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# Impact of Herbicides on Non-Target Organisms in Sustainable Irrigated Rice Production Systems: State of Knowledge and Future Prospects

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## 1. Introduction

Feeding the 9 billion people expected to inhabit our planet by 2050 will be an unprecedented challenge for all the mankind (Ash et al., 2010). Nevertheless, producing enough food for the world's population in 2050 will be easy, but doing it at an acceptable cost to the planet will depend on research into everything, from high-tech seeds to low-tech farming practices (Anonymous, 2010). Research into rice (*Oryza sativa* L.), the most important food crop in the developing world and a staple food for more than half of the world's population, is crucial for the development of strategies that will increase global food security (Normile, 2008). Appropriate integrated management of parasitic rice weeds is thus expected to increase in importance due to their general invasive nature and their abilities to adapt to changing conditions such as those imposed by more and more unpredicted global climate changes. Modern sustainable paddy cultivation worldwide involves extensive use of agrochemicals such as insecticides, fungicides but especially herbicides. Herbicide demand has unique characteristics compared with other common productive inputs in rice culture systems such as land, labour, seeds and chemical fertilizers (Yamamoto & Nakamura, 2003). The goal of herbicide use is to kill or stunt weed infestation allowing the rice to grow and gain a competitive advantage (Monaco et al., 2002). The use of rice herbicides has been expanding enormously worldwide over the past 20-40 years. However, herbicides are considered a "two-edged sword" (Kudsk & Streibig, 2003) or the "reverse of the coin" (Jurado *et al.*, 2010; chapter 1, this book), since the subsequent dispersion of herbicide compounds and their degradation products in rice fields and adjacent areas with strong ecological value still threatens the integrity of ecosystems, thus resulting in serious global environmental concern (Olofsdotter et al., 1998). One of the major issues about environmental herbicide contamination in wetland rice fields is its bioaccumulation in ecosystem primary producers

and its subsequent propagation through trophic chain. Therefore, reliable legislation and risk assessment tools are needed to carry out the monitoring of herbicide residues in autochthonous living organisms inhabiting rice fields.

The main objective of this review is to compile and summarize relevant update information on herbicide weed control and consequent impacts of these agrochemicals on rice field organisms as well as on the environment, mainly in European and particularly Mediterranean countries. For additional information, though some of them are being not necessarily updated, the readers are reported to the pioneer works of Padhy (1985), Pingali & Roger (1995) and Roger (1996). A considerable amount of research has been published since these previous reviews and this chapter summarizes only the main findings related to impacts of herbicides on the ecology of sustainable rice field agroecosystems in the new policy context. Moreover, we have chosen examples of herbicide effects on non-target rice field organisms that, in our view, best illustrate different effects and environmental impacts. It is not our intention to suggest that these are the sole examples or the most suitable herbicides. Clearly, there are numerous and elegant examples of herbicide effects on non-target rice field organisms that fall outside of the small, but in our opinion acceptable, number of cases we have chosen to present.

## 2. Herbicide use and predominant weeds in irrigated rice fields

According to the immense worldwide literature database quested, herbicides are the most frequently detected chemical pollutants in water. Moreover, herbicides account for more than 80% of the total consumption of pesticides utilized for crop protection, with a total spending of about € 110 million.year[-1] (Ferrero & Tinarelli, 2007). Herbicides[1] are chemicals used to manage unwanted plants in the ecosystem, plants that are commonly referred to as "weeds". Weeds are the most important biological constraint to increasing yields wherever rice is grown and every rice researcher and farmer must be provided with a great amount of useful scientific information on weed control. Unlike the periodic outbreaks of insect pests and plant diseases, rice weeds are ever-present and threatening (Olofsdotter et al., 1998).

The application of different rice field herbicides varies with the region. For instance, in North America and Western Europe, due to high costs of labour, the chemical control of weeds is heavily done with herbicides, contrasting to East Asia and Latin America where herbicides are much less used (Carvalho, 2006). In European rice fields, weeds are considered the most noxious organisms affecting rice production. It has been estimated that without weed control, at a yield level of 7 to 8 t.ha^{-1}, yield loss can be as high as about 90% (Ferrero & Tinarelli, 2007 and references therein).

At present, rice is grown in all continents under various environmental conditions which can be separated into four main ecosystems: irrigated lowland, rainfed lowland, upland and flood prone (De Datta, 1981; Ferrero & Tinarelli, 2007). The cultural practices, weed species present, and control methods are somewhat different in these systems (Monaco et al., 2002). In this review we are only concerned about sustainable irrigated lowland rice production systems (both seeded and transplanted), which account for about half of the world's rice harvested area and provide 75% of total rice production (Ferrero & Tinarelli, 2007).

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[1] Herbicides were preferably named by their common names in the text, but sometimes registered trademarks are cited and their use is not free for everyone. In view of the vast number of trademarks, it was not possible to indicate each particular case and contribution. The authors accepted no liability for this.

Sustainable irrigated rice should be perceived here as a prolonged existence and functioning of several important interrelated elements of irrigated rice culture (Sutawan, 2005). In direct-seeded fields flooding cannot be used until crop establishment, so post-emergence weed control is essential.

## 3. Impact of herbicides on soil and water non-target organisms in wetland rice fields

Besides weeds, herbicides can act upon other species, causing serious side effects on non-target rice field inhabiting organisms. Moreover, herbicide residues contaminate soils and water, remain in the rice crop, enter the food chain, and finally are ingested by humans with rice foodstuffs and water (Liebman, 2001). Traditionally, paddy fields are home-ecosystems to many species. In early 1991 Kenmore (as cited in Clay, 2004) wrote that "*Rice ecosystems often have more than 700 animal species per hectare in highly intensified fields in the Philippines and over 1000 so far described in Asian species of higher trophic level predators and parasitoids.*" Besides the application of ever-increasing quantities of synthetic fertilizers, the increasing application of pesticides, mainly herbicides, has led to the disappearance of much of this biodiversity. In addition, non-target survivors have been continuously threatened by these xenobiotics.

### 3.1 Effects on non-photosynthetic microorganisms

Frequently, non-photosynthetic microorganisms undergo stress conditions caused by herbicide application. For instance, the metabolism of the Gram-negative bacteria *Stenotrophomonas maltophilia*, sometimes present in rice field irrigation channels (Reche & Fiuza, 2005), could be negativelly affected by some rice field herbicides (Lü et al., 2009). These authors showed that a mixture of quinclorac and bensulfuron-methyl (BSM) induced the activity of the antioxidant enzymes superoxide dismutase and catalase of a *S. maltophilia* strain (WZ2), thus demonstrating the induced oxidative stress caused by the herbicides. The effect of BSM on a soil microbial community in a model paddy microcosm was studied by Saeki & Toyota (2004). BSM did not affect bacterial numbers remarkably, either in the overlying water or in the surface paddy soil, but the nitrification potential was significantly suppressed was significantly suppressed.

The composition of culture-independent microbial communities and the change of nitrogenase activities under butachlor application to paddy soil were investigated by Chen et al. (2009). The results of their work showed that the nitrogen-fixing ability was suppressed shortly after butachlor application but was augmented after 37 days both in upper and lower soil layers. A significant variation on microbial community shift was also demonstrated, favouring the diazotrophic microorganisms within the general bacterial communities imposed by butachlor, which may be a reason for the boosting nitrogen-fixation ability in paddy soils. Other effects of butachlor applied at 5.5 µg.g$^{-1}$ to 22.0 µg.g$^{-1}$ to microbial populations inhabiting dried paddy soil resulted in a decline of actinomycetes number and and an increase of bacteria and fungi, but fungi were easily affected by butachlor compared to bacteria, particularly at higher butachlor concentrations (Min et al., 2001).

