EFFECT OF HERBICIDE USE ON SOIL MICROBIOLOGY

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Modern rice-growing technologies—increasing cropping intensity, using optimum crop management practices, planting improved varieties, applying fertilizers, protecting the crop with pesticides—have increased yields markedly. These same technologies also have changed traditional rice-growing environments markedly. One change is less diversity of vertebrates and invertebrates in ricefields (Roger et al 1991). Other environmental impacts, whether short- or long-term, are far from being fully assessed.

The most intense pressure being exerted on the microbial, faunal, and floral communities of ricefields comes from the increasing use of fertilizers and pesticides. These agrochemicals significantly impact the composition and population dynamics of microorganism and invertebrate communities. Greater understanding is needed of how agrochemicals, especially pesticides, may affect soil fertility through their effects on the populations of microorganisms and invertebrates that recycle and translocate nutrients, and make them available to a crop. The issue is whether, as use of pesticides increases, the efficacy of soil microorganisms will be reduced by population shifts toward species more efficient in pesticide degradation.

In recent years, pesticide use in less developed countries has tended to increase faster than it has in the industrialized countries, and that trend is causing concern (Moody 1990). In most rice-growing countries, insecticides have been the dominant class of pesticides used (Van der Valk and Koeman 1988). In the Philippines, for example, 55-60% of the pesticides used before 1980 were insecticides, 20-25% were fungicides, and 5-16% herbicides. While that relative pattern still exists, herbicide use is increasing rapidly as labor availability in many agricultural areas is depleted by rapid urbanization (Moody 1990).

We undertook a bibliographic survey to identify studies that measured the impact of pesticides on nontarget microorganisms and soil and water invertebrates in ricefields (Roger et al 1994). A computerized data base was established and quantitative data on the fate of pesticides and their effects on microorganisms in rice soils were tabulated and analyzed. We used this data base to assess the relationships between herbicides and nontarget soil microorganisms and aquatic invertebrates in rice environments.

PESTICIDE FATE AND IMPACT

The bibliographic data base on pesticide fate and impact on microorganisms and invertebrates in rice environments contains about 600 references. Quantitative data collected from 63 articles on pesticide fate, 71 articles on impact on nonphotosynthetic microorganisms, and 149 articles on impact on photosynthetic microorganisms were tabulated and general trends analyzed (Roger et al 1994). Quantitative data on pesticide impact on soil and water invertebrates were only available in some dozen articles, not enough to be tabulated.

The general characteristics and limitations of this data base, and specific aspects that pertain to herbicide studies, include the following:

- Most pesticide studies conducted in ricefields, with ricefield soil, or with organisms isolated from ricefields, were published during the 1980s. More recent studies are scarce, which implies that current knowledge is somewhat outdated. Part of the information deals with superseded formulations, and little is known about recently released compounds and formulations. Table 1 lists the number of papers in the data base that deals with the impacts on microflora of the herbicides applied on California rice in 1990 (Crosby 1996). No information is available for two of the herbicides; for the others, most studies deal with cultures of microalgae and cyanobacteria tested in the laboratory.
- Most of the available information on the fate of different pesticides in ricefield soil is based on experiments with 20-100 g of unplanted soil. Such experiments underestimate chemical degradation because of the absence of rhizospheric effect and because of variations of environmental conditions (light, wind, temperature, redox potential). Only about 70 of 200 references presented data on herbicides.
- Quantitative estimates of the effects of pesticides on microorganisms in ricefields are given in 240 references. Those studies assessed impacts using the classical methodology of soil microbiology, including enumeration and measurement of activity (Greaves et al 1978). Such quantitative analyses and interpretation should be accepted with caution, for the following reasons:
  - The organisms and pesticides studied do not constitute a representative sample of the numerous combinations occurring in ricefields. Data for photosynthetic microorganisms (149 references) are mostly on herbicides and cyanobacteria, and are more abundant than those on nonphotosynthetic microorganisms (71 references), which deal mostly with insecticides (Roger et al 1994). The impact of herbicides on photosynthetic microorganisms is found in 64 studies testing 54 formulations; only 16 studies, testing 22 formulations, deal with nonphotosynthetic microorganisms.

Table 1. Number of papers dealing with the impact of herbicides[a] on microorganisms in California ricefields, 1990.

Herbicide	Algae and cyanobacteria			Bacteria		
	Lab[b]	Pot	Field	Lab	Pot	Field
Bensulfuron methyl						
Copper sulfate	2	-	6	-	-	-
2,4-D	21	-	2	-	1	-
Glyphosate	2	-	-	-	-	-
MCPA	5	-	1	1	-	-
Molinate	3	1	1	1	1	
Pendimethalin	-	-	-	-	-	-
Propanil	7	1	3	2	-	-
Thiobencarb	5	1	1	1	-	-
Total	45	2	14	5	2	0

[a]Listed by Crosby (1996). [b]Lab = laboratory.

  - Most studies are laboratory experiments with cultures of microorganisms or with only a few grams of soil. Less than 8% of the quantitative studies were conducted in ricefields. Experiments with microbial cultures give an index of a strain's sensitivity to pesticides, but the results cannot be extrapolated to field conditions where the initial concentrations of pesticides are likely to decrease rapidly, as their degradation is hastened by soil microflora, nonbiological decomposition, leaching, volatilization, and soil adsorption. Concentrations of pesticides that affect microorganism growth depend on the initial microbial population, its nutrient status, the method of pesticide application, and the degradation products. These factors markedly differ in vitro and in situ. For example, little agreement was found between the responses of microbial species to glyphosate in incubated soil samples and in pure culture (Wardle and Parkinson 1990).
  - Many studies used pesticide concentrations higher than those resulting from the recommended level for field application. Such experiments may overestimate pesticide persistence because degradation is slowed at high concentrations, as has been shown with trifluralin (Parr and Smith 1973) and molinate (Deul et al 1978). The recommended levels of traditional pesticides range from a few hundred grams to a few kilograms of active ingredient per hectare. The median recommendation for herbicides (2.5 kg ai ha^{-1}) is higher than it is for fungicides (1.7 kg ai ha^{-1}) and insecticides (1.1 kg ai ha^{-1}) (Roger 1990). Experimental results should be interpreted in the context of the range of pesticide concentrations that can be expected in farmers' fields after application. If a herbicide applied on a nonflooded field stays within the surface 2 cm of soil, 2.5 kg ai ha^{-1} would correspond to 15 mg kg^{-1} dry weight of soil, for a bulk density of 1 mg m^{-3}. If a water-

