure 11.2. Effect of N and Pesticides on the Dynamics of Major Components of the Zooplankton in Experimental Plots, IRRI, 1990 DS

Notes: Figures on the left side present average values (five replicated plots) of treatments with:

- no fertilizer,
- 55 kg N/ha broadcast,
- 55kg N/ha deep-placed, and
- and 110 kg N/ha broadcast.

Figures on the right side show the effects of four levels of pesticide in plots receiving 110 kg N/ha.

- 1 × 0.1 = 1 application of 0.1 kg ai/ha carbofuran
- 2 × 0.3 = 2 applications of 0.3 kg ai/ha carbofuran + 1 application of 0.375 kg ai/ha butachlor
- 5 × 0.3 = 5 applications of 0.3 kg ai/ha carbofuran + 1 application of 0.375 kg ai/ha butachlor
- 5 × 0.5 = 5 applications of 0.3 kg ai/ha carbofuran + 1 application of 0.375 kg ai/ha butachlor

Table 11.4. Summary of Snail Population Densities (nb/sq m) for Individual Plots in Each Treatment After Transplanting in 1990 and 1991 DS

	Dry Season 1990			*Dry Season 1991*		
Treatment	*Min.*	*Mean*	*Max.*	*Min.*	*Mean*	*Max*
Populations (number/sq m):						
0 kg N/ha	17	370	1,533	0	275	1,042
110 kg N/ha	0	77	533	0	42	117
110 kg N/ha + pesticides	17	167	633	0	67	283
Fallow	—	—	—	107	550	2,042
Biomass (kg/ha):						
0 kg N/ha	50	250	1,060	0	190	590
110 kg N/ha	0	90	380	0	60	470
110 kg N/ha + pesticides	0	200	580	0	80	310
Fallow	—	—	—	90	300	1,080

Source: Simpson, Roger, Oficial, and Grant (1994c, 1994a, 1994b).

Floodwater biology was markedly affected by N-fertilizer broadcasting and only incidentally by pesticides. N-fertilizer broadcasting markedly stimulated floodwater primary productivity, favoring the growth of unicellular algae, which resulted in the proliferation of algivorous aquatic arthropods. Pesticides had no marked effect on these organisms when considered at the crop cycle level.

Aquatic oligochaetes exhibited a significant negative response to pesticide application in the first DS crop cycle; however, this effect was not reproducible over two successive DS crops.

Pesticide impacts were (1) usually not observed in the presence of broadcast fertilizer, and (2) incidental and limited in plots where N-fertilizer had no or limited effects on floodwater ecology (i.e., N-fertilizer either not applied or deep-placed). However, in farmers' fields, pesticides are usually not used in the absence of fertilizer.

11.3.2. Rice-Fish Culture Experiments

In both experiments, measurements were performed at four regular intervals during the crop cycle. The measurement at the beginning of the crop served as reference. As pesticides were applied only at transplanting, their effect was expected to be more obvious during the early part of the crop cycle. Therefore, Table 11.5, which summarizes the results of pesticide effects in the two experiments, presents the analysis of the measurements performed about one month

Table 11.5. Effects of Pesticide Treatment on Selected Biological Indicators and Yields, and Significance (p) of the Differences Observed in Two Rice-Fish Culture Experiments[a]

		1st crop (WS)				*2nd crop (DS)*			
		Pesticide			*Fish*[b]	*Pesticide*			*Fish*
Variable		–	+	*p*	*Effect*	–	+	*p*	*Effect*
Dissolved O_2 (ppm) (average 0–23 DT)		7.4	13.5	0.01		13.8	14.9	0.47	
Dissolved O_2 (ppm) (average crop cycle)		7.0	8.7	0.02		9.6	9.5	0.88	
Bulk density of surface soil	2nd	0.75	0.70	0.60		0.65	0.65	0.86	
(g cu m)	mean	0.703	0.743	0.34		0.724	0.718	0.80	–
N content of surface soil	2nd	0.126	1.130	0.83		0.131	0.135	0.80	
(%)	mean	0.122	0.124	0.84		0.122	0.130	0.45	+
N change in surface soil[c]	2nd	0.024	0.033	0.23		0.014	0.018	0.25	+
(%)	mean	0.022	0.030	0.03	+	0.010	0.015	0.06	+
Avail. N in surface soil	2nd	5.04	5.48	0.40		5.39	3.83	0.38	
(ppm NH_4-N dry soil)	mean	4.17	4.67	0.55		3.51	3.46	0.89	
Avail. N in surface soil	2nd	7.48	7.54	0.80		6.05	5.00	0.04	
(kg/ha)	mean	5.84	5.81	0.80		4.81	4.88	0.84	
Flush-N in surface soil	2nd	11.40	9.70	0.46		11.40	11.60	0.93	
(ppm NH_4-N dry soil)	mean	8.04	8.36	0.71	+	9.00	9.36	0.64	+
Flush-N in surface soil	2nd	16.40	13.00	0.10		14.73	15.11	0.32	
(kg/ha)	mean	11.22	12.19	0.20	+	12.72	13.15	0.70	