### 3.2 Effects on photosynthetic microorganisms
### 3.2.1 Effects on microalgae

Besides nonphotosynthetic organisms, on the first level of rice field trophic chain we can find the phytoplankton, microscopic algae which are the food for the next steps in trophic chain.

Planktonic green algae are the ones generally used as test species for the first tier aquatic risk assessment of herbicides (Ishihara, 2009). Wetland contamination could result in a die-off of most algal species present, causing a severe decline in this food source; alternatively, certain species or groups of algae could be selectively inhibited (Ferraz et al., 2004).

An *in vitro* toxicity bioassay screening conducted by Marques et al. (2008) on surface waters and sediment elutriates proceeding from River Pranto waters, which irrigate Western Portugal's rice fields, as well as on the irrigated rice fields in "Quinta do Seminário" (Soure, Portugal), revealed that *Pseudokirchneriella subcapitata* was more sensitive to the overall physico-chemical conditions in natural samples than *Chlorella vulgaris*, its growth being inhibited under water samples from both sites. In addition, these authors found that water samples, mainly those from the main irrigation/drainage channel of the rice fields, were more deleterious to microalgae than those from River Pranto or any of the elutriates. Surprisingly, the qualitative chemical analysis done by these researchers did not reveal the presence of the herbicides applied in the field like molinate and propanil. These two herbicides plus MCPA were used in microbiotests in order to assess the comparative toxicity effects of herbicide active ingredients (a.i.) *versus* commercial products (c.p.) on the green algae *Raphidocelis subcapitata* (Pereira et al., 2000). It was demonstrated in laboratory that water samples fortified with each active ingredient caused higher toxic effects than the respective formulated product, mainly with molinate and propanil. A study carried out by Leitão et al. (2007) in an irrigated rice field ecosystem in the river Sado basin, a characteristic Mediterranean lowland basin located in southwestern Portugal, a highly acute toxicity (80%) was detected on *P. subcapitata* in June after propanil (0.2 µg a.i.L$^{-1}$) application to paddy fields. Sabater & Carrasco (1998) found that species of isolated microalgae responded differently to molinate concentrations tested in laboratory: at 44.6, 50.2, 3.2, and 1.12 ppm, the growth of *Chlorella saccharophila*, *C. vulgaris*, *Scenedesmus acutus*, and *Scenedesmus subspicatus* was strongly inhibited after 96 h, respectively, whereas no growth was observed at 69.8 ppm for *C. vulgaris* and 2.2 ppm *S. subspicatus.* This study showed also that the two *Chlorella* species were considerably more tolerant than the two *Scenedesmus* species isolated from Spanish rice fields. Vendrell et al. (2009) tested the acute toxicity of glyphosate on *S. acutus*, *S. subspicatus*, *C. vulgaris* and *C. saccharophila* isolated from samples collected at Albufera lake in Valencia (Spain), one of the most important rice areas in Europe with a very rich flora and fauna. Although glyphosate is not applied to Valencian rice fields, its massive spraying in other agricultural crops surrounding the Albufera National Park (ANP), a protected ecosystem where rice is cultivated in harmony with local fauna and flora, is still a common practice. In a microplate bioassay, the authors demonstrated the acute toxicity induced by glyphosate on the four *Chlorophyceae* and the herbicide concentrations eliciting a 50% growth reduction over 72 h (EC$_{50}$) ranged from 24.5 to 41.7 mg.L$^{-1}$, while a 10% growth inhibition was achieved with 1.6 to 3.0 mg.L$^{-1}$, difficult to find both in the paddy field and in the ANP lake. Therefore, it could be concluded from this study that glyphosate is not a dangerous herbicide for the ANP ecosystem due to its low microalgae toxicity at low glyphosate concentrations. Also in the ANP ecosystem, Ferraz et al. (2004) assessed the sensitivity of the same aforementioned four algal species to propanil and mefenacet using single species short-termed (72 h) toxicity tests. The 72 h-EC$_{50}$ of propanil and mefenacet ranged from 0.29 to 5.98 mg.L$^{-1}$, and from 0.25 to 0.67 mg.L$^{-1}$, respectively for the four algal species.

The relative toxicity of hydroquinone on submerged aquatic weed green musk chara (*Chara zeylanica* Willd.) was investigated by Pandey et al. (2005) to explore possible use of the

phytotoxin as herbicide management of the weed. It was found that hydroquinone was phytotoxic to *C. zeylanica* at 0.01 mM and lethal at 0.075-0.10 mM, resulting in death after 3-12 days. Moreover, it is important to highlight that this phytotoxin has a short life in the environment and a promissory potential for weed management.

### 3.2.2 Effects on cyanobacteria

The nitrogen-fixing cyanobacteria form a prominent component of microbial population in rice paddy fields, since they significantly contribute to fertility as natural biofertilizers (Fernández Valiente et al., 2000; Singh & Datta, 2007). The influence of herbicides on cyanobacteria has been extensively reviewed in many studies (Padhy, 1985; Pingali & Roger, 1995), though most have been restricted to laboratory cultures (Whitton, 2000). Furthermore, one of us, together with the research team colleagues of the Biology Department of the Autonomous University of Madrid, has contributed significantly to the comprehension of herbicide action mechanisms on Mediterranean rice field cyanobacteria (Leganés & Fernández-Valiente, 1992; González Tomé, 1996). Generally, cyanobacteria are quite sensitive to herbicides, because they share many of the physiological features of higher plants, which form the site of herbicide action (Whitton, 2000). Leganés & Fernández-Valiente (1992) showed that $N_2$-fixing cyanobacteria were mostly relatively tolerant to 2,4-D, at least under field conditions. Differences have been found between the tolerance to herbicides of cyanobacteria and the one of other organisms (Whitton, 2000). For example, a study performed in liquid culture showed that hexazinone was more toxic to green algae, diatoms and duckweed than to cyanobacteria, whereas green algae were more tolerant to diquat than cyanobacteria and diatoms (Peterson et al., 1997).

Certain rice field herbicide resistant strains of cyanobacteria have been isolated and characterized in laboratory studies, but their outdoor survival, competence and biofertilizer potentials have only recently been characterized. In a research undertaken by Singh & Datta (2007), four natural strains of *Anabaena variabilis* that showed multiple herbicide resistance to arozin, alachlor, butachlor and 2,4-D, were inoculated in growing rice plants, being demonstrated that mutant strains had stable resistance to herbicides under outdoor conditions in flooded soils.

Butachlor can boost *Anabaena sphaerica* biomass and accelerate the amount of nitrogen fixation (Suseela, 2001). Working with butachlor and also with arozin, alachlor and 2,4-D, (Singh & Datta, 2005) developed an immobilization technique with Ca-alginate that provides protection to diazotrophic cyanobacteria inoculants against the growth-toxic action of the herbicides. These authors tested the effect of graded concentrations (2 to 25 mg.L$^{-1}$) of the four common rice field herbicides on immobilized free-living isolates of *Nostoc punctiforme*, *Nostoc calcicola*, *A. variabilis*, *Gloeocapsa* sp., *Aphanocapsa* sp. and on a laboratory strain of *Nostoc muscorum*. It was demonstrated that: 1) *A. variabilis* showed maximum natural tolerance towards all the four tested herbicides; 2) all cyanobacterial isolates showed progressive inhibition of growth with increasing dosage of herbicides in both free and immobilized states; 3) arozin was more toxic to cyanobacterial growth compared to the other three herbicides; and 4) at herbicidal lethal concentrations, Ca-immobilized cells showed prolonged survival times when compared to their free-living counterparts, suggesting that immobilized $N_2$-fixing cyanobacteria could be used as better inocula delivery system for enhancing rice agriculture.

