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CHALLENGES AND OPPORTUNITIES FOR SUSTAINABLE RICE-BASED PRODUCTION SYSTEMS

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The year 2004 was designated by the United Nation as the International Year of Rice (IYR).
To celebrate the International Year of Rice, the Medrice Network organized an International Conference aimed at examining main aspects which may shape the development of rice-based production systems in the temperate climate areas.
The Conference focused on several scientific and technical issues of rice cultivation, spanning from agronomy and crop management, environment and ecology, genetics and breeding, grain quality and nutrition.
The Conference was convened at the Faculty of Agriculture, University of Torino (Italy) and was also one of the international events organized to celebrate the sixth century of foundation of the University of Torino.

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Session 1

AGRONOMY

Full papers

PESTICIDES APPLICATION REDUCTION IN INTEGRATED RICE PRODUCTION IN SOUTHERN SPAIN

Aguilar-Portero M

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Abstract
In 1990 the European Council established the basic strategies and technical guidelines of Integrated Production. Andalusia (Southern Spain) has been pioneer in Europe by preparing the *Specific Regulations of Rice Integrated Production* (December 1997). These Regulations structured its rice area in Rice Integrated Production (RIP) Groups. Each Group covers about 500 ha and is advised by a Technician, who supervises the agronomical practices.
The regulations, specially the phytopathological ones, have brought a decrease in the pesticides application, mainly in the case of insecticides (reduced to 1/3 of the traditional applications).
It is significant the fungicides application reduction, due to the RIP Technicians recommendations based mainly in the air humidity and temperature data. Regarding herbicides, the reduction is not significant.
The subsidies for Rice Integrated Production depend on the farm size. They are set in about 240$/ha (for the first 40 ha), 144$/ha (next 40 ha) and 70$/ha (for the rest of the farm surface). The Integrated Production surface has grown gradually (98% in 2003).

Keywords
Integrated Rice Production; pesticides reduction.

Introduction

Of the 450.000 ha given over rice culture in Europe, almost a half of it goes to Italy and nearly a 23% to Spain. Portugal, Greece and France, in this order, are the rest of the european rice producing countries. In Southern Spain 38.000 ha are dedicated to rice culture.
The Andalusian rice area is located at the final stretch of the Guadalquivir river. Its Mediterranean climate is characterised by warm and dry summers with clear and long days. The soil is of sedimentary origin, clayey, saline and poorly drained. The Andalusian rice area is characterised as well by the use of modern agronomic practises, such as the use of airplanes or laser levelling.

Specific Regulations of Rice Integrated Production

Integrated Production is defined as a farming system that produces high quality food and other products by using natural resources and regulating mechanisms to replace polluting inputs and to secure sustainable farming (Table 1).

Table 1 Main agronomic practices in Rice Integrated Production Regulations.

	Compulsory	Prohibited	Recommended
Land preparation	Laser land levelling at least once every three years	Tillages no more than 20 cm deep in order to avoid salt to flow upwards	
Seeding	Immediately after flooding	Seeding after May the 30th	Seeding between April the 20th and May the 20th
	Use of disinfected and Certified Seed	Seeding rates over 200 kg/ha	Seeding rates: Indica type: 165 kg/ha Japonica: 180 kg/ha
Fertilization	Soil analysis	Nitrogen fertilization after panicle differentiation. Nitrogen fertilization over the following doses: Indica type: 145 kg/ha Japonica: 125 kg/ha	
Irrigation	Water analysis		Water recycling if water salt content allows to do it
Integrated control	Estimating the risks in pest control as established in the Integrated Pest Control Strategies Protecting the auxiliary fauna Use of active matter included in the RIP Regulations The machinery used in the phytosanitary treatments must be periodically checked and calibrated	Use of pest control calendars	
Harvest	Analysing grain samples to detect phytosanitary residues, regarding Spanish Legislation of LMR (Level of Maximum Residues)		Leaving the paddies flooded for the next 2-3 months after harvest, considering the possible benefit to waterfowl

Results

The rice farmer has accepted not to apply both excessive seeding and nitrogen rates. Stands out the high level of water recycling and the recent implantation of an intermittent flooding system instead of a continuously flooding system. This has brought an appreciable saving of water and electrical energy.