soluble herbicide is distributed in 10 cm water and 10 cm puddled soil, 1 kg ai ha^{-1} would correspond to 1 mg kg^{-1} of soil. The field situation is probably closer to the lower value. Our data base lists 1,045 articles that report quantitative data on the effects of pesticides on photosynthetic microorganisms. In 638 of the studies, effects were measured at concentrations higher than twice the recommended level for field application. This is probably because the studies were designed to establish the lethal concentration for strains rather than to measure possible effects in the field. Such data are of little value in drawing conclusions, except when no effect was recorded.

MICROBIAL DEGRADATION OF HERBICIDES

Pesticide degradation in ricefields is accelerated by the reducing conditions caused by submersion and by the temperature and pH ranges that favor microbial activity (Ponnamperuma 1972). As a result, pesticides often persist longer in nonflooded soils than they do in flooded soils (Sethunathan and Siddaramapa 1978). In a data base on the half-life of pesticides in rice soils, only 8 of 45 tests reported shorter half-lives in nonflooded than in flooded soils (Roger et al 1994). Herbicides with faster degradation in flooded soils include trifluralin (half-life >4 d in flooded soil and >20 d in nonflooded soil [Parr and Smith 1973, Willis et al 1974]), pyrazoxyfen (half-life <10 d in flooded soil and 3-34 d in nonflooded soil [Arita and Kuwatsuka 1991]), and MCPB-ethyl (half-life 2 d in flooded soil and 3 d in nonflooded soil [Asaka and Izawa 1982]). Some herbicides degrade faster in nonflooded, upland soils than they do in flooded, lowland soils. These include molinate (half-life 4-160 d in flooded soil, 8-25 d in nonflooded soil [Deuel et al 1978, Thomas and Holt 1980, Imai and Kuwatsuka 1982]), thiobencarb (half-life 45 d in nonflooded soil, 100 d in flooded soil [Ishikawa et al 1976, Nakamura et al 1977, Duah-Yentumi and Kuwatsuka 1980]), and MCPA (4-chloro-2-methylphenoxyacetic acid [Duah-Yentumi and Kuwatsuka 1980]). The persistence of MCPA, 2,4-D, and 2,4,5-T about half as long under moist as under flooded conditions was explained by the need of an aerobic microflora to rapidly degrade phenoxy acid herbicides (Sattar and Paasivirta 1980).

Herbicide-degrading microflora in ricefields

In dryland soils, bacteria and fungi are considered to be the organisms primarily responsible for pesticide degradation. In wetland soils, bacteria are the major agent (although fungi are involved in wetland soils [Rao and Sethunathan 1974], they probably are less important than bacteria). Microalgae may play a significant role in wetland soils, either directly by favoring photooxidation through the oxygen they release in floodwater or indirectly when their photosynthetic activity causes water pH to vary 1-2 units during the day, to favor chemical decomposition of pesticides. So far, the contribution of microalgae to pesticide decomposition in ricefields has been demonstrated mostly for insecticides. Photolysis of the insecticide methyl parathion was much faster in water containing algae than in distilled water (Zepp and Schlotzhauer 1983). The importance for pesticide degradation of the increase of water pH caused by algae is obvious for the insecticide carbofuran, whose hydrolysis was more than 700 times faster at pH 10 than at pH 7 (Seiber et al 1978). The cyanobacterium *Anacystis nidulans* can convert the phenylcarbamate herbicides propham and chlorpropham to their corresponding anilines (Wright and Maule 1982). A significant role of rice rhizospheric bacteria in pesticide degradation was also demonstrated for the insecticide parathion. After 2 wk in an unplanted, flooded soil, less than 5.5% of ^{14}C-labeled parathion was evolved as $^{14}CO_2$; in a planted, flooded soil, 22.6% was evolved (Rajasekhar Reddy and Sethunathan 1983a). Similar data are not available for herbicides.

Pesticide-decomposing microorganisms isolated from ricefield soils belong to the genera *Arthrobacter, Bacillus, Clostridium, Flavobacterium, Micrococcus, Mycobacterium, Pseudomonas,* and *Streptomyces* (Roger et al 1994). They can degrade pesticides by using them as their sole carbon sources, through cometabolism, or by synergy. Most of the species that have been isolated degrade insecticides; two species have been isolated that degrade pentachlorophenol (PCP), *Mycobacterium* sp. (Suzuki 1983a,b) and *Pseudomonas* sp. (Watanabe 1973). Several species have been characterized that degrade MCPA. *Flavobacterium peregrenum* and *Arthrobacter* spp. produced 4-chloro-2-methylphenol as the major metabolite, and *Aspergillus niger* produced 4-chloro-5-hydroxy-2-methylphenoxyacetic acid (Soderquist and Crosby 1975).

Relative importance of microbial degradation

The usually faster degradation of pesticides in nonsterilized than in sterilized (autoclaved) soils and, in some cases, faster degradation in soil on second exposure to a given pesticide demonstrate the importance of microbial degradation of pesticides (Roger et al 1994). Several pathways of degradation/dissipation may be involved for a single pesticide—adsorption, transport (volatilization, percolation, runoff), and transformation processes (photolysis, chemical hydrolysis, biodegradation). If a chemical is very volatile, it may not have a long enough residence time for significant degradation to occur, even though the rate of degradation could be relatively rapid if the chemical is maintained in contact with the soil. The relative importance of microbial decomposition will vary, depending on the nature of the herbicide, the environmental conditions, and the method of application.