High bensulfuron-methyl concentrations (8-10 ppm) inhibited the growth and photosynthesis of over 50% in *A. variabilis* and *Nostoc commune* rice field isolated;

nitrogenase activity decreased by 94-98% in *A. variabilis* and by 85-86% in *N. commune* after 24 hours' incubation with 10 ppm and 20 ppm of the herbicide, respectively (Kim & Lee, 2006). Ahluwalia et al. (2002) proved that the incorporation of relatively higher doses (> 5 µg.mL$^{-1}$) of diquat into *N. muscorum* and *Cylindrospermum* sp. cultures could be highly toxic, thereby reducing their chlorophyll *a* (Chl*a*) content and contributing to a progressive decrease in growth which culminates in complete lysis of the cells with the increasing level of the herbicide. The highest concentration tested (15 µg.mL$^{-1}$) has been found to be algicidal for both cyanobacteria. At this concentration, the same authors demonstrated that paraquat supplemented into culture medium containing *Cylindrospermum* sp. also had an algicidal effect (Kaur et al., 2002).

## 3.3 Effects on invertebrates

Herbicides are responsible for a general reduction in the numbers of invertebrates within agricultural landscapes, which in turn compromises food supply for higher taxa (Stoate et al., 2009). Populations of copepods, cladocerans and ostracods fluctuate during the paddy-growing season in response to flooding, field drainage, ploughing and other practices (Tarazona & Dohmen, 2007 and references therein). In order to better understand the toxic results obtained in field conditions, particularly the influence of herbicide formulations on the toxic effects on immobilized forms of the crustaceans *Daphnia magna*, *Thamnocephalus platyurus* and *Artemia salina*, Pereira et al. (2000) performed different laboratory microbiotests with water samples fortified with the active ingredient and the respective formulated or commercial product of some rice field herbicides usually applied in Portuguese paddies *viz.* molinate, MCPA and propanil. The results obtained after the bioassays suggested that: 1) the organic solvents and surfactants present in some of the tested formulations affected the toxicity of the sample; 2) except for propanil, water samples fortified with formulated herbicides seemed to be more toxic than the respective active ingredient solutions on the tested crustaceans.

In a 6-day bioassay carried out by Faria et al. (2007) with the macroinvertebrate *Chironomus riparius* larvae, both in laboratory and *in situ* (from high and low contaminated rice fields and in the adjacent wetland channel) during molinate and propanil treatments, the larvae were not affected by the respective herbicide concentrations in water and sediments. In laboratory experiments, Burdett et al. (2001) used *Chironomus tepperi* midge larvae to assess the relative toxicity of formulated molinate, clomazone and thiobencarb applied to Australian rice crops. Whereas clomazone had no effect on *C. tepperi* at concentrations up to 0.288 mg.L$^{-1}$, molinate significantly increased its development time at anticipated field concentrations (AFC; 3.6 mg.L$^{-1}$) and above. Thiobencarb reduced emergence success of the adults at 0.0625 times the AFC and it decreased male adult size and increased development time for males and females at 0.125 times the AFC. The authors of this study also assessed the non-target effects of the herbicides on the aquatic invertebrate communities through shallow experimental ponds using commercial application rates and, one week after treatment, they saw that only thiobencarb had a significat effect, supressing populations of chironomids, calanoids and cyclopids. Besides *C. tepperi*, Wilson et al. (2000) conducted some laboratory acute and chronic toxicity tests on the herbicide benzofenap using adults of the aquatic snail *Isidorella newcombi*. Whereas in 24-h acute bioassays the midge larvae did not show significant mortality at 1.2 mg a.i. L$^{-1}$ [double the maximum recommended levels for field application (RLFA) expected in rice fields at the permitted rate of 2 L.ha$^{-1}$], no

significant snail mortality was recorded in the acute bioassays at concentrations up to 76 mg a.i. L$^{-1}$ (120 times the maximum RLFA); it was concluded that benzofenap does not represent a significant risk to these invertebrates in downstream environments when applied to rice fields at the permitted rate of 2 L.ha$^{-1}$.

The high acute toxicity effects (100%) of propanil (0.2 µg a.i.L$^{-1}$) on *D. magna* were demonstrated by Leitão et al. (2007) in rice field floodwaters from river Sado basin. These authors also study the relationship between native fauna composition and the ecotoxicological variables throughout rice crop season. The analysis of variance partition with the accepted RDA (redundancy analysis) model showed that the macroinvetebrate distribution was strongly correlated with the ecotoxicological parameters (higher toxicity) in 27.5% of total variation and 17.7% with floodwater characteristics. It was demonstrated that macroinvertebrates' assemblages inhabiting rice fields tend to be different in their richness and abundance according to the paddy sediment and water characteristics.

In a study performed by Uno et al. (2001), the accumulative characteristics of herbicide residues in the organs of two bivalve mollusks, *Corbicula leana* and *Anodonta woodiana*, were examined during rice planting seasons of 1992 and 1994, using a small artificial stream under natural conditions. It was shown that thiobencarb accumulated in *C. leana* at extremely high levels in the midgut gland (12.45 and 15.70 µg.g$^{-1}$, in 1992 and 1994, respectively) and in the gonad (15.80 and 16.40 µg.g$^{-1}$, in 1992 and 1994, respectively); these levels were about 100 times as high as those of *A. woodiana.*

### 3.4 Effects on vertebrates

As we saw before, during the aquatic phase of rice plant growth, many organisms other than rice colonize the paddy fields, but fish from source rivers, in particular, are sometimes seen as detrimental to the rice harvest (Williams, 2006) and could be affected by herbicide pollution as described next.

### 3.4.1 Effects on fish

The effect of herbicides on estuaries, rivers, and fragile coastal zones are all reflected on the reduction of fish catch (Clay, 2004). In a recent 90-day experiment carried out by Moraes et al. (2009) with the objective of evaluating the effects of herbicide commercial formulations on acetylcholinesterase (AChE), thiobarbituric acid-reactive substances (TBARS), catalase (CAT) and metabolic parameters in teleost fish (*Leporinus obtusidens*) exposed to field concentrations of clomazone (376 µg.L$^{-1}$) and propanil (1644 µg.L$^{-1}$) on Brazilian rice paddy waters, it was shown that AChE activity decreased in the brain and muscle, whereas TBARS levels decreased in brain, muscle and liver tissues and liver CAT decreased after exposure to both herbicides. The results obtained by these authors suggest that environmentally relevant rice field herbicide concentrations are toxic to *L. obtusidens.* Crestani et al. (2006) suggested that alanine aminotransferase and aspartate aminotransferase activities could be used as early biomarkers of clomazone fish toxicity, since enzyme activities were significantly elevated in silver catfish's (*Rhamdia quelen*) liver after 12 to 24 hours' clomazone exposure to nominal concentrations used in Brazilian paddy fields (0.4-0.7 mg.L$^{-1}$). Working also with *R. quelen*, Miron et al. (2005) demonstrated significantly short-term (96 h) effects of exposure to environmentally relevant nominal concentrations of clomazone (LC$_{50}$ = 7.32 mg.L$^{-1}$) and quinclorac (LC$_{50}$ = 395 mg.L$^{-1}$) on AChE activity in brain and muscle tissues of silver catfish, but the fish fingerlings survived at metsulfuron-methyl concentrations as high as 1200 mg.L$^{-}$

[1]. They suggested that AChE activity could be used as an early biomarker for studies on fish toxicity, since enzyme's activity increased by 98 and 179% in fish's brain after quinclorac and metsulfuron methyl exposure, respectively.