It is quite frequent leaving the fields flooded after harvest until three months before seeding, aiming to decrease the proliferation of weeds, and considering the possible benefit to waterfowl.

The straw incorporation (about 20% of the farms), instead of burning, is a practise which becomes slowly widespread.

The maximum residue level in grain is another regulated parameter.

The agronomic regulations, specially the phytopathological ones, have brought a decrease in the pesticides application, a fact specially significant in the case of insecticides (Figure 1).

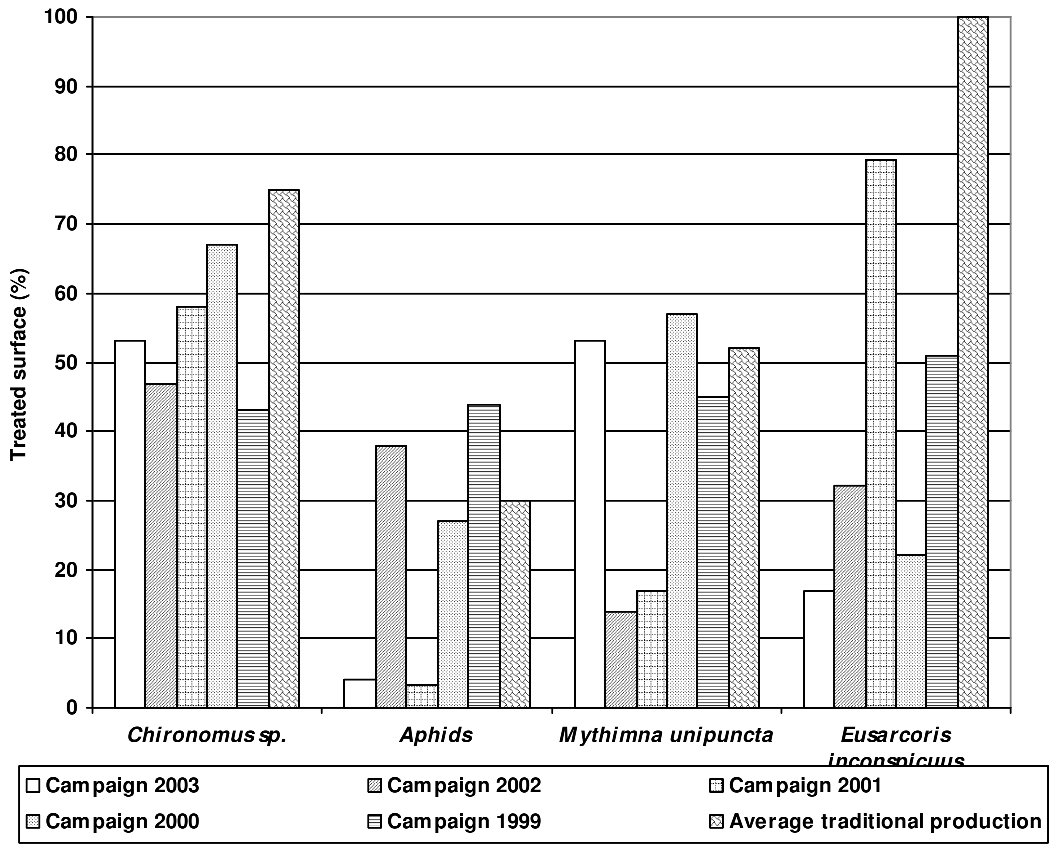

Figure 1 Integrated vs traditional production. Insecticide application evolution. Seville, 1999-2003.

We consider significant the reduction in the application of fungicides, thanks to the Rice Integrated Production (RIP) technicians recommendations on the application of chemical treatments, depending basically on the air humidity and temperature.

Regarding herbicides, the difference between both integrated and traditional production methods is not significant, although the compulsory laser levelling must be considered together with a list of permitted herbicides. Only officially registered pesticides with a minor impact on the environment, a higher efficiency and a lower toxicological degree are permitted, and their intended use has to be respected. Unselective pesticides with long persistence, high volatility, leachable or with other major detrimental characteristics are prohibited.