Table 11.5. (Continued)

Variable		1st crop (WS) Pesticide –	+	p	Fish[b] Effect	2nd crop (DS) Pesticide –	+	p	Fish Effect
N_2-fixing cyanobacteria	2nd	9.00	6.40	0.30		3.20	4.30	0.21	
(CFU/sq m x 10–4)	mean	6.70	5.40	0.40		2.30	3.40	0.16	
Oligochaetes	2nd	3,068	81	0.01	–	4,538	1147	0.01	
(number/sq m)	mean	1,130	118	0.01		2,390	689	0.18	
Fish yield[d] (kg/ha)		199	179	0.36		233	202	0.63	
Rice yield[d] (t/ha)		3.82	4.16	0.18		4.12	4.88	0.10	

a. Each value is the average of measurements in four replicated plots performed at three regular intervals after treatments were applied. 2nd refers to the second measurement: mean refers to the average of the second, third, and fourth measurements (see text). Surface soil refers to the 0–2 cm layer. Soil N, bulk density, available-N (ppm NH_4-N and kg/ha), and flush-N (ppm NH_4-N and kg/ha) were also measured in lower soil (2–10 cm) but showed no effect of pesticide treatment.

b. Only significant effects are indicated; + = positive effect, – = negative effect at $p < 0.05$.

c. Difference in N content between the upper (0–2 cm) and the deeper (2–10 cm) soil layer.

d. The analysis of the data in the larger experimental desings of twenty-four plots and eight rice-fish managements indicated significant positive effects of pesticide application on rice yield and negative effects on fish yield.

after transplanting (second measurement) and the average value of the second, third, and fourth measurements performed after the treatments were applied.

Both experiments showed a quite large variability among replicated plots. Variability in rice yield was due to a marked incidence of golden snails, which damaged or destroyed patches of newly transplanted seedlings. Variability of fish yield was due to the development of a predator fish (*Ophicephalus striatus*) in some plots. Total N, available-N, and flush-N measurements also exhibited a high variability among replicated plots. In upper soil, the coefficients of variation of total soil N estimates ranged from 12.9 to 37 percent and averaged 18.3 percent; the coefficients of variation of flush-N measurements ranged from 15 to 28 percent and averaged 20 percent. The average of the three measurements performed during the crop had a lower variability than individual measurements, but coefficients of variation were still high, ranging from 12.2 to 18.8 percent and averaging 15.8 percent. This situation was not exceptional as observed by App et al. (1984). It may partly explain why little significant differences were observed between plots receiving and not receiving pesticides, and call for caution when interpreting the data.

Pesticide application caused a statistically significant increase of the photosynthetic activity in the floodwater at the beginning of the WS crop cycle (Table 11.5). During the DS, photosynthetic activity in floodwater was still higher in plots where pesticide were applied, but the difference was not statistically significant.

No significant effect of pesticide application was observed on the total-N, available-N, and flush-N content of surface soil. In order to alleviate the effects of the large variability among replicated plots, variance analysis was performed on (1) difference between each of the second, third, and fourth measurement and the first measurement, and (2) differences between measurements in the upper and the lower soil layer. Over sixty-three variance analysis performed on individual (second, third, and fourth) measurements or their average, p values lower than 0.05 were obtained in only eight cases and p values lower than 0.10 in twelve cases. The only significant effect observed in both seasons indicated that, on the average, pesticide application increased the difference in N content between the upper and the lower soil layers. This might reflect the increase in floodwater primary production in plots where pesticide was applied. Significant effects observed in surface soil on available-N, and, possibly, on flush-N were observed only for one season and might have been incidental.

No significant effect of pesticide application was observed in the total-N, available-N, and flush-N measurements and related calculated variables performed on the deeper soil layer.

The most consistent effect of pesticide application was a significant inhibition of aquatic oligochaetes, which was observed in both seasons.

There was no significant effect on populations of N_2-fixing cyanobacteria, and fish and rice yields when analysis was performed on twelve plots. However, the analysis of the data in the larger experimental designs (twenty-four plots and eight managements for rice-fish culture) indicated a significant positive effect of pesticide application on rice yield and a negative effect on fish yield.

The effects of fish stocking were more often significant than those of pesticide application ($p < 0.05$ in sixteen cases over sixty-three variance analysis). Fish stocking significantly increased N content and microbial biomass of the surface soil and decreased the bulk density of the surface soil (Table 11.5).