### 3.4.2 Effects on amphibians

In a recent study performed by Kang et al. (2009) about *Bombina orientalis*, a worldwide common amphibian that frequently spawns in rice fields where massive application of herbicides occurs, the deleterious effects of molinate on embryonic survival and development abnormality in *B. orientalis* embryos were demonstrated. A statistical significant decrease in embryonic survival was detected at mouth open stage after exposure to 100 μM molinate (46.8% *vs.* 81.1% in control) and then in the tadpole stage at 50 μM molinate (35.9% *vs.* 68.9% in control). The authors thus concluded that molinate at 50 μM (the lowest observed effective dose; LOED) was detrimental for survival and development following zygote transcription after midblastula transition in frog embryos, causing severe development abnormities like bent trunk, neurula with yolk plug, bent tail, tail dysplasia, ventral blister, eye dysplasia, thick-set body and cephalic dysplasia. Acute toxicity tests were carried out by Saka (1999), on five species of Japanese amphibian larvae to assess the risk posed by thiobencarb. The 24, 48, 72 and 96 h-$LC_{50}$ (median lethal concentration) values ranged from 0.9 to 6.5 mg.$L^{-1}$ of thiobencarb. Moreover, in all tested species, the newly hatched larvae seemed to be slightly more resistant to the herbicide than well-developed larvae, which led the authors to conclude that thiobencarb residues in paddy water can be lethal to amphibians through larval development.

## 4. Legislation and assessment of contamination risks by rice field herbicides

Concerns about environmental protection have increased over the years from a global viewpoint. As the criteria especially for standard risk assessments are well harmonized amongst OECD member states (Streloke, 2007), there is only a need to give a short overview here. Needless to say, there is a raft of regulations and standards to negotiate in testing any herbicide. Diffuse pesticide pollution of water bodies and potentially adverse effects on aquatic communities gave rise to current legal formulations of the European Union (EU) such as the Council Directive 91/414/EEC concerning the placing of plant protection products on the market (EEC, 1991) or the Water Framework Directive (WFD) (EC, 2000) that presents the concept for the sustainable use of water resources by integrated river basin management (Schriever & Liess, 2007). Where appropriate, these Directives call on other European legislation in related areas such as test methods, classification and labelling, and maximum residue levels (MRLs) (Hussey & Bell, 2004). The WFD intends to provide an overall framework for a cleaner and safer water ecosystem, particularly regarding surface freshwater and ground water bodies (*i.e.* lakes, streams, rivers, estuaries, coastal waters etc.). The Directive 91/414/EEC defines the principles and procedures to be used for authorization of plant protection products, and its annexes outline the basis for coordination or harmonization of data requirements and regulatory decisions. The implementation of this Directive has led to an EU-wide regulatory process for evaluating the safety of herbicides to humans and the environment, whilst leaving the responsibility for approval of plant protection products in individual countries to member states. The Directive has established a positive list (Annex 1) that lists those herbicides that have been judged to be "without unacceptable risk" to people or the environment, and all new active molecules proposed for

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use as herbicides within the EU must be deemed acceptable according to the Directive. Article 5 of the Directive requires that the use of plant protection products and their residues should not have any harmful effects on human or animal health or on groundwater, or have any unacceptable influence on the environment. In this respect, all existing active ingredients (about 800) introduced into the market prior to 2000 had transitional approval, pending their re-evaluation using modern toxicological and environmental protocols with a view to inclusion in Annex 1. Identification of products to be supported for re-registration was sought by 2002, and evaluation of those using modern toxicological and environmental protocols is required by 2012. Approved substances will be listed in Annex 1 of the Directive (EEC, 1991; Vogelezang-Stoute, 2003; Hussey & Bell, 2004; Carlile, 2006).

The risk to the environment covers the fate and behaviour of an active ingredient (*i.e.*, exposure) as well as its possible effects on non-target organisms (EEC, 1991; Benfenati et al., 2007). It should be stressed that the Directive 91/414/EEC and associated technical annexes are constantly under revision.

For reasons of preventive health protection and protection of the environment, the use of plant protection products has to be limited to the minimum level compatible with effective crop protection. The MRLs (in the EU) or tolerances (in the USA) are established for crops and food commodities (Siebers & Hänel, 2003). In countries with no national legislation, the MRLs are set by the Codex Alimentarius Commission, an international body that aims to protect the health of consumers (Granby et al., 2008). Groundwater contamination has also received increasing attention over the last few years as most of the drinking water is drawn from wells (Vidotto et al., 2004). In the last few years, great activity in regulating the level of herbicide in water has been carried out in the EU. The maximum allowable concentrations in groundwaters (for drinking and any other use) are set to 0.1 $\mu g.L^{-1}$ for any individual chemical and 0.5 $\mu g.L^{-1}$ for total herbicide load (Gan & Bondarenko, 2008).

In Europe, regulations and regulatory methods to assess and control the impact of herbicides in the aquatic environment aim to protect the ecosystem and public health, while monitoring contamination levels and any potential negative effects; in addition to the REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals) law (EC 1907/2006) (EU, 2006), regarding chemicals and their safe use, which entered into force on 1st June 2007, there are specific regulations towards protecting health and ensure quality of all water resources such as the Drinking Water Directive (DWD, Council Directive 98/83/EC) (EC, 2007; Nielsen et al., 2008; Adrián et al., 2009). Environment quality standards for 33 priority substances in surface aquatic bodies have been recently established in the Directive 2008/105/EC (EC, 2008), offspring of the WFD (EC, 2000), and about a third of the priority substances covered by this directive are pesticides (Köck et al., 2010). For additional aspects relating to regulatory authorities and legislation in many other parts of the world besides the EU, readers are reported to the works of Marrs & Ballantyne (2004), Carlile (2006), Racke (2007), Zimdahl (2007), and Nielsen et al. (2008).

It is important to stress that the ecotoxicological assessment of rice herbicides is particularly complex due to the specificities of this crop where aquatic and terrestrial communities are mixed in a human-managed wetland-type agro-bio-ecosystem. According to Tarazona & Dohmen (2007), any assessment must cover at least three main aspects: 1) potential effects on the paddy community; 2) potential effects on associated wetlands and water bodies, and 3) potential risks for vertebrates feeding on the paddy. The initial low tier assessment may be conducted using the generic approach used for other crops. For higher tier risk assessment it is recommended to develop a specific conceptual model for identifying the key elements that should be addressed (Tarazona & Dohmen, 2007).

## 5. Environmental exposure and contamination

Since rice is an irrigated crop, the use of pesticides during cereal growth may affect the quality of the surrounding aquatic environment (Pereira et al., 2000). Irrigation control after the application of herbicides could be the most important approach to avoiding environmental impact (Yamamoto & Nakamura, 2003). Several herbicides may enter aquatic environments through spray drift or runoff events, drainage or leaching, resulting in contamination of surface waters and groundwaters (Cerejeira et al., 2003; Faria et al., 2007), thus affecting phytoplankton and delicate autochthonous living organisms and also human health (Srivastava et al., 2010).