Conclusions

The Rice Integrated Production Regulation (RIPR) has brought a significant advance in cultivation methods, more friendly with the environment, and carrying no lessening of the crop profitability.

These regulations have carried out significant inputs reductions, specially in case of insecticides (33%).

INTEGRATED MANAGEMENT OF FALSE SMUT DISEASE OF RICE IN INDIA

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Abstract

False smut (*Ustilaginoidea virens*) has recently become an important problem in Punjab,India and may be of concern to livestock and humans due to its ustilotoxins. The rice cvs. PR 103 and PR 106 were found to have a high level of genetic resistance to the disease. Of the nine fungicides evaluated , Tilt (Propiconazole) and Contaf (Hexaconazole) at 0.1% each and Blitox (Copper oxychloride) at 0.3% ,when applied during the boot stage, significantly controlled false smut and improved grain quality. However, Blitox reduced grain yield which may be due to the copper toxicity. A supplemental dose of nitrogen , applied during the boot stage, aggravated the disease and reduced fungicide efficacy. The disease can be managed by integrating various options.

Keywords

Rice; false smut; cultivars; resistance; growth stage; nitrogen; integrated management

Introduction

False smut (*Ustilaginoidea virens* (Cooke)Takah) disease of rice has recently become an important disease problem in the Punjab state of north west India and caused widespread concern in 2001 and 2002 in a commercial cultivar PR 116 when environmental conditions were very favourable for the disease. False smut infection in the field is known to occur at the rather short period just before heading (Ikegami,1960). False smut primarily affects quality since the fungus produces brown smut balls that contaminate rice grain at harvest. Moreover ,the recent discovery of ustiloxins – a phytotoxin and mycotoxin, produced by the pathogen, may be of concern to livestock and humans (Koiso et. al. 1992, 1994).This has increased the need to monitor and control the disease. Although ,resistant varieties are the best method of control, the false smut reactions of rice cvs. grown in Punjab are not known. Hence , identification of rice cvs. with reduced susceptibility to false smut would offer an additional component in the integrated control of the disease. Although, copper fungicides are known for controlling false smut (Kannaiyan and Rao,1976), there are reports of copper showing phytotoxicity and reducing grain yields (Cartwright et. al.2000). This paper reports the reactions of rice cvs.to false smut and evaluation of new triazole fungicides, at different growth stages and normal and supplemental nitrogen application, for integrated management of false smut disease.

Materials and Methods

141 advanced cultivars /lines of rice were sown in May,2001,2002 and 2003 at the farms of PAU, Regional Research Station ,Gurdaspur in a loam soil. Forty days old seedlings of the different cvs. were transplanted in 6 rows of 4m length at 20*15 cm spacing in the puddled field in a randomized complete block design with four replications and plots received 50 kg nitrogen/acre(as urea) in three split doses. Plots were treated with rice herbicide Butachlor 50

EC at 1200 ml/acre,2-3 days after transplanting,for weed control.Plots were spray inoculated with conidia/ chlamydospores of U virens collected from spore balls from commercial fields. Conidia were suspended in water at 2.5*107 spores /ml and applied to plots during the boot stage in the evenings.The environmental conditions were very favourable for disease development in 2001 and 2002 which resulted in severe natural and artificial infection and facilitated differentiation of resistant and susceptible reactions of these cvs./lines. Data on disease incidence(no. of smut balls/plot) and severity (no. of smut balls/infected panicle) were recorded just before maturity.

Nine fungicides viz; Fujione 40 E (Isoprothiolane),Tilt 25EC (Propiconazole),Contaf (Hexaconazole) ,Folicur (Tebuconazole), Hinosan (Edifenphos), Bayleton (Triadimefon), Benlate50WP (Benomyl),Bavistin 75WP(Carbendazim), Blitox (Copper oxychloride), Kasu-B(Kasugamycin) and Sixer(Carbendazim + mancozeb) were evaluated as two foliar sprays (the first spray applied during the boot stage and the second 15 days later), after natural and artificial infection in the field, during 2001-2003 in the rice cv. PR 116- highly susceptible to false smut. Some of these effective fungicides were also evaluated at two different growth stages i.e boot stage and 50 % panicle emergence and also under normal recommended nitrogen level and higher nitrogen level where a supplemental dose of nitrogen was applied at the boot stage. Data on disease incidence and severity were recorded just before maturity of the crop and the grain yield recorded after harvest and analysed statistically.