For a number of herbicides, soil sterilization markedly reduced degradation, indicating that under the experimental conditions, degradation was mostly a microbial process. Those herbicides include thiobencarb (Nakamura et al 1977), butachlor (Chen 1980), MCPB-ethyl (Asaka and Izawa 1982), 2,4-D and 2,4,5-T (Yoshida and Castro 1975), and molinate (Imai and Kuwatsuka 1982). Studies of the dissipation of MCPA indicated that microbial decomposition was greater than photodecomposition, and that volatilization was of little importance (Soderquist and Crosby 1975). Dissipation of the rice herbicides thiobencarb and molinate was attributed primarily to volatilization, although some microbial decomposition was demonstrated (Crosby 1983). In a mass balance for molinate dissipation, 75-85% was attributed to volatilization and less than 1% to aqueous microbial metabolism (Soderquist et al 1977).

An interesting example is trifluralin, for which the relative importance of microbial degradation may vary markedly. In the presence of ultraviolet radiation, trifluralin is rapidly photodecomposed. It can also be extensively lost by volatilization, depending on its concentration and mode of application and on the moisture content of the soil (Bardsley et al 1968). Volatilization is reduced under flooded conditions (Parr and Smith 1973). In soil and water, trifluralin is decomposed by physicochemical and microbiological processes. Under aerobic conditions, it degrades by a pathway involving sequential dealkylation of propyl groups, and under anaerobic conditions, by a pathway involving initial reduction of the nitro groups (Parr and Smith 1973). Decomposition is usually faster under anaerobic conditions. A study of the relationship between Eh and the rate of trifluralin degradation used a system for controlling redox potential in soil suspensions. Oxygen exclusion by soil flooding initiated rapid trifluralin degradation only when the Eh decreased below a critical range between +150 and +50 mV (Willis et al 1974). Depending on experimental conditions, physical and chemical processes (Probst and Tepe 1969) or microbiological processes (Parr and Smith 1973) predominate. Trifluralin was not significantly decomposed after 20 d in a sterilized soil, but had almost completely disappeared in the same, unsterilized soil enriched with alfalfa meal (Parr and Smith 1973).

The pesticide concentrations among replicated samples in field trials of pesticide persistence often are extremely variable. For example, the propanil remaining in four soil samples from the same field 40 d after its application ranged from 0.2 to <0.01 ppm (Kearney et al 1970). This may have been caused by several factors, including heterogeneity in soil properties and degradation by heterogeneously (log normally) distributed microflora (Roger et al 1991).

Effect of repeated pesticide application

Repeated application of the same pesticide on the same field has been reported to increase the growth of related, specific decomposing microorganisms and cause its rapid inactivation. Several bacteria that have the ability to degrade a given pesticide were isolated from the soil and water of ricefields previously treated with the pesticide. This has been reported for a number of insecticides, including gamma-BHC, diazinon, and aldicarb (Roger et al 1994). The available data for herbicides deal with PCP and thiobencarb. Watanabe (1973, 1977) isolated PCP-decomposing and PCP-tolerant bacteria from soils. He observed a 1000-fold difference in the number of PCP-decomposing microorganisms between treated and untreated soils (Watanabe 1978). Data for thiobencarb are somewhat contradictory. Nakamura et al (1977) reported that repetitive application of thiobencarb did not lead to an increase in thiobencarb-degrading microflora. However, Moon and Kuwatsuka (1985) reported that when thiobencarb was repeatedly applied to a soil, the lag time for dechlorination decreased from 20 d to 10 d to 2 d due to the multiplication of specific facultative anaerobes that degrade thiobencarb. Those anaerobes rapidly decreased or disappeared when thiobencarb was absent.

Effect of pesticide combinations

When two pesticides are applied simultaneously, one can inhibit the microorganisms responsible for the degradation of the other or modify the physicochemical conditions in a way that reduces the degradation of both. In a model ecosystem, the combination of the insecticide methyl parathion with atrazine substantially increased the persistence of both pesticides in the water and soil phases of the system (Au 1979). On the other hand, soil incorporation of thiobencarb with simetryn or propanil had no significant effect on the degradation rate of any of the pesticides (Nakamura et al 1977).

IMPACT ON MICROALGAE AND CYANOBACTERIA

Microalgae and cyanobacteria are major components of the photosynthetic aquatic biomass that develops in ricefield floodwater. Other components include macrophytic algae and vascular macrophytes. The average primary production reported in ricefield floodwater over a crop cycle ranged from 0.5 to 1 g C m^{-1} d^{-1} (Roger and Kurihara 1991). Cyanobacteria and microalgae trap both atmospheric C and N and C and N evolved from the soil, and, when reincorporated, help to reduce nutrient losses into the soil. They affect N fertility of ricefields through the following ways:

- Photodependent biological N_2 fixation (BNF) by cyanobacteria, which contributes 5-20 kg N ha^{-1} per crop from free-living forms and 30-60 kg N ha^{-1} per crop from symbiotic forms associated with azolla used as green manure (Roger et al 1993).
- N immobilization and recycling through death or grazing, followed by decomposition, N accumulation at the soil surface, and translocation to deeper soil by soil fauna (Roger and Kurihara 1991).
- Replenishment of the soil microbial biomass and available N, as shown by positive correlation with chlorophyll-type compounds (Watanabe and Inubushi 1986).
- Provision to the rice plant of an average 30% of the total N contained in cyanobacteria, algae, and aquatic plants incorporated into soil and 20% of those decomposing at the soil surface (Roger and Kurihara 1991).
- Induction of N losses by NH_3 volatilization (2-60% of the N applied), partly due to microalgae which deplete CO_2 in floodwater, which increases its pH and the concentration of volatile NH_3 (Fillery et al 1986).

In transplanted rice, cyanobacteria and microalgae are not considered weeds of major economic importance. In direct-seeded rice, however, they are detrimental at germination because they compete for light, form a membranaceous mat restricting penetration of the rice roots into the soil and the gaseous exchange between soil and water, and have detrimental mechanical effects on rice when their epiphytic growth either pulls seedlings down or lifts and uproots them when the water level varies (Smith et al 1977, Noble and Happey-Wood 1987).