11.3.3. Surveys in Farmers' Fields

Available-N, microbial biomass, N_2-fixing cyanobacteria, and aquatic oligochaetes were quantified for two consecutive seasons, at the beginning and at the end of the crop cycle (when short-term effects of pesticide application were not expected to occur), in thirty-two farms of the Laguna area where pesticides were used, and thirty farms of the Lucban, Quezon area where no pesticide other than rodenticides were used. The rationale of this study was to take advantage of extensive data on pesticide utilization collected during a survey conducted by the Social Sciences Division of IRRI, to try to identify long-term effects of pesticides on soil and water biology by (1) comparing farms where pesticides were or were not used and (2) studying correlations between biological variables and pesticide use.

11.3.3.1. Comparison Between Sites. Results of analyses showed significant differences in the mean values of all the quantified variables in Laguna and Quezon areas, including N-fertilizer application and soil properties (Table 11.6). Therefore, significant differences in soil physicochemical properties between both areas strongly limited the utilization of Quezon area as a no-pesticide control for measurements performed in Laguna area where pesticide was used.

Flush-N, available-N, and densities of cyanobacteria and aquatic oligochaetes were significantly lower in Quezon area, where no pesticide was used, than that in Laguna area. However, these differences were most probably attributable more to soil properties and environmental conditions than to pesticide use.

Published data on microbial biomass estimate in wetland soils are too scarce to allow comparison with those collected during the survey. Soils in Laguna area are more fertile than in Quezon area, as indicated by available-N and P contents and rice yield. A higher microbial biomass in Laguna soils might be associated with these trophic properties. No conclusion regarding pesticide effects could be drawn.

Table 11.6. Comparison of Agrochemical Use, and Average Soil and Biological Properties in Laguna and Lucban, Quezon Areas

	Laguna Area		*Quezon Area*		
	Mean	*Range*	*Mean*	*Range*	*p*
Agrochemical use:					
Pesticides (kg ai/ha/crop)	1.5	(0.5–2.5)	0.0	(0.0–0.0)	**
Fertilizer N DS (kg/ha/crop)	103	(33–184)	35	(8–92)	**
Fertilizer N WS (kg /ha/crop)	67	(0–134)	22	(7–60)	**
Soil physicochemical properties:					
pH	6.37	(5.7–7.5)	5.6	(4.7–6.1)	**
C (%)	2.23	(1.48–3.35)	2.55	(1.99–3.17)	**
N (%)	0.22	(0.15–0.31)	0.28	(0.19–0.60)	**
C/N	10.0	(7.5–11.7)	9.3	(5.3–11.2)	**
Available P (Olsen) ppm	31.3	(2.7–81)	8.2	(2.2–71)	**
Active Fe	1.13	(0.34–2.36)	3.59	(1.61–4.57)	**
Bulk density, 0–2 cm (g dw/cu cm)	0.59	(0.45–0.75)	0.43	(0.28–0.61)	**
Bulk density, 0–10 cm (g dw/cu cm)	0.67	(0.50–0.89)	0.54	(0.33–0.56)	**
Biological properties:					
Available-N, 0–2 cm (kg/ha)	8.0	(4.4–27.7)	5.6	(3.2–8.3)	**
Available-N, 0–10 cm (kg/ha)	27.2	(16–89)	19.3	(10–33)	**
Flush-N, 0–2 cm (kg/ha)	18.7	(9.8–33.7)	8.4	(5.1–14.0)	**
Flush-N, 0–10 cm (kg/ha)	84.7	(51–134)	44.0	(22–72)	**
Cyanobacteria (1,000/sq cm)	87	(30–300)	17	(0.7–94)	**
Aquatic Oligochaetes (1,000/sq m)	6.2	(0.1–18.3)	1.2	(0–16.8)	**

Note: Values are the average of four measurements at the sequencing and the end of the WS and DS.

Cyanobacteria abundance in both sites was within the range most commonly encountered in ricefields (10^3–$10^5/cm^2$). As in most ricefields, populations were dominated by *Nostoc* spp. and genera forming mucilaginous colonies of definite shape (Roger, Santiago-Ardales, Reddy, and Watanabe, 1987b). On the average cyanobacteria were about five times more abundant in Laguna than in Quezon soils. This might be explained by the higher pH and available P contents of the Laguna soils. Positive correlations between (1) cyanobacteria abundance and (2) soil pH and available P have been reported for a broad range of soils (Roger, Santiago-Ardales, Reddy, and Watanabe 1987b). In addition, reports in the literature indicate an enhancement of cyanobacteria growth by insecticide application. None of the observations made allowed to conclude that differences observed between cyanobacteria populations in the two sites were attributable to pesticide, because both insecticides (often reported to favor cyanobacteria), and herbicide (often reported to be detrimental to cyanobacteria) were used in most farms of Laguna.