In a recent review about the ecological status of agricultural systems across the European Union, Stoate et al. (2009) reported to various studies about the presence of herbicide contamination, some exceeding the 0.1 µg.L$^{-1}$ EU limit, in both surface waters and groundwaters in areas occupied by intensive agriculture, rice fields included. Silva et al. (2006) detected residues of chlorfenvinphos, cycloxydim, 3,4-dichloroaniline, MCPA, molinate, oxidiazon, profoxydim, and propanil in 62% of 171 water samples collected from 22 groundwater wells used for public supply, domestic supply, and irrigation purposes in oriziculture areas of "Baixo Sado" region. In this study, 6% of total samples presented maximum concentration levels of at least one of the compounds above 0.1 µg.L$^{-1}$ and molinate was the most frequently detected (55%), particularly with maximum concentration levels above the maximum limit.

### 5.1 Surface water bodies

During three years (2000 to 2003), Marchesan et al. (2007) detected clomazone, propanil and quinclorac in water samples during the rice growing season in the Vacacaí and Vacacaí-Mirim Rivers, located in Rio Grande do Sul State (Brazil) and concluded that river water contamination by rice herbicides was probably caused by rice water management used in the fields. In a study designed by Castro et al. (2005) to monitor molinate accumulation in surface waters and underground waters during herbicide application in rice fields of central Portugal (valley of River Pranto), it was concluded that the thiocarbamate was rapidly dissipated in the environment, reaching levels as high as 3.9 µg.L$^{-1}$ and 15.8 µg.L$^{-1}$ in underground and in the river receiving tail waters, respectively (the legally recommended limits are bellow 5 µg.L$^{-1}$). Some common rice field herbicides, *e.g.* propanil and molinate have been detected in surface waters of Portugal's river basins (Tejo, Sado and Guadiana) and in groundwater samples collected from wells in seven different areas in the Tejo and Sado basins (Cerejeira et al., 2003). Clomazone dissipation in water samples from irrigated rice cultures was analyzed by Carlomagno et al. (2010) in a water management study implemented in Uruguay during the 2008-2009 harvest season. As expected, early irrigation of rice plots was accompanied by increasing concentrations of clomazone in water layers: 77 ng.mL$^{-1}$ in the day of flooding to 129 ng.mL$^{-1}$ after 6 day flooding. The high levels of clomazone attained in this study are a matter of concern, since contamination of drainages and rivers near rice paddies holds the potential for unacceptable levels of clomazone in drinking waters.

### 5.2 Groundwater systems

Rice fields are considered a flood control and a groundwater-charging area. Therefore, the risk of rice herbicides contaminating the groundwater cannot be neglected. Depending on

the soil type and metereological conditions, among other abiotic and biotic factors and application rates, thiobencarb poses less risk for groundwater when applied to flooded rice fields than other herbicides. In a 2-year study done by Phong et al. (2009), the concentrations of thiobencarb were in the hundreds of µg.kg$^{-1}$ in the top soil layer (0-5 cm) and became significantly lower in tens of µg.kg$^{-1}$ in deeper soil layers (5-10 and 10-15 cm) with a short life time (17 days) in the top layer, 69 days in the 5-10 cm layer and 165 days in the 10-15 cm layer. In another study, it was found that bentazon is one of the most frequently detected herbicide in groundwater in Northern Italy, even though its use is either prohibited or reduced in many districts in this country (Garagna et al., 2005).

## 6. Herbicide effects on non-target cyanobacteria: the case study of Lower Mondego River Valley, Portugal

In the European Union (EU), rice is cultivated in about 410 000 ha, mostly located in the Mediterranean countries. It is mostly grown in concentrated areas such as the Po valley in Italy, the Rhône delta in France, and the Thessaloniki area in Greece. In Spain, rice cultivation is scattered in several areas such as the Aragon area, the Guadalquivir valley, the Ebro delta and Valencia Albufera (Ferrero & Tinarelli, 2007). In Portugal, the approximately 25 000 ha of rice cultivation area is concentrated mainly in three regions: the Tagus and Sorraia valleys (10 000 ha), the Mondego (8000 ha), and the Sado and Caia (7000 ha) river valleys (Calha et al., 1999; Silva et al., 2006). In all European countries rice is cultivated in permanent flooded conditions, with short periods during which soil is dried to favour rice rooting (in the early stages) or weed control treatments. The conventional irrigation system is also known as a "flow-through" system, because water is usually supplied in a series from the topmost to the bottommost basin and is regulated by floodgates by means of removable boards. Water circulation implies that effects of the herbicide application can also be observed in areas different from that of direct application. Moreover, paddy flooding favours the flow of solutes from the surface to the subsoil waters and may result in significant exchanges between these two aqueous bodies (Vidotto et al., 2004). Because of the high water demand for maintaining flooded conditions in the rice fields, Mediterranean rice-growing areas are restricted to the lower parts of river basins and near or directly on river deltas (Comoretto et al., 2008).

The management of Portuguese rice ecosystems has adapted to Mediterranean climate conditions: they are dry in the winter, serving as cattle grazing prairies, while in the summer they are flooded up to 15-30 cm, with water coming from nearby dams or rivers, thus becoming temporary aquatic habitats managed with a variable degree of intensity; the paddies of rectangular or similar shaped flooded fields comprised mostly rice plants, surrounded by dry bunds (levees) rich in weeds, connected by irrigation canals and ditches that serve as contiguous aquatic habitats (Lima, 1997; Leitão et al., 2007).

The Lower Mondego River Valley is located in the central-western region of Portugal (40º 10' N, 8º 41' W, 5 m). The valley consists of about 15 000 ha where the main agricultural crop is "paddy" rice, which occupies about 60% of the usable area (Cabral et al., 2001). Non cultivated areas, such as swamps, are usually located in the perimeter of the valley and exhibit a flourished fauna and flora, whereas drainage canals, which are widespread across the whole valley, also constitute a biological reservoir (Anastácio et al., 1999). Additional geologic, hidrogeologic and climate details of the valley were described by Andrade & Stigter (2009). Rice cultivation starts normally with seeding from the beginning of April to the end of May, which depends on water availability for field flooding, the choice of rice

variety (early or late) and climatic conditions. The growing cycle continues until September-October, when rice is harvested. The rice cropping is supported by an organized system of irrigation-drainage canals around the paddies and the water flow is controlled by small and simple dams, constructed in strategic points to provide the entrance or discharge of water. Local rice fields are abundantly fertilized and a variety of pesticides, including herbicides, are intensively and sometimes indiscriminately used in weed control programs. The application of agrochemicals occurs mainly during the end of April up to June, but additional amounts of fertilizers or pesticides are added along the whole cropping season, depending on the type of culture demands and on the rice crop regional conditions (Marques et al., 2008).