Pesticides have three major effects on ricefield microalgae and cyanobacteria. Some preferentially affect green algae and promote cyanobacteria growth, as has been observed with the algicides simetryn (Yamagishi and Hashizume 1974) and algaedyn (Almazan and Robles 1956). Insecticides over the short term increase microalgae by temporarily decreasing populations of invertebrates that graze on algae. Insecticides also have a selective effect on cyanobacteria by causing the recruitment of algal grazers, which results in the dominance of strains that form mucilaginous macrocolonies resistant to grazing.

General trends

As photosynthetic organisms, cyanobacteria and algae can be expected to be more sensitive than other microorganisms to herbicides, especially the photosynthetic inhibitors. Several unicellular eukaryotic algae most common in ricefields (*Chlorella, Chlamydomonas, Euglena)* have been shown to be sensitive to photosynthetic inhibitor herbicides (Arvik et al 1973). Quantitative data obtained at concentrations corresponding to the recommended level of field application are mostly estimates of the inhibitory effect of herbicides on cyanobacteria cultures; experiments with soil in vitro and in situ make up less than 10% of the data (Table 2). Results confirm, however, that among pesticides not aimed at controlling algae, herbicides are most detrimental to cyanobacteria and algae, causing partial or total inhibition in 67% of the in vitro tests and in 42% of the in situ or soil tests at recommended levels of field application. These values also confirm that pesticide effects are more marked in vitro than in situ.

Table 2. Effects of pesticides on photosynthetic ricefield microorganisms (cyanobacteria and microalgae) at concentrations corresponding to recommended field application.

Nature of data	Data (no.)	Data (%) corresponding to different levels of inhibition				
		None	<50	50	>50	100
All data	407	39	19	26	2	14
All data in situ or with soil	39	62	8	3	3	26
Algicides (3 tested)	33	3	0	67	0	30
Fungicides (22 tested)[a]	30	40	10	7	0	43
Herbicides (57 tested)	252	33	25	28	2	12
Herbicides in situ or with soil	24	59	8	4	4	25
Insecticides (28 tested)	97	67	11	14	3	4
Insecticides in situ or with soil	10	90	10	0	0	0

[a]Several fungicides are used as algicides.

Effects on photodependent BNF and biofertilizers

In the wake of interest in biofertilizers which developed in the 1980s, a number of studies dealt with the impact of herbicides on free-living and symbiotic N_2-fixing cyanobacteria indigenous to or inoculated in ricefields.

Indigenous cyanobacteria. Herbicides can inhibit cyanobacteria and photodependent BNF. Laboratory experiments showed that PCP—a pesticide used both as an insecticide and a herbicide—was inhibitory to cyanobacteria and diatoms when applied on the surface, but not when incorporated into the soil (Ishizawa and Matsuguchi 1966). In field studies, CNP (2,4,6-trichlorophenyl 4-nitrophenyl ether) inhibited photodependent BNF (Matsuguchi 1979) and several formulations used in ricefields reduced algal growth (Srinivasan and Ponnuswami 1978). Some herbicides seem to affect the N_2-fixing ability of cyanobacteria specifically; the inhibitory effect of butachlor on N_2-fixing strains growing in an N-free medium was markedly decreased or reversed by inorganic N sources (Kashyap and Pandey 1982). Whereas many herbicides seem to be most detrimental for photodependent BNF, several species of cyanobacteria tolerated 100-500 ppm of 2,4-D, a level much higher than that recommended for field application. This suggests that this herbicide might be compatible with cultural practices aimed at promoting cyanobacteria growth as biofertilizer (Venkataraman and Rajyalakshmi 1971, 1972).

Algal inoculation. Although numerous experiments have dealt with inoculating ricefields with N_2-fixing strains of cyanobacteria (Roger 1990), almost no field trials have tested the interaction between pesticides and algal inoculation. Kerni et al (1983, 1984) reported that butachlor applied at 5-30 kg ha^{-1} in inoculated plots had no effect. El-Sawy et al (1984) tested the interaction between cyanobacteria inoculation and four herbicides in a pot experiment. When algal inoculation was effective, herbicide application mostly had no effect or a positive effect on plant characteristics and soil N at 40 d after transplanting. Negative effects were observed with propanil in only 2 out of 16 cases. Srinivasan and Ponnuswami (1978) reported that recommended levels of field application of Saturn (thiobencarb), Basalin, and TOK had no effect on the production of blue-green algae in cyanobacteria inoculum multiplication plots; Sirmate, Fernoxone, Stam F 30 (propanil), and Weedone (2,4-D) decreased growth by 15-40%.

Azolla involves a symbiosis between a N_2-fixing cyanobacterium and an aquatic fern traditionally used as green manure for rice (Roger et al 1993). Information on the effects of pesticides on BNF by azolla is limited. Insecticides decrease pest incidence, which usually favors azolla growth (Satapathy and Singh 1987). Herbicides more often have a detrimental effect. Holst et al (1982) tested the effect in vitro of 15 herbicides on growth and N_2 fixation of *Azolla mexicana*. Bipyridinlium and phenolic herbicides were the most detrimental; at 0.1 ppm, they caused up to a 75% reduction in N_2 fixation and nitrate reduction. Chloramben and the fungicide benomyl at 10 ppm caused 84-99% reduction in N_2 fixation without affecting nitrate reduction or growth. Simazine at 10 ppm stimulated nitrate reduction 20-fold, causing a 99% reduction in N_2 fixation. Growth and N_2 fixation were reduced by other benzoic, triazine, dinitroaniline, and urea herbicides tested at concentrations between 0.1 and 10 ppm. Naptalam was the only herbicide tested that had no effect on growth or N_2 fixation at 10 ppm. In a field test, preemergence herbicide applied about 1 wk before azolla inoculation had only limited effects on azolla; postemergence herbicides were more detrimental (Singh and Singh 1988).

Nevertheless, simultaneous application of herbicides and biofertilizer might not be a sound practice because both free-living cyanobacteria and azolla form a mat that covers the surface of the floodwater and reduces the growth of weeds (Roger et al 1993).