Aquatic oligochaete population in both regions were dominated by *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*. Some species were recorded in one region and not in the other, but similar number of species were recorded in both regions. Species diversity was low, and average Simpson's diversity indices were not significantly different between both regions. The range of population densities was similar in both regions (0 to 45,000/sq m), but in Quezon most populations were below 2,500/sq m and the average was significantly lower than in Laguna. Median densities were 190 and 4,050/sq m, respectively. A lower aquatic oligochaete population in Quezon area might be attributed to the general soil acidity and the usually long lasting dry fallow period in this area. The organic matter and the water contents of the soils in Quezon area were higher than threshold values (1.75 percent carbon and 80 percent water dw.basis) that seems to limit the growth of aquatic oligochaetes (Simpson, Roger, Oficial, and Grant, 1993b). Populations of oligochaetes were denser in soils where pesticides were applied, but none of the observations made allowed researchers to draw conclusions regarding the effects of pesticide use on the relative abundance of aquatic oligochaetes in the two sites.

Because the comparison of biological variables in areas where pesticides were used or not used was refrained by significant differences in soil properties between both areas, another approach was tried by restricting the study to the Laguna area and analyzing correlations between biological variables and pesticides use in the thirty-two farms surveyed in this area.

11.3.3.2. Relations Between Pesticide Use and Biological Characteristics in Laguna Area

Pesticide Use in Laguna Area. All farmers used pesticides at various levels, at least during the DS (Table 11.7). The nature of insecticides was extremely variable: during the survey of biological properties of the soils, fifteen formulations were used during the DS and eight during the WS. As a result, each formulation was used by a few farmers only. Brodan was most frequently applied (in nine farms during the DS and in ten farms during the WS). The rates of utilization of a given insecticide were quite variable, with ratios between maximum and minimum rate ranging from one to 40. This extremely high ratio (considering that there is one recommended level of application) was due to one farmer, who applied a "cocktail" of several insecticides at reduced level. For the two formulations of herbicides applied, the ratio between maximum and minimum rate of application was about 4.5. Molluscicides were used in four farms during the DS and in twelve farms during the WS.

Table 11.7. Commercial Pesticides Used, Number of Users, and Quantity of ai Applied per Crop (kg ai/ha) in Laguna Area During the Biological Soil Survey

	Dry Season				*Wet Season*			
	Number of	*Rate of Utilization*			*Number of*	*Rate of Utilization*		
Pesticide	*Users*	*Maxi*	*Mini*	*Mean*	*Users*	*Maxi*	*Mini*	*Mean*
Insecticides:								
Azocord + Azodrin	11	0.67	0.01	0.25	6	0.33	0.10	0.25
Brodan	9	0.53	0.10	0.25	10	0.42	0.11	0.21
Decis	2	0.01	0.01	0.01	2	0.02	0.00	0.01
Diagran	1	—	—	0.67	0	—	—	—
Endox	1	—	—	0.58	0	—	—	—
Folidol	3	0.83	0.02	0.39	1	—	—	0.21
Furadan	4	0.37	0.01	0.20	4	0.46	0.32	0.39
Hytox	1	—	—	0.21	3	0.29	0.11	0.20
Lorsban	0	—	—	—	4	0.64	0.21	0.45
Thiocarb	2	0.02	0.01	0.02	0	—	—	—
Thiodan	8	0.66	0.01	0.31	6	0.22	0.10	0.15
Trebon	3	0.20	0.07	0.12	0	—	—	—
Sevin	1	—	—	0.95	0	—	—	—
Super gran	1	—	—	0.70	0	—	—	
Vindex	1	—	—	0.01	0	—	—	—
Herbicides:								
2,4-D	1	—	—	0.33	9	0.40	0.07	0.16
Machete	25	0.71	0.16	0.40	22	0.65	0.14	0.34
Solnet	1	—	—	0.01	0	—	—	—
Molluscicides:								
Aquatin	3	0.07	0.09	0.08	8	0.18	0.10	0.11
Brestan	1	—	—	0.29	4	0.39	0.10	0.19

	Summary of Quantities of ai Used					
	Insecticide		*Herbicide*		*Molluscicide*	
	DS	*WS*	*DS*	*WS*	*DS*	*WS*
Maximum	1.76	1.06	0.71	0.74	0.29	0.53
Minimum	0.00	0.00	0.00	0.00	0.00	0.00
Average	0.47	0.27	0.37	0.29	0.02	0.05
Standard deviation	0.34	0.27	0.16	0.17	0.06	0.10

The quantities of ai of individual pesticides used per cropping season averaged about 0.3 kg/ha/crop and did not exceed 1 kg/ha/crop. The total quantities of ai used during a cropping season in a field ranged from 0.5 to 2.5 kg/ha. Most values were between 1 and 2 kg/ha. More pesticide was used during the DS than during the WS (Table 11.7). During the DS, when a wider range of pesticide was used, the number of different formulations used per field varied from one to eight and averaged three. Five farmers utilized more than three different pesticides.