## 6.1 Herbicides: overall use and selection
Almost all the major weeds in the Mondego Valley are grasses (family *Poaceae*) and, given the efficiency of the herbicides used, red rice (*O. sativa* L.) is undoubtedly the adventitious plant which poses more problems, followed by *Leersia oryzoides* (L.) Sw., which is spreading fast. Other important weeds found in the area are the *Poaceae* barnyard grass [*E. crus-galli* (L.) Beauv.] and early watergrass [*Echinochloa oryzoides* (Ard.) Fritsch]; the *Ponteridaceae* roundleaf mudplantain [*Heteranthera reniformis* Ruiz & Pavón] and ducksalad [*Heteranthera limosa* (Sw.) Willd.]; the *Cyperaceae* triangle (*Scirpus maritimus* L.) and dirty dora (*C. difformis* L.); the *Alismataceae* water-plantain (*A. plantago-aquatica* L.); and the algae *Sphaeroplea annulina* (class *Chlorophyceae*) and *Chara foetida* (class *Charophyceae*) (CACMV, 2010).
Most of the herbicides used in rice are selective, and the time of application relative to the growth stage of the crop and the weeds, along with proper water management, is critical for crop safety and good weed control (Monaco et al., 2002). The recommended herbicides for integrated weed management (IWM) are depicted in Table 1. Only a short overview of the more important characteristics of herbicides is described. For more detailed and specific information about herbicide properties and modes of action, the reader is reported to more extensive and specialized literature such as Tomlin (2000), Aizawa (2001), Stenersen (2004), Mackay et al. (2006), and Krieger (2010). It is important to mention that some active ingredients were already excluded from the *EU Pesticides database* (http://ec.europa.eu/ sanco_pesticides/public/index.cfm; last updated on 03/08/2010; last accessed on 3 November 2010) but we still include them here just for information purposes, since some local farmers still use them and because of the holistic approach of this review.

## 6.2 Ecotoxicological impacts of Basagran® (*a.i.* bentazon) and Ordram® (*a.i.* molinate) on non-target rice field cyanobacteria
Since 2005 our research group has been developing various relevant studies about the effects of two most commonly used rice field herbicides – Basagran® and Ordram® – on native non-target rice field cyanobacteria. In one of those studies, the effects of the two selective herbicides recommended for IWM on rice were laboratory-assessed in *Anabaena cylindrica* during a short-term experiment of 72 h (Galhano et al., 2009). The results obtained in this work revealed that both herbicides had a pleiotropic effect on the cyanobacterium at the range of concentrations tested (0.75-2 mM). Cyanobacterial growth (expressed as dry weight, D.W.) as well as photosynthetic pigments [Chl*a*, carotenoids (Car), phycobiliproteins (PBP)] were more adversely affected by molinate than bentazon commercial formulations. *A. cylindrica* growth of over 50% was observed soon after 48 h with 1.5-2 mM of Ordram. The protein content increased with both herbicides although the

| Weeds/ Active Ingredient | Form. | Concent. (g a.i. ha⁻¹) | TC | Recommended application conditions |
|---|---|---|---|---|
| **Monocotyledonous** | | | | |
| Cycloxydim (authorized only at national level) | EC | 200 | Xi | Only for red rice control. Rice preplant and with red rice in the 1-2 leaves stage |
| Cyalofop-butyl | EC | 300 | Xn; N | In rice post-emergence and grass weeds with 1-3 leaves |
| Molinate (cancelled at national level in 28/04/2010) (*) | FG | 4500 | Xn; N | In rice preplant, pre-emergence or early post-emergence, with grass weeds in the 2-leaves stage |
| Oxadiazon | EC | 300 - 400 | Xn; N | Apply after soil preparation and flood the soil after application. In rice preplant (5–8 days after application) |
| Profoxydim[(1)] (pending chemical in the *EU Pesticides database*) | EC | 100 – 150 | C; N | In rice post-emergence; apply 100 - 120 g from the 4-leaf stage to tillering, and 100 - 150 g from the beginning to the middle of tillering |
| **Monocotyledonous (families *Poaceae, Alismataceae, Cyperaceae, Pontederiaceae*) and Dicotyledonous** | | | | |
| Azimsulfuron | WG | 20 - 25 | N | In rice post-emergence, from the 2-3 leaves stage to tillering; with grass weeds from 2-leaves stage to the beginning of tillering and other weeds to the 4-6 leaves stage |
| Bensulfuron-methyl | WG | 51 - 60 | Is | In early rice post-emergence and weeds with 2-3 leaves |
| Bensulfuron-methyl + Metsulfuron-methyl | WG | 40 + 1.6 - 50 + 2 | Is | In early rice post-emergence and weeds with 2-3 leaves; it does not control grassy weeds |
| Bentazon (cancelled at national level in 16/04/2008) (*) | SL | 1440 - 2400 | Xn; N | In rice post-emergence from the 3-leaves stage to tillering, and weeds with 3-5 leaves |
| Bispyribac-sodium | SC | 25 - 30 | Xi; N | In rice post-emergence, from the 3-leaves stage to tillering; with grass weeds from 2-leaves stage to the beginning of tillering, and other weeds with 4-6 leaves |
| MCPA (ester) | SL | 630 - 1050 | Xn | In rice post-emergence and with weeds in active growth (4-6 leaves) |

| | | | | |
|---|---|---|---|---|
| MCPA (potassium salt) | SL | 800 - 1200 | Xn | In rice post-emergence and with weeds in active growth (4-6 leaves) |
| Penoxsulam | OD | 40 | Xi; N | In rice post-emergence from the 2-3 leaves stage to stalk stage, and weeds with 1-4 leaves |
| Propanil (not included in the *EU Pesticides database*); (cancelled at national level in 30/09/2010) (*) | EC SC WG | 3600 - 9000 3600 - 8640 3600 - 9000 | Xn; N Xn; N Xn; N | In rice post-emergence and grass weeds with 3-4 leaves; apply lower and higher doses in nurseries and sowed or planted rice, respectively |
| **Ridges (Monocotyledonous and Dicotyledonous)** | | | | |
| Glyphosate (ammonium salt) (some formulations were cancelled at national level in 14/08/2008) (*) | SG | 272 - 3600 | Is; N | Apply when weeds are well developed by using a bell glass during crop cycle or after harvest; apply lower and higher doses to control annual and perennial weeds, respectively |
| Glyphosate (isopropylammonium salt) (some formulations were cancelled at national level in 26/08/2010) (*) | SL | 540 – 3600 | | |
| **Algae** | | | | |
| Copper sulphate (some formulations will be cancelled at national level in 31/02/2012) (*) | XX | 750 - 1875 | Xn; N | As soon as algae became perceptible; apply by spraying or by placing jute bags in the entrance of irrigations ditches |
| Copper and calcium sulphate (some formulations will be cancelled at national level in 30/04/2011) (*) | WP | 1700 | | |

Table 1. Herbicide active ingredients and chemicals recommended in plant protection to control rice field weeds (adapted from: DGADR, 2009). Abbreviations: Form., formulation [EC, emulsifiable concentrate; FG, granules; OD, oil dispersion; SC, irritant; SG, water soluble granules; SL, aqueous liquid solution; WG, exempt of classification; XX, others; WP, wettable powder]; Concent., concentration; TC, toxicological classification [C, corrosive; Is, exempt; N, nocive and dangerous to the environment; Xi, irritating or sensitizing; Xn, nocive]. Observations: [1]It must be applied in a mixture with 0.75 L.ha$^{-1}$ of the commercially available wettable adjuvant Dash HC® (adjuvant concentration should not exceed 0.5% in low volume applications); [2]Treated rice for human consumption should be cleaned; (*) DGADR, 2010. *Cancelamento de AVs e APVs (Circular 16/2004) – Exhaustive Cancellation List of All Active Ingredients in Portugal Since 01/01/2001 (Last Review: 10 June, 2010).* Direcção-Geral de Agricultura e Desenvolvimento Rural; Ministério da Agricultura, do Desenvolvimento Rural e das Pescas, 23 pp. (in Portuguese).