Field experiments

Little impact of herbicides on algae and cyanobacteria has been reported from field experiments in the tropics. Arvik et al (1971) found no change in the composition of algal flora within 18 mo after the application of a 1:4 commercial mixture of 4-amino-3,5,6-trichloropicolinic acid (picloram) and 2,4-D at the recommended field level. Srinivasan and Ponnuswami (1978) found no significant effect or only moderate inhibition of cyanobacteria by seven herbicides applied at recommended field levels. Singh et al (1986) studied the effect of butachlor, thiobencarb, and 2,4-D on N_2-fixing cyanobacteria and found that the recommended rates did not result in major changes in composition of the algal population. All herbicides increased the proportion of *Nostoc;* propanil reduced the proportion of *Anabaena* in the algal population.

In temperate climates, some inhibitory effect of herbicides has been reported. Benthic algae decreased with most applications of herbicides (oxadiazon, bentazon, thiobencarb, simetryn) in Japan (Takamura and Yasuno 1986). Heterocystous and nonheterocystous cyanobacteria and microalgae were affected differently by repeated use of simazine (4 kg ha^{-1}) in Italy, with heterocystous affected more severely. Simazine also reduced species diversity, which was very evident in the case of heterocystous cyanobacteria (Tomaselli et al 1987).

Resistance of algae and cyanobacteria

Resistance to pesticides is common among microalgae and cyanobacteria, and that resistance can adapt to increased concentrations of pesticides (Sharma and Gaur 1981). When several strains are tested for sensitivity to pesticides, some that are resistant to recommended field levels usually are identified (Gadkari 1987). Spontaneous mutants resistant to monuron and blitox have been isolated (Vaishampayan 1984 and 1985, Vaishampayan and Prasad 1982). A study of various classes of herbicides showed that s-triazines and substituted ureas could alter phytoplankton composition by selective inhibition of certain species (Hawxby et al 1977). Because sensitivity to any particular herbicide may vary considerably among algal strains, herbicide application might cause shifts in dominant strains within the algal/cyanobacterial community rather than a decrease of the entire algal biomass.

Bioconcentration in algae and cyanobacteria

Although bioconcentration of pesticides in food chains has been demonstrated in many ecosystems, the issue has received little attention in ricefield studies. Available data only refer to possible pesticide accumulation in vitro by the cyanobacteria common in ricefields (Das and Singh 1977, Kar and Singh 1979). The ability of microalgae to accumulate herbicides in freshwater environments has been demonstrated (Wright 1978). Algae and cyanobacteria also are known to accumulate heavy metals, which has implications in terms of copper- or tin-based pesticides. Invertebrate grazers that feed on phytoplankton that contains high concentrations of pesticide or metal could suffer. Bioconcentration of pesticides in phytoplankton and zooplankton is important when the ricefield ecosystem is considered as a possible environment for aquaculture (rice-fish, rice-shrimp).

IMPACTS OF HERBICIDES ON CHEMOTROPHIC MICROORGANISMS

Chemoautotrophic and chemoheterotrophic microorganisms are the agents of nutrient recycling and maintenance of soil fertility. The N fertility of rice soils results in part from the balance among activities of populations that perform the following tasks (Roger et al 1993):

- Transform organic N into forms available to rice (mineralizing microflora, ammonifiers, and nitrifiers),
- Provide inputs of N through BNF (heterotrophic N_2 fixers in the bulk of soil and the rhizosphere), and
- Cause gaseous losses of N (denitrifiers).

At the levels of inorganic fertilizer usually applied in ricefields, most of the N absorbed by the plant originates from the soil, where it is released by the turnover of a microbial biomass; it represents only a small proportion of the total soil N (Watanabe et al 1988).

General trends

In contrast to experiments with microalgae and cyanobacteria which were conducted primarily with laboratory cultures, tests of pesticide effects on nonphotosynthetic microflora and their activities were performed primarily in small-scale experiments with soil or in situ at concentrations corresponding to the recommended level of field application. The data base contains 606 records obtained at those concentrations, although most of the studies deal with insecticides. Studies with herbicides (a mere 102 records) only allow us to identify very general trends (Table 3). Insecticides affected the microflora or its activities less often (no effect in 68% of the studies) than fungi-

Table 3. Effects of pesticides on nonphotosynthetic ricefield microorganisms at concentrations corresponding to recommended field application.

Group	Data (no.)	Data for each effect (%)*				
		All negative	Negative trend	No effect	Positive trend	All positive
All data	606	8	12	60	11	9
Fungicides	58	5	0	50	24	21
Herbicides	102	13	23	30	21	14
Insecticides	440	6	11	68	7	8
Biological N_2 fixation	176	2	23	31	26	19
Fungicides	25	0	0	20	52	28
Herbicides	26	0	23	23	35	19
Insecticides	125	2	27	35	18	17

*Most experiments are bacterial counts and activity measurements performed several times after application. Each experiment was as follows: no effect—no significant difference between treatment and control; all negative/positive—for all measurements the treatment was statistically lower/higher than the control; negative trend—various effects; positive trend—various effects. Adapted from Roger et al (1994).

cides (no effect in 50% of the studies) and herbicides (no effect in 30% of the studies). Data from field experiments (Sato 1987, Mandal et al 1987, Roger et al 1994) are not numerous enough to be tabulated.

Changes in microbial populations

Reports of experiments where microbial populations were counted after herbicide was applied usually do not present a statistical analysis of the data. Microbial enumerations in soil are highly variable, making it difficult to assess the significance of data not supported by statistical evaluation. In many cases, the differences between microbial enumerations in treated and control soils were less than threefold, which indicates a need for caution in interpreting the results (Roger et al 1993). For example, among 14 microbial groups (aerobic bacteria, actinomycetes, fungi, ammonifiers, ammonium oxidizers, denitrifiers, aerobic and anaerobic N_2 fixers, aerobic and anaerobic P solubilizers, sulfate reducers, cellulose decomposers, and iron precipitators) counted 3 wk after the application of linuron to a flooded soil, only ammonium oxidizers and sulfate reducers exhibited changes in densities more than three times that of the control (Sivasithamparam 1970).