Because of the variability of the nature of pesticides used in Laguna area, no fully satisfactory quantification of overall pesticide utilization among farms could be established.

The first approach considered the quantity of ai of insecticide, herbicide, molluscicide, and total pesticides applied per hectare during the DS and WS of the soil survey. This method of calculation was biased because the quantities of ai required to be active vary among pesticides; some, such as Decis, require very low levels. Cluster and principal component analysis using ai of pesticides and number of applications as descriptors did not show any grouping that would have permitted to compare sets of farms with low, medium, and high pesticide use.

The second approach, intended to express both the intensity and the history of pesticide use in the farm by considering the cost of pesticides groups (insecticide, herbicide, molluscicide, and their sum) applied per hectare in each field during the two seasons of the soil survey and the five previous cropping seasons. Indeed this method was biased by commercial aspects, but one can expect such an estimate to be less dependent upon the quantity of ai required to be active because what is roughly priced in a commercial formulation is the quantity of ai necessary for an efficient application.

In a first approach, all possible correlations between (1) the measurements performed at individual sampling times, or their combinations, or their means, and (2) the estimates of ai of pesticide groups applied in both seasons of the soil survey (WS, DS, sum), and the number of applications (WS, DS, and sum) were tested. In total, 420 individual correlations were studied corresponding to the cross tabulation of

- Twenty-eight sets of data on biological variables:
  Aquatic oligochaetes and cyanobacteria: (4 estimates + average) × 2 = 10
  Flush-N: (5 estimates + average) × (upper soil, lower soil, average) = 18
- Fifteen sets of data on pesticide application:
  Insecticide: (WS, DS, sum = 3); Herbicide: 3; Molluscicide: 3; Sum pesticides: 3; = 12
  Number of applications (WS, DS, and sum) = 3

In a second approach, data were aggregated or selected in order to study cross-correlations between a limited number of values representative of the average physicochemical status, biological status, and the long-term utilization of pesticide in each field.

Soil physicochemical properties recorded from two independent sampling (WS and DS) were highly correlated ($r > 0.95$ for pH, C, and available P; $r > 0.8$ for N). Therefore, average values were used. The two expressions of bulk density were highly positively correlated to each other and both were highly negatively correlated with soil humidity. Carbon and N content were also highly positively correlated to each other. Therefore, only bulk density (g dry weight/cu cm) and total soil N were considered. Bulk density, available-N, and flush-N measurements performed in the 0–2 and 2–10 cm layers were highly positively correlated to each others and highly correlated to their respective sums or average in the 0–10 cm layer. Therefore, only their sums or average value in the 0–10 cm layer were considered.

For biological variables, measurements performed at individual sampling times (transplanting and crop maturity in the DS and WS) were highly positively correlated with overall farm means ($p < 0.01$). Therefore, only mean values were used to study correlations with pesticide use.

The correlations between aggregated/selected data and both estimates of pesticide use (ai and cost) are presented in Table 11.8.

Results and Discussion. (1) *Soil properties*: Laguna soils showed a range of variation in their physicochemical properties (Table 11.6) that allowed to recognize five groups by cluster and principal component analysis, using pH, C, N, P, and active Fe as descriptors. Groups corresponded to the geographical distribution of the fields (data not shown). Average contents in C (2.23 percent), N (0.22 percent), and available-P (30 ppm) indicated an average fertility higher than that of Asian and Philippine rice soils. For comparison, Kawaguchi and Kyuma (1977) reported average contents of 1.4 percent C, 0.13 percent N, and 21 ppm available P in 410 rice soils of tropical Asia. pH was positively correlated with soil organic matter content (C and N) and negatively with Olsen P (Table 11.8). This is not a general feature of rice soils but a characteristic of the set of soils studied.

Significant correlations were found between (1) total pesticide applied and soil pH ($r = -0.356$, $p < 0.05$), (2) molluscicide and available-P, (3) herbicide with available-P and bulk density, and (4) total pesticide and bulk density (Table 11.8). Indeed, such correlations are either spurious, occurring by accident, or highly indirect. They have to be kept in mind when interpreting correlations between biological variables and pesticide use.