effect was more remarkable with the highest concentration of Ordram. Concerning to carbohydrate content, it was shown that Ordram increased this organic fraction whereas Basagran decreases it. Photosynthesis ($P_m^{Chl}$) and dark respiration ($R_d^{Chl}$) normalized to Chl*a* were inhibited by both herbicide formulations in a time- and dose-response manner within the experiment time, and higher Ordram concentrations full stopped $O_2$ evolution after 48 h. Pointing to safety environmental precautions, the findings obtained with our study suggest the reduction or even ban of molinate from the agro-ecosystem rice field because of its strong inhibitory action on soil autochthonous microflora, mainly on the important diazotrophic primary producers, the cyanobacteria. Together with pulse-amplitude-modulation (PAM) fluorimetric routine parameters, namely maximum photosystem II (PS II) quantum efficiency (Fv/Fm), effective quantum efficiency of PS II for a light-acclimated sample ($\Phi_{PSII}$), and photochemical quenching (qP; proportion of light excitation energy converted to photochemical act by the active PS II reaction centres), part of the results published by Galhano et al. (2009) were previously presented to the overall scientific community in the 15th European Bioenergetics Conference held in Dublin, from 19 to 24 July 2008 (Galhano et al., 2008). Relevant findings related to Basagran and discussed in the meeting are displayed in Figure 1 carpet-plot.

But we wanted to go further on and test the effects of the herbicides on other cyanobacteria strain isolated and subsequently identified by suitable molecular biology tools (Galhano et al., 2010a). So, the next step was the assessment of toxicity of Basagran and Ordram on *Nostoc muscorum*, an abundant and well characterized cyanobacterium from Mondego River Valley rice fields. Once again, in a short-term exposure experiments during 72 h with a concentration range from 0.75 to 2 mM, the toxicity of commercial formulations on growth and some biochemical and physiological parameters cited before were studied (Galhano et
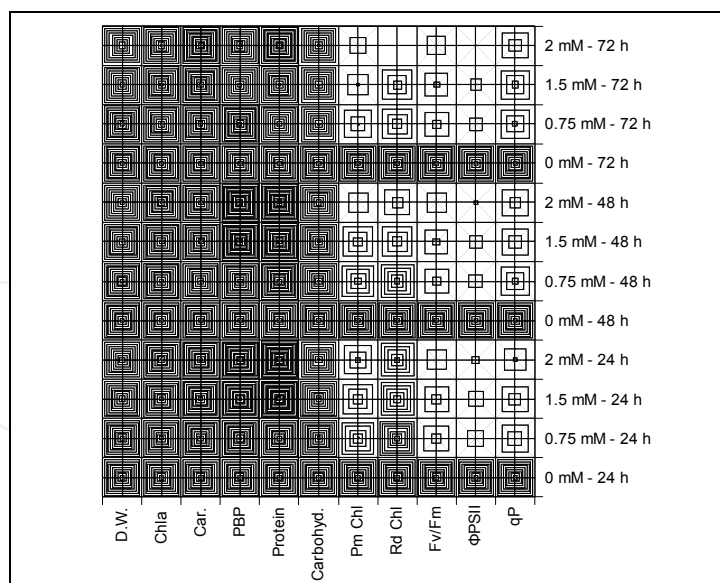


Fig. 1. Effect of Basagran on dry weight (D.W.), chlorophyll *a* (Chl*a*), carotenoids (Car), phycobiliproteins (PBP), protein, carbohydrates, photosynthetic rate ($P_m^{Chl}$), dark respiration rate ($R_d^{Chl}$) and fluorescence parameters of *A. cylindrica* after exposure for 24, 48 and 72 h. Relative values are means ± SE of at least three independent experiments. The plotted values are visualised by the number of the contour lines, with successive lines corresponding to values differering by 0.05 (here above zero, *i.e.* bigger than the control).

al., 2010d). The results almost entirely obtained in this study confirmed the mode of action of both herbicides on cyanobacteria: 1) molinate was more toxic than bentazon to growth, respiration, Chl*a*, Car, and PBP contents; 2) protein content increased with both herbicides but the effect was mostly evident at higher molinate concentrations (1.5-2 mM); 3) the herbicides had contrasting effects on carbohydrates content – molinate increased it whereas bentazon caused a decrease of this organic fraction; 4) both photosynthesis and respiration were inhibited by Ordram and Basagran.

At this point of our research we were intrigued about the insufficient information on the biochemical mode of action of both herbicides at cellular level, particularly concerning bentazon. We knew from previous works that bentazon acts as an inhibitor of photosynthesis by blocking the electron transfer flow in the PS II and $CO_2$ fixation. The blockage of PS II induced by bentazon in the presence of light induces secondary effects on several metabolic pathways, such as the production of singlet and triplet Chl energized states, as well as various reactive oxygen species (ROS) like *e.g.* the singlet oxygen (Macedo et al., 2008). The very recent reviews of Latifi et al. (2009) and Pospíšil (2009) on oxidative stress science that came to us almost at the same time were very exciting and inspiring, thus contributing to the next follow up step. Therefore, by interlinking these reviews, we hypothesized that bentazon, like most environmental stresses *e.g.* heavy metals, high light, UV-B, heat, salinity and drought, induced the production of ROS in cyanobacteria, causing
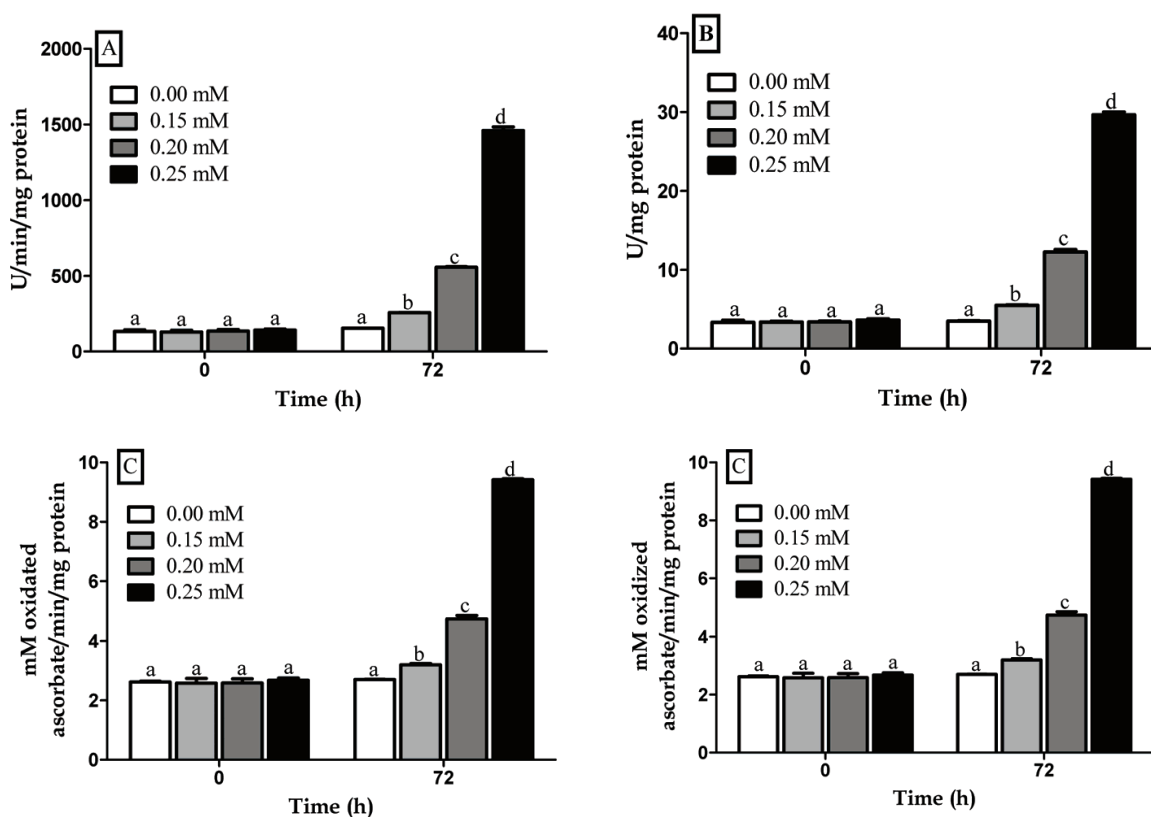