When changes in populations were considered significant, they were usually not long lasting. In one field experiment, counts at 4, 11, 18, and 25 d after application of preemergence herbicides (Goal, TOK E-25, Saturn, and Machete) indicated only a slight initial depression of total microflora, bacteria, actinomycetes, and fungi populations; recovery occurred within a few days. No prolonged effect of herbicides on microflora was observed (Mandal et al 1987). Immediately after thiobencarb was applied to a rice soil, total viable bacteria and populations of Gram-negative, ammonifying, nitrate-reducing, and denitrifying bacteria increased and populations of ammonium-oxidizing and nitrite-oxidizing bacteria decreased. The changes did not persist and the general dynamics of microbial populations during the crop cycle was not affected (Sato 1987). At 30 °C and pH 6.8, 6 ppm butachlor had no significant effect on populations of fungi and actinomycetes, but possibly increased total populations of bacteria for about 2 wk (Chen 1980).

Effects on soil nutrients and enzymes

In a pot experiment using unplanted flooded soil, Ordram (molinate) reduced NH_4 availability and the decomposition of organic matter, and increased P and K availability for the 80-d duration of the experiment (Russo 1970). However, algal development in the pots may have interfered with the process. It was suggested that the effects observed on NH_4 and K availability were possibly correlated with stimulation/inhibition of soil microorganisms, while P availability may have been affected by the capacities of the active chemical groups of the pesticides to substitute for P ions freed from stable combinations of the soil constituents.

Measurements of enzymatic activities in pot and flask experiments showed either an absence of effect or a slight and nonlasting inhibition of amylase, dehydrogenase, invertase, and urease by 2,4-D, atrazine, basalin 48-EC, butachlor, and oxyfluorfen (Chendrayan and Sethunathan 1980, Palaniappan and Balasubramanian 1985, Baruah and Mishra 1986). In a West Bengal ricefield soil, 10 kg MCPA ha^{-1} was needed to decrease cellulolytic populations (De and Mukhopadhyay 1971); the recommended field application is only 0.28-2.25 kg ha^{-1}.

Effects on nitrogen cycle

Ammonification. Thiobencarb (Sato 1987) and butachlor or mixtures of butachlor and diphenylether-type herbicides (nitrofen, chlornitrofen, and chlomethoxynil) (Chen 1980) had no significant effect on ammonification when applied at recommended field levels. Ten times the recommended rate of thiobencarb was needed to significantly affect ammonification (Sato 1987).

Nitrification. Various in vitro experiments with unplanted soil report either no or negative effects of herbicides on nitrification. At 30 °C and pH 6.8, butachlor or mixtures of butachlor and three diphenylether-type herbicides applied at recommended field level and at 10 times the recommended level had no significant effect on nitrification (Chen 1980). Propanil at 3.14 and 5.3 kg ha^{-1} decreased populations of nitrifiers (De and Mukhopadhyay 1971). Three weeks after linuron application, growth of nitrifiers was reduced, but population density recovered within 3 mo (Sivasithamparam 1970). Propanil and bifenox applied at the recommended field level inhibited nitrification during the first 10 d of incubation (Turner 1979). Their effectiveness varied with soil type. After 60 d of incubation, only bifenox still retarded nitrification. That both herbicides have the potential to retard nitrification should be recognized when N transformations in soils are studied, or their effect on plants grown in soil is evaluated, but it is unlikely that they significantly affect N transformation at recommended levels of field application.

Negative effects of herbicides on nitrification cannot necessarily be considered detrimental because reducing nitrification also reduces N losses by denitrification. Identifying efficient and economically feasible nitrification inhibitors has been an objective of the research on microbial management in ricefields (Roger et al 1993).

Denitrification. Denitrification is little affected by pesticides, probably because the complex and versatile denitrifying microflora can metabolize or resist a wide range of substrates. High levels of pesticide are needed to inhibit denitrification (Roger et al 1994). This probably explains why current research aiming at decreasing N fertilizer losses focuses on urease and nitrification inhibitors rather than on denitrification inhibitors (Roger et al 1993). Pesticides tested for their effects on denitrification are mostly fungicides and insecticides. Data for herbicides indicate no significant effect of PCP on denitrification of nitrate applied to flooded soil (Mitsui et al 1962, Mitsui et al 1964). On the other hand, a significant decrease in denitrifier population and denitrification was observed after 10.5 kg ha^{-1} of propanil and 2.25 kg ha^{-1} of MCPA were applied to a West Bengal ricefield soil (De and Mukhopadhyay 1971).

N_2 fixation. N_2-fixing microorganisms and BNF are more affected by pesticides than are other populations and activities (Table 3). With 25% of the negative effects and 45% of the positive effects, BNF seems quite versatile in its response to pesticides. Even the same pesticide could exhibit a negative or positive effect, depending on the soil type; the insecticide gamma-BHC stimulated BNF in alluvial and acid sulfate soils but inhibited it in other soils (Nayak and Rajaramamohan Rao 1980). These results were attributed to the differential responses of specific groups of N_2-fixing organisms to the pesticides, depending on soil type. When significant, the effects of pesticides on nonphotodependent BNF were more often positive than negative.

Reports of experiments on herbicide effects on heterotrophic BNF are scarce, most were conducted as laboratory incubations with a few grams of soil (Sivasithamparam 1970, Nayak and Rajaramamohan Rao 1982, Jena and Rajaramamohan Rao 1987). Jena and Rajaramamohan Rao studied the effect of herbicides thiobencarb and oxadiazon and insecticide carbofuran on three flooded soils. Carbofuran alone or in combination with herbicides had a clear stimulatory effect, up to 150%, on BNF (estimated from acetylene-reducing activity measurements after 30 d of incubation). Herbicides applied alone mostly had only a moderate effect, an average of 15% over 18 values. Similar results were obtained for nitrofen, at concentrations close to the recommended field level (5 µg g^{-1}). It stimulated N_2 fixation in a submerged rice soil under laboratory conditions and synergistic stimulatory effects were evident when it was applied in combination with the insecticide carbofuran (Nayak and Rajaramamohan Rao 1982).

Field experiments

Field experiments on the impacts of herbicides on microorganisms in ricefields have consisted of enumerations of soil microflora after application of thiobencarb (Sato 1987) or preemergence herbicides (Mandal et al 1987). Both experiments indicated either an absence of effect or a transitory change of population densities, followed by recovery within 2 or 3 wk.