(2) *Microbial biomass*: Flush-N was positively correlated with soil pH and

Table 11.8. Correlations Between Soil Properties, Selected Biological Variables, and Two Estimations of the Intensity of Pesticide Use in Thirty-Two Ricefields of the Laguna Area

	pH	*N*	*C/N*	*Average P*	*Active Fe(iron)*	*Bulk Density*	*Average N*	*Flush N*	*Cyano.*	*Oligochaete*	*Insecticide*	*Herbicide*	*Molluscide*	*Pesticide*
pH	+1.000										—	—	−0.295	—
N	**+0.445**	1.000									—	+0.260	—	+0.277
C/N	—	—	1.000								—	+0.260	—	—
Average	−0.294	**−0.506**	−0.297	1.000							—	**−0.483**	—	−0.260
Act.Fe	**−0.646**	**−0.480**	—	+0.293	1.000						—	—	—	—
BD	−0.304	**−0.638**	**−0.430**	**+0.576**	+0.390	1.000					−0.252	−0.339	—	−0.383
Average N	—	—	**−0.690**	+0.318	—	**+0.577**	1.000				+0.291	−0.254	—	—
Flush N	+0.367	+0.289	**−0.497**	—	—	—	+0.338	1.000			—	—	—	—
Cyano.	—	—	**−0.528**	—	—	**+0.347**	**+0.530**	**+0.514**	1.000		+0.301	—	—	+0.260
Oligochaete	—	**+0.510**	**+0.530**	−0.305	—	**−0.752**	**−0.683**	−0.316	−0.421	1.000	+0.201	+0.254	—	+0.339
Insecticide	−0.269	—	—	—	—	—	—	—	—	+0.334	1.000			
Herbicide	−0.325	—	—	—	+0.292	—	—	—	—	—		1.000		
Molluscide	−0.322	—	—	+0.364	—	—	—	—	−0.293	+0.282			1.000	
Pesticide	−0.356	—	—	—	—	—	—	—	—	+0.337				1.000

Note: Levels of significance of the correlations: $r = 270$, $p = 0.10$; $r = 0.339$, $p = 0.05$; $r = 0.425$, $p = 0.01$ ($n = 32$).
Correlation coefficients with $p < 0.15$ are not presented; those with $p < 0.05$ are underlined, those with $p < 0.01$ are in bold.
The upper right area of the table presents correlations with pesticide use expressed as ai applied per hectare during the two crops of the biological survey, the lower left area presents correlations with pesticide use expressed as cost per hectare of pesticide applied during the two crops of the biological survey and the five preceeding crops.

negatively with the C/N ratio of the soil (Table 11.8) indicating that neutral to slightly alkaline soils with a well-decomposed organic matter might have a larger microbial biomass than acidic soils with a less decomposed organic matter. The weak correlations between flush-N, available-N, and total N confirmed that total N is not the best index of N-fertility of wetland soils (Watanabe, De Datta, and Roger, 1988).

Among the 270 individual coefficients of correlation between flush-N and pesticide use, only five indicated a significant correlation at $p < 0.05$. This was observed for

- Flush-N in the 0–2 cm soil layer at the beginning of the WS with the quantity of ai of insecticide applied during (1) the previous DS ($r = 0.353$; $p < 0.05$), and (2) the DS and WS of the soil survey ($r = 0.389$; $p < 0.03$).
- Average flush-N value in the 0–2 cm soil layer with the quantity of ai of insecticide applied during the DS ($r = 0.374$; $p < 0.04$).
- Flush-N in the 2–10 cm soil layer at the beginning of the DS with the quantity of ai of (1) insecticide applied during the *next* (!) WS ($r = 0.421$; $p < 0.05$), and (2) herbicide applied during the previous DS ($r = -0.501$; $p < 0.01$).

The four correlations between flush-N and insecticide were positive, while the correlation with herbicide was negative.

Positive correlations between insecticide use and microbial biomass in the upper soil layer are in agreement with the known positive effect of insecticides on microalgae, in relation with grazer control. A negative effect of herbicides is in agreement with their possible inhibitory effect on both microalgae and microflora (see chapter 10). However, the very low number of significant individual correlations (less than 3 percent) and the absence of significant correlations between aggregated/selected data (Table 11.8) indicate that effects were rather incidental and not long lasting or not strong enough to be established with the method of estimation of microbial biomass.

(3) *Cyanobacteria*: Roger, Santiago-Ardales, Reddy, and Watanabe (1987b) reported positive correlations between N_2-fixing cyanobacteria populations and soil pH and available P content in a large sample of 102 rice soils. In this study, cyanobacteria showed no correlation with soil pH or available P (Table 11.8) probably because of the relatively narrow range of pH of the soils (most of the soils pH were between 5.8 and 6.5) and their relatively high available P content. The correlation between cyanobacteria and available-N ($r = 0.530$) and flush-N ($r = 0.514$) may indicate that cyanobacteria contribute to N-fertility even in soils receiving N-fertilizers. A significant negative correlation ($r = -0.421$, $p > 0.02$) was found between aquatic oligochaete populations and cyanobacteria (Table

11.7), which may reflect the burrowing action of aquatic oligochaetes on organic material accumulating at soil surface (Grant and Seegers, 1985).