Conclusions

Among the 141 cvs./lines of rice evaluated only for one year , 65(46%) lines remained free from false smut, under natural field infection, whereas 76(53.9%) lines were found to be susceptible to highly susceptible. None of the 11 released cvs. of rice was free from the disease but these cvs. differed greatly in their reactions to false smut (Table 1).The disease was much higher in 2001 and 2002 as compared to 2003 and was more or less uniform across plots and replicates in this study. The cv. PR 116 was consistently the worst affected in all the three years of testing and was rated as the most susceptible cultivar. Among the three early heading rice cvs. viz; PR 103, PR 115 and Sharbati which had almost the same heading date(26/27 August), the cv. PR 103 was the least affected.

All the other rice cvs. which came to heading between 12-16 th September, were much less affected, as compared to the most susceptible cv. PR 116. The cv. PR 106 which had the same heading date(15 September) as the most susceptible cv. PR116,was the least affected in all the three years of testing. The cvs. PR 103 and PR 106 which consistently showed a very low level of infection, as compared to the most susceptible cv. PR 116, in all the three years of testing , may possess a high degree of genetic resistance to false smut and these multi-year tested disease reactions can be relied.

Table 1. Reactions of rice cultivars to false smut after natural/ artificial infection at Gurdaspur, during 2001-2003.

Cultivar	Heading Date	Disease Incidence* No.Smut balls/plot				Disease Severity* No. Smut balls/panicle			
		2001	2002	2003	Mean	2001	2002	2003	Mean
PR 103	27 Aug.	0.2	2.0	4.2	2.8	0.25	0.66	1.36	0.76
PR 106	15 Sept.	4.5	19.3	6.7	10.2	1.06	1.24	1.04	1.11
PR 108	13 Sept.	20.2	102.3	8.5	47.0	1.98	2.2	0.93	1.70
PR 111	08 Sept.	8.0	22.0	1.0	10.3	1.36	1.4	0.25	1.0
PR 113	12 Sept	40.2	90.0	4.0	44.7	2.33	1.84	0.91	1.7
PR 114	15 Sept.	65.7	132.6	9.0	69.1	2.36	2.05	1.85	2.0
PR 115	27 Aug.	2.2	76.0	11.7	29.9	0.82	2.06	1.20	1.36
PR 116	15 Sept.	447.7	528.3	80.2	352.1	3.47	2.96	1.60	2.68
PR 117	13 Sept.	-	-	2.0	-	-	-	0.75	-
PR 118	16 Sept.	-	-	4.7	-	-	-	1.43	-
Jaya	12 Sept.	38.2	60.0	3.0	33.7	1.69	1.76	1.00	1.48
Inderasan	12 Sept.	42.5	66.6	3.5	37.5	-	1.81	0.90	1.36
Sharbati	26 Aug.	-	-	22.2	-	-	-	1.51	-
CD 5%		212.2	98.3	3.38		1.26	0.764	0.564	

* *Mean of four replications*

In field trials on chemical control, the cultivar PR 116 developed severe false smut in 2001 and 2002 as compared to 2003.All the nine fungicides evaluated in 2001 (Table 2), other than Fujione 40E and Bavistin, considerably reduced false smut incidence when applied during the boot stage,in comparison with the control. Treatments with Blitox (0.3%) and Tilt (0.1%) were the most effective, closely followed by folicur,Contaf,Hinosan,Benlate and Bayleton. Fujione at all the three rates (0.1- 0.3%) tested, was ineffective. Although reduced yields were obtained with all the fungicides, treatment with Blitox, in particular, adversely affected grain yield. This yield reduction may be due to the phytotoxicity of copper to flag leaf/panicles which warrants further studies. Cartwright et. al. (2000) have also reported similar findings.

Table 2. Evaluation of fungicides for the control of False smut of rice after natural/artificial infection in the field in 2001 .