Soil microbial biomass is regarded as a major channel through which nutrients are transferred to rice (Watanabe et al 1988). Field surveys on 32 rice farms in the Philippines showed no correlation between the intensity of pesticide use in farmers' fields, including the specific use of herbicide (as estimated from surveys of use over several previous cropping seasons) and the soil microbial biomass estimated at the beginning and end of the crop cycle (Roger et al 1994).

IMPACT ON INVERTEBRATES

The dominant soil and water invertebrates in ricefields are ostracods, copepods, cladocerans, rotifers, insect larvae, aquatic insects, mollusks, oligochaetes, and nematodes (Roger and Kurihara 1991). They have agricultural significance as nutrient recyclers, rice pests, and rice pest predators, and medical significance as vectors of human and animal diseases.

Microcrustaceans and larvae of mosquitoes and chironomids are ubiquitous primary consumers which recycle nutrients from the photosynthetic aquatic biomass. They usually proliferate about 2 wk after the peak of phytoplankton abundance (Kurasawa 1956) and may cause the disappearance of microalgae blooms within 1-2 wk. Ostracods have the potential to recycle 20 kg N ha^{-1} per crop. Primary consumers that feed on cyanobacteria may inhibit photodependent BNF or cause the dominance of mucilaginous colonial forms that are less susceptible to grazing than are noncolonial forms, but are less active N_2 fixers (Roger and Kurihara 1991).

Oligochaetes, especially tubificidae, are a major component of the zoobenthos that ensure nutrient exchange between soil and floodwater and increase soil N uptake by rice plants. Populations in ricefields range up to 40,000 m^{-2} (0-700 kg fresh weight ha^{-1}) (Simpson et al 1993a,b).

Aquatic invertebrates also have detrimental effects in rice-based ecosystems. Mosquitoes are vectors of diseases, including malaria and Japanese encephalitis (Roger and Bhuiyan 1990). Chironomids and ostracods feed on rice seedling roots, but this effect is limited in time and space (Clement et al 1977, Barrion and Litsinger 1984). Species of large snails that graze on rice seedlings have been recognized as an important rice pest in tropical countries and Japan. Other species (*Bilinus* spp., *Biomphalaria* spp., *Limnea* spp.) are detrimental as vectors of bilharziosis (Roger and Bhuiyan 1990).

Most information on the impacts of pesticides on nontarget invertebrates deal with insecticides applied alone or in combination with herbicides. Thiobencarb is the herbicide most often tested.

Floodwater invertebrates

Application of thiobencarb to experimental ricefields in Japan drastically reduced populations of cladocerans, odonatans, midges, and mosquito larvae. Resurgence of midges, cladocerans, and mosquito larvae occurred rapidly, to densities higher than those of the controls (Ishibashi and Itoh 1981). Simpson et al (1994a,b) studied the impact of carbofuran and butachlor applications on the population dynamics of floodwater invertebrates in Philippine ricefields. While significant effects were observed on ostracod, copepod, cladoceran, chironomid, and mosquito larvae populations, the impacts were relatively small, transient, and inconsistent. They concluded that, at realistic application rates, carbofuran and butachlor did not affect floodwater invertebrates in the context of crop cycle population dynamics.

Snails are not usually affected directly by conventional rice pesticides, but their populations may increase because of reduced competition. Ishibashi and Itoh (1981) observed larger snail populations after harvest in fields treated with thiobencarb than in untreated fields. Simpson et al (1994c) found little evidence that indigenous snail populations were affected by butachlor applications.

Soil invertebrates

Aquatic oligochaetes and nematodes dominate the soil fauna in wetland ricefields. The effects of pesticides on nontarget nematodes, however, have received little attention. Ishibashi and Itoh (1981) found no effect of thiobencarb on average populations of saprophytic and parasitic nematodes in a Japanese ricefield. Of 16 insecticides and 3 herbicides (2-4-D, butachlor, and pretilachlor) applied to ricefields in the Philippines, only the insecticides monocrotophos and ethofenprox had limited impact on parasitic nematodes (Prot and Mathias 1990). Information about herbicide impacts on populations of aquatic oligochaetes in ricefields also is scarce. The recent disappearance of aquatic oligochaetes from some Japanese ricefields is thought to be associated with the use of some herbicides. This would explain the reappearance of oligochaetes soon after PCP was replaced by NIP (2,4-dichlorophenyl p-nitrophenyl ether), CNP (4-nitrophenyl 2,4,6-trichlorophenyl ether), and thiobencarb. This conclusion has been supported by laboratory tests (Kurihara and Kikuchi 1988). A survey of aquatic oligochaetes in farmers' fields in the Philippines did not find differences among populations associated with differential pesticide use (Simpson et al 1993b).

Biodiversity

It is generally accepted that crop intensification and use of agrochemicals decrease biodiversity and provoke "blooms" of certain organisms. However, quantitative data on aquatic invertebrate diversity in ricefields are rare, and the limited amount of data that are available were obtained by different methods of sampling, over different time frames, from different locations. The studies do not specifically refer to herbicides but to pesticide use in general.

The only reference on the diversity of aquatic invertebrates in traditional ricefields is a 1975 study in Thailand, where 183 species (protozoans excluded) were recorded in one field within 1 yr (Heckman 1979). In a 2-yr study in Selangor, Malaysia, 39 invertebrate taxa were recorded in ricefields where pesticides were applied (Lim 1980). A single sampling in four Californian ricefields recorded 10-21 taxa (Takahashi et al 1982). Surveys of 18 sites in the Philippines (IRRI 1985) and India (Roger et al 1987) found population dominance inversely proportional to diversity. Ostracods, chironomids, and mollusks dominated the invertebrate community at most sites, and a few species attained exceptionally high densities at some sites. The highest number of taxa recorded at a site was 26; the lowest, 2. The marked decrease in number of taxa recorded since 1975 might be taken as a rough indication of a decrease in species richness. This agrees with, but does not demonstrate, the generally accepted concept that crop intensification has reduced biodiversity in ricefields (Roger et al 1991). A decrease of biodiversity also could be attributed to the disappearance of permanent reservoirs of organisms in the vicinity of the fields (Fernando et al 1980).