Among the seventy-five individual coefficients of correlation between cyanobacteria abundance and pesticide use that were calculated, only five significant values ($p < 0.05$) were observed. This was for

- Populations of cyanobacteria at the beginning of the WS with the quantity of ai of (1) insecticide applied during the previous DS ($r = 0.410$; $p < 0.02$), (2) pesticide applied during the previous DS ($r = 0.440$; $p < 0.01$), and (3) insecticide applied during the DS and WS of the soil survey ($r = 0.374$; $p < 0.04$).
- Populations of cyanobacteria at the end of the DS with the quantity of ai of (1) herbicide applied during the previous DS ($r = 0.507$; $p < 0.01$), and (2) pesticide applied during the previous DS ($r = 0.448$; $p < 0.015$).

In addition positive correlations with a level of significance between 0.05 and 0.10 were found for

- The total quantity of ai of insecticide applied during the DS and WS of the soil survey and (1) populations of cyanobacteria at the beginning of the WS, (2) populations at the end of the DS,
- Average populations of cyanobacteria and total ai of insecticide applied during the DS and WS of the soil survey.

Correlations between aggregated/selected data (Table 11.8) showed a significant effect of insecticides (and to a lesser extent total pesticides) estimated as ai applied during the DS and WS of the soil survey. But these correlations were not significant when pesticide use was estimated from the cost over the last seven cropping seasons. Both methods of estimation of pesticide use indicated an inhibitory effect of molluscicides.

These results partly confirm a positive (but not strongly marked) effect of insecticides on populations of cyanobacteria (see chapter 10). Data on molluscicide seems to indicate an inhibitory effect of the formulations applied, but, because of the restricted number of farms where molluscicide was used, such data need further confirmation.

(4) *Aquatic oligochaetes*: The abundance of aquatic oligochaetes was positively correlated with C and N content in soil, and negatively with bulk density (Table 11.8). The first correlation is in agreement with the observation that organic matter incorporation increases aquatic oligochaete populations (Roger and Kurihara, 1991). The negative correlation with bulk density most probably reflects the requirement of aquatic oligochaetes for wet soils rather than their

mechanical effect on soil. In drying soils, no aquatic oligochaetes were usually recorded in the 10 first centimeters of soil. The negative correlation between aquatic oligochaete and flush-N could be a correlation at the second level resulting from (1) the negative correlation between aquatic oligochaetes and bulk density, and (2) the positive correlation between bulk density and flush-N. However, the hypothesis of a decrease of microbial populations by a direct or indirect action of aquatic oligochaetes cannot be rejected. In particular, aquatic oligochaetes were negatively correlated with cyanobacteria, which were highly correlated with flush-N.

Among the seventy-five individual coefficients of correlation between aquatic oligochaetes abundance and pesticide use, eight significant values ($p < 0.05$) were observed. This was for

- Populations at the end of the WS with (1) the number of pesticide applications r = 0.436; $p < 0.014$), (2) the total quantity of ai of pesticide applied during the previous DS ($r = 0.424$; $p < 0.017$), (3) the total quantity of ai of insecticide ($r = 0.354$; p < 0.05), (4) the number of pesticide applications ($r = 0.443$; $p < 0.012$), and (5) the total quantity of ai of pesticide applied during the DS and WS of the soil survey ($r = 0.431$; $p < 0.015$).
- Populations at the end of the DS with the quantity of ai of molluscicide applied during the previous DS ($r = 0.440$; $p < 0.037$).
- Average populations during the survey with (1) the quantity of ai of herbicide applied during the DS ($r = 0.400$; $p < 0.024$), (2) the number of pesticide applications during the DS ($r = 0.392$; $p < 0.027$), and (3) the number of pesticide applications during the DS and WS of the soil survey ($r = 0.408$; $p < 0.02$).

In addition, correlations with a level of significance between 0.05 and 0.10 were found for average populations and the quantity of ai of pesticide applied during (1) the DS ($r = 0.339$; $p < 0.062$), and (2) the DS and WS of the soil survey ($r = 0.339$; $p < 0.062$).

Correlations between aggregated/selected data (Table 11.8) showed only correlations at levels lower than $p < 0.05$.