Fig. 2. Effect of molinate on the activities of (A) SOD, (B) CAT, (C) APX, and (D) GST of *A. flos-aquae* after exposure to bentazon for 0 and 72 h. The values (%) relative to controls are means ± SE of three to six independent experiments. Results of the one-way ANOVA factorial analysis. Values with a common letter are not significantly different according to Tukey's test (P<0.05).

oxidative damage. The obtained results confirmed the formulated hypothesis, since the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione *S*-transferase (GST) increased in a time- and Basagran dose-response manner and were higher than those in the control samples after 72 h for a concentration range of 0.75 to 2 mM. On the basis of these data and information available elsewhere we proposed a hypothetical mechanistic model for Basagran-induced oxidative stress on *A. cylindrica* (Galhano et al., 2010c). A similar trend was attained with the active ingredient of Basagran *i.e.* bentazon in an *Aphanizomenon flos-aquae* strain isolated from "Quinta do Seminário" rice fields, also in the Baixo Mondego River Valley region (Fig. 2). Acute and short-term (72 h) experiments also demonstrated that the active ingredient caused a time- and concentration (0.15-0.25 mM) dependent decrease of *A. flos-aquae* growth, photosynthetic pigments, total protein, carbohydrates, photosynthesis and respiration (Galhano, V., et al., 2010; unpublished results).

Meanwile, we did not forget the antioxidant system machinery response of cyanobacteria exposed to the formulation Ordram in acute and short-termed experiments (72 h). Contrary to Basagran, the thiocarbamate dramatically decreased the activity of all antioxidative stress enzymes tested in a time- and concentration-dependent manner within a concentration range of 0.75 to 2 mM. The collapse of both enzymatic (*e.g.* SOD, CAT, APX, GST) and non-enzymatic (*e.g.* glutathione pool and total carotenoids) antioxidants was evident after 72 hours' exposure to the highest Ordram concentrations. A radical decline in saturated fatty acids was also observed in this study (Galhano et al., 2010b).

## 7. Conclusions and future prospects

The present environmental concerns about rice field herbicide residues in water, soil and rice foodstuffs will probably not vanish in the next years. Not enough has been done to reduce the risks caused by herbicide pollution in rice agriculture. Even though the switch to low-dose agents has significantly reduced herbicide consumption, most surface water and groundwater samples still contain herbicides, sometimes at levels harmful for human health and for environment. At present, it is not possible to assess the future risks to human beings and to environment caused by the hazardous properties of herbicides used today and the trends will vary from country to country (OECD, 2008). Notwithstanding, to guarantee minimal negative side-effects on rice field ecosystems other than the soil-plant systems, herbicides should have no or low toxicity, except for the target weeds. Improved formulations will be needed to reduce off-target deposition, improve retention on target, and enhance uptake and translocation (Arias-Estévez et al., 2008). As many European rice fields are located in natural parks or environmentally protected areas, the future of rice in the European countries will most likely be interlinked to the development of new varieties and environmentally and economically sustainable methods of production, which allow to increase rice yield and quality by improving water and fertilizer efficiency and minimizing the use of crop protection products like herbicides (Ferrero, 2007; Ferrero & Tinarelli, 2007). At present there is a very considerable literature related to herbicide effects on the flora and fauna of rice fields but a conspicuous lacuna related to rice field biodiversity affected by this kind of xenobiotics still exists. Ecological studies contrasting intensive irrigated rice systems reported in this review with the traditional rainfed rice lands have not been carried out so far. As a result of a heavy dependence on selective herbicides for weed control in rice fields worldwide, a serious problem has emerged in the last years: several weed species have

evolved resistance to herbicides, including the most pernicious grass weed, *Echinochloa* spp., becoming resistant to rice gramicidines (Olofsdotter et al., 1998; Vidotto et al., 2007; Gressel & Valverde, 2009). So far, some mechanisms of evolved resistance of rice weedy species are still unknown (Dyer & Weller, 2005). Therefore, in future weed management programs, Gressel & Valverde (2009) recommended the use of transgenic herbicide-resistant rice cultivars to better achieve the control of weeds that have evolved herbicide resistance.

Interestingly, some organisms that have evolved in natural ponds near rice fields are now being used as biological control agents in rice culture, as for example in Japan, where the problem of annual weeds infesting paddy fields has been countered by the introduction of several species of tadpole shrimp (*Triops* spp.) which agitates the soil surface, uproots weed seedlings, creates turbid water (which compromises photosynthesis) and consumes weed buds (Williams, 2006). Therefore, nowadays, as in the future, the commonly accepted best approach to manage rice field weeds is to follow an IWM strategy that includes good land preparation, good water management, a competitive crop, and a judicious herbicide use (Monaco et al., 2002; Upadhyaya & Blackshaw, 2007; Demont et al., 2009).

The use of biological control agents (natural enemies and pathogens) (Castle et al., 2006) and/or biologically-based products (allelochemicals) (Belz, 2007) should also have to be implemented. The use of bioherbicides are also interesting alternatives for use in rice IWM programs (Charudattan et al., 2002; Kendig et al., 2003). A suitable and periodic water quality monitoring together with the improvement of good agricultural practices will be advisable in future IWM programs.

More important yet, rice researchers and farmers must go one step further and rapidly to an era of precision farming, which helps to reduce the cost of production and improve productivity on an ecologically sustainable basis. They should launch a movement for achieving an evergreen revolution in rice farming systems based on ecologically sustainable and location-specific precision farming technologies (Pretty, 2005; Swaminathan & Rao, 2009). Dr. Norman E. Borlaug, The Nobel Prize in Peace of 1970, called it the "Blue Revolution" of the 21st century to complement the so-called "Green Revolution" of the 20th century in order to feed the growing world population (Borlaug, 2004).

In conclusion, we must say that nowadays, not only the scientific community, but also the general public, including rice farmers and extension workers, should be aware of the need for a continual review of rice field herbicides once they have been authorized to be lunched into the market, mainly due to their unpredictable effects on both the environment and human health. We think that this timely and up-to-date review can significantly improve the information in this research area and contribute to a better understanding of the effects of rice field herbicides on non-target organisms, which inevitably will lead to a rationalization of their use in future integrated weed management programs.

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**Herbicides and Environment**

Edited by Dr Andreas Kortekamp

Herbicides are much more than just weed killers. They may exhibit beneficial or adverse effects on other organisms. Given their toxicological, environmental but also agricultural relevance, herbicides are an interesting field of activity not only for scientists working in the field of agriculture. It seems that the investigation of herbicide-induced effects on weeds, crop plants, ecosystems, microorganisms, and higher organism requires a multidisciplinary approach. Some important aspects regarding the multisided impacts of herbicides on the living world are highlighted in this book. I am sure that the readers will find a lot of helpful information, even if they are only slightly interested in the topic.

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