LONG-TERM EFFECTS

Available information indicates the possibility of detrimental impacts of herbicides on soil fertility and the microbial metabolism of herbicides over the long term.

Change in herbicide metabolism

Repeated application of a single pesticide has been reported to cause changes in the pattern of its metabolic decomposition. This has been observed for the insecticide parathion (Sudhakar-Barik et al 1979) and the herbicide thiobencarb (Moon and Kuwatsuka 1984). Such changes in degradation pathways could lead to agricultural problems. Thiobencarb usually is detoxified by hydrolysis, but its repeated application to flooded soil favors the multiplication of anaerobic bacteria that decompose thiobencarb by reductive dechlorination. That reaction results in the formation of a phytotoxic compound (S-benzyl N, N-diethylthiocarbamate) that causes dwarfing in rice (Moon and Kuwatsuka 1985).

Impacts on soil microbial biomass

Several long-term experiments evaluating continuous pesticide applications have resulted in declining rice yields over time (Cassman and Pingali 1995). The reasons are not fully understood, but one factor might be intensive hand weeding and herbicide use combined with a dense rice canopy that could restrict the growth of the photosynthetic aquatic biomass. That, in turn, would restrict the replenishment of soil microbial biomass and N fertility. Pesticides, including herbicides, also might be involved in decreasing populations of aquatic oligochaetes (Simpson et al 1993a) and the translocation of the nutrients accumulating at the soil surface to a deeper soil layer. Little data are available to substantiate this hypothesis, but in experiments at IRRI that totally prevented photosynthetic activity in the floodwater of planted fields by covering them with black cloth, soil microbial biomass was reduced 22% after 2 yr (IRRI 1989).

CONCLUSION

Most of the literature on pesticide fate and its impacts in wetland rice was published between 1970 and 1985. Since then, the number of studies has decreased precipitously. Most studies have dealt with the effects of insecticides on heterotrophic microorganisms and invertebrates, and of herbicides on cyanobacteria. In studies on the side effects of herbicides (and pesticides in general), data were generated primarily in the laboratory with microbial cultures or small samples of unplanted soil, using relatively high concentrations of pesticides. This makes extrapolation to field conditions questionable. Pure culture studies may not have much relevance, since the net effect on the microbial community is more important than the effect on an individual microorganism. This is particularly true for activities such as denitrification and N_2 fixation that are carried out by a broad range of taxonomically different microorganisms (Ray and Sethunathan 1988). Several researchers have developed small-scale models (microcosms) of ricefields or aquatic ecosystems to study and/or predict the bioaccumulation and dissipation of various pesticides applied to flooded ricefields (Higashi 1987). Such methods offer an interesting tool for detailed pesticide studies under controlled conditions, but they have not yet involved the study of the microbial component of the ecosystem. No field experiments have studied the impact of herbicide application on microorganisms and invertebrates over several crop cycles in a flooded soil. Studies of the microbial degradation of herbicides and their influence on microflora and nontarget invertebrates in flooded ricefields, hitherto mostly restricted to short-term laboratory experiments, should be performed under more realistic field conditions and cultural practices, over a long term.

The information on relationships between herbicides and nontarget microorganisms and invertebrates in wetland ricefields is not only biased by experimental designs that constrain extrapolation to field conditions, but also is too fragmentary to draw any conclusions other than general trends.

Microbial degradation is one of the main factors that affect herbicide persistence in flooded soils. Its importance varies quite broadly, depending upon the herbicide formulation, the mode of application, and the environmental conditions. While pesticides in general persist longer in nonflooded than in flooded soils, there is no obvious trend for herbicides. Trifluralin, pyrazoxyfen, and MCPB-ethyl persisted longer under nonflooded conditions; molinate, thiobencarb, and MCPA persisted longer under flooded conditions. Findings on the buildup of the degrading microflora after repeated application of herbicides in flooded soils are poorly documented, and this area needs further investigation.

In laboratory experiments, herbicides affected soil microflora and its activities more often than did fungicides or insecticides. However, when applied on soil at recommended levels, herbicides rarely had a detrimental effect on microbial populations or on their activities. When significant changes were observed, populations or activities usually recovered within 1-3 wk. This seems to partially confirm the common belief that pesticides applied at recommended levels and intervals are seldom deleterious to the beneficial microorganisms and their activities (Wainright 1978). While herbicides might have only temporary effects, when applied repeatedly they could lead to the promotion, depression, or disappearance of components of the microbial community, and promote a new equilibrium. This could bring about changes in the rate or pattern of microbial decomposition of the herbicides that might be detrimental. This aspect needs further investigation.

Invertebrates seem to be more sensitive to pesticides than are microorganisms. Combined use of insecticides and herbicides can lead to floodwater blooms of individual species (especially primary consumers) that might be detrimental. Aquatic oligochaetes in soil are at least partly inhibited by some herbicides, which might affect nutrient translocation and soil fertility. Greater understanding of floodwater ecology is needed as a basis for developing agricultural practices that maintain a biological equilibrium in the ricefield ecosystem. In particular, practices are needed that will decrease pesticide use and conserve the natural predators of rice pests and disease vectors. In order to develop cultural practices that favor the conservation of invertebrate predators—a major component of integrated pest and vector management—more knowledge on the long-term impact of herbicides on ricefield invertebrate populations is needed.

It is important to remember that impacts of pesticides on the soil-floodwater ecosystem can be significant without being detrimental. For example, a shift in algal community structure may not affect soil fertility, provided that aquatic primary production is unchanged. We should be cautious in identifying the nature of impacts, which should be considered in the context of ecosystem equilibrium, not in isolation. It would be as unwise to underestimate as to overestimate the significance of pesticide impacts in soils. Underestimation could cause avoidable ecological damage. Overestimation could restrict the judicious use of pesticide when appropriate. However, current knowledge on the long-term impacts of herbicide use in ricefields is fragmentary. Investigation is needed to establish how herbicide application over the long term may affect primary production in floodwater, soil microbial biomass and microbial populations, populations of invertebrates responsible for nutrient recycling and translocation, and populations of invertebrate predators of rice pests and vectors of human diseases.

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NOTES

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