All correlations with pesticide use were positive whereas previous experiments rather indicated a possible negative effect of pesticides, especially insecticides. In fact there was a high chance for correlations observed to be spurious. The quantity of insecticide, herbicide and total pesticide applied were, by accident, negatively correlated with bulk density (Table 11.8) whereas aquatic oligochaetes were strongly negatively correlated with bulk density. Positive correlation between herbicide application and total N and C/N of the soil, and between populations of aquatic oligochaetes and total N and C/N might have also contributed to a spurious positive correlation between pesticide application and populations of aquatic oligochaetes.

Other Approaches. 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 five farmers were tested. None of the sixty tests performed indicated a significant difference.

11.4. Conclusion

Field experiments under controlled conditions were conducted at rates of agrochemicals currently used in farmers' fields. Survey was conducted in fields where farmers applied their usual practices.

Soil microbial biomass, estimated through flush-N measurements, was studied in two experiments in controlled plots and in the farmers' field survey. Data did not show any significant effect of pesticides on a short- or long-term basis. However, caution is needed when interpreting these results because of the high variability of flush-N measurements and the limited knowledge on the significance of the method.

N_2-fixing cyanobacteria were enumerated in all experiments and the field survey. A few individual observations in field experiments showed a positive effect of insecticide application in plots where N-fertilizer was not broadcast in the floodwater. In the field survey, a weak correlation ($0.10 > p > 0.05$) between cyanobacteria abundance and the quantity of ai of insecticide applied was also found. These results are in agreement with published data reporting a positive effect of insecticides on cyanobacteria through grazer control, but they indicate effects less marked than in many experiments where pesticide levels higher than the recommended dose applied in the absence of N-fertilizer were often used (see chapter 10).

Primary production in the floodwater, studied in two experiments in controlled plots, was also favored by insecticide application. This effect was limited to the early stage of the crop cycle. This observation was in agreement with result of the farm survey showing a weak positive correlation ($p < 0.10$) between soil available-N and insecticide application.

Intensive sampling in experimental plots showed that N-fertilizer and the presence of rice plant affected more floodwater biology than pesticides do. In particular, N-fertilizer broadcasting had a clear stimulatory effect on floodwater primary productivity, favoring the growth of unicellular algae, which resulted in the proliferation of algivorous aquatic arthropods. Pesticides had no long-lasting effects on these organisms when considered at the crop cycle level. No pesticide effect was usually observed on zooplankton in the presence of broadcast N-fertilizer. In plots where no N-fertilizer was applied, significant effects on zooplankton were observed, but they were incidental and limited. However, in farmers' fields, pesticides are usually not used in the absence of fertilizer.

Aquatic oligochaetes exhibited a significant negative response to pesticide application in three of the four crop cycles studied in experimental plots. Field surveys did not confirm a long-term negative effect of pesticide use on these populations. However, aquatic oligochaetes seems to be a most sensitive indicator of pesticide effect in wetland soils than aquatic arthropods, N_2-fixing cyanobacteria and soil microbial biomass. The hypothesis that pesticide use, by reducing oligochaete populations, might reduce the translocation of recycled nutrients accumulating at the soil-water interface into the deeper soil was not confirmed by flush-N measurements in the deeper soil layer.

The results summarized in this chapter seem to confirm the common belief that pesticides applied at recommended levels and intervals in ricefields are seldom deleterious to the beneficial microorganisms, invertebrates, and their activities.

In the intensive surveys in experimental plots, only a low percentage of measurements performed at individual sampling times indicated statistically significant differences attributable to pesticide use (Tables 11.2 and 11.5). In the field survey aiming at identifying long-term effects of pesticides significant correlations were relatively less numerous than in experimental plots. Among 420 individual correlations between pesticide use and biological variables tested, only eighteen (4.3 percent) were significant at $p < 0.05$. Among twenty-four correlations between aggregated/selected data (Table 11.8) one was significant at $p < 0.05$ and eight were significant at $0.10 > p > 0.05$. Among those, six were doubtful because of a few artificial correlations between pesticide use and soil properties.

Significant effects of pesticides on measured biological variables were not frequent, not very marked—except for aquatic oligochaetes—and not long lasting. However, because of the variety of pesticide used by farmers, the two methods of estimation of pesticide use intensity utilized in the soil survey were biased, and caution is needed when interpreting the results. In addition, results presented are fragmentary, and more detailed studies are definitely needed before drawing conclusions on the long-term effects of pesticide use on microorganisms and invertebrate populations in ricefields. Data presented raise the problem of the methodological approach to assess these effects. In particular, the results of the field survey demonstrate the limitations of studies performed under realistic but uncontrolled conditions and the need for long-term trials in experimental fields.

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Notes

1. As indicated on the package. Indeed, the authors disagree with the utilization of tablespoons for preparing pesticide solutions.

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