Treatment	Rate %	No. Smut * balls/plot	Grain yield* (kg/plot)
Fujione	0.1	141.5	5.05
Fujione	0.2	113.0	5.15
Fujione	0.3	129.5	5.40
Hinosan	0.1	11.5	5.00
Tilt	0.1	3.0	5.55
Folicur	0.1	9.5	5.55
Contaf	0.1	11.0	5.25
Bavistin	0.1	133.0	5.50
Benlate	0.1	27.0	5.15
Bayleton	0.1	34.5	5.80
Blitox	0.3	1.0	4.00
Control	-	99.5	5.05
CD 5%		NS	NS

* *Mean of four replications*

In 2002 and 2003, the fungicide Tilt, closely followed by Contaf, consistently reduced false smut incidence and severity in both the years and was found to be the most effective fungicide for controlling false smut but was inconsistent in increasing grain yields (Table 3).The application of a supplemental dose of nitrogen , during the boot stage, aggravated the disease and reduced fungicide efficacy, as compared to the normal nitrogen level (Table 4). The efficacy of Tilt in reducing disease incidence was reduced from 93.2% under normal – N to 49.14% under supplemental nitrogen but increased grain yields were obtained with higher nitrogen . Application of Contaf and Kasu-B became ineffective when additional nitrogen was applied. Manibhushan Rao (1964) also reported highest false smut infection when fertilizer was applied at the time of flowering.

Table 3 Chemical control of false smut of rice by foliar spray of fungicides during 2001-2003.

Treatment	%	No. Smut balls /plot*			No. Smut balls/panicle*		Grain Yield (kg/plot)		
		2001	2002	2003	2003	2003	2001	2002	2003
Hinosan	0.1	11.5	92.0	20.3	1.68	1.59	5.00	4.36	4.47
Tilt	0.1	3.0	11.6	3.7	1.43	1.08	5.55	4.05	4.75
Contaf	0.1	11.0	75.0	8.2	1.79	1.26	5.25	4.30	4.72
Kasu-B	0.1	-	68.3	-	1.98	-	-	3.96	-
Kasu-B	0.2	-	117.0	-	2.38	-	-	4.33	-
Sixer	0.1	-	94.0	-	2.07	-	-	4.16	-
Control	-	99.5	171.0	31.6	2.17	1.49	5.0	4.30	4.37
CD 5%		NS	NS	10.4	NS	0.3	NS	NS	0.3

* *Mean of four replications*

Table 4 Efficacy of fungicides for controlling false smut under normal and supplemental nitrogen application in 2002.

Treatment %	No. Smut balls /plot*		No. Smut balls/panicle*		Grain Yield (kg/plot)	
	Nor.-N	Supple.-N	Nor. –N	Supple. -N	Nor.-N	Supple.-N
Hinosan 0.1	92.0(46.1)	182.6(37.3)	1.68	2.48	4.36	4.75
Tilt 0.1	11.6(93.2)	148.3(49.1)	1.43	2.08	4.05	5.23
Contaf 0.1	75.0(56.1)	329.6(13.0)	1.79	2.24	4.30	4.85
Kasu-B 0.1	68.3(60.0)	270.0(7.4)	1.98	2.58	3.96	4.73
Control -	171.0 -	291.6	2.17	2.78	4.30	4.45
CD 5%	NS	NS	NS	NS	NS	

** Mean of four replications. Figures in parenthesis denote % efficacy of fungicides.*

Application of Tilt and Hinosan was more effective when applied during the boot stage as compared to the heading stage whereas reverse was true with Contaf and Kasu-B, which were more effective when applied at heading stage (Table 5).Application of Blitox, at both the growth stages, was ineffective in this trial. The reduced efficacy/ineffectiveness of the fungicides in this trial may be due to the high disease pressure under higher nitrogen level and the heavy rainfall which occurred within a few hours of their application. Although all the fungicides in this trial increased grain yields, the differences were non-significant.

Table 5 Efficacy of fungicides, at different growth stages ,for controlling false smut of rice in 2002.

Treatment %	Growth Stage	No. Smut* balls/plot	No. Smut* balls/panicle	Grain Yield* (Kg/plot)
Hinosan 0.1	boot	182.6	2.48	4.75
	heading	248.6	2.73	4.93
Tilt 0.1	boot	148.3	2.08	5.23
	heading	211.3	2.06	5.18
Contaf 0.1	boot	329.6	2.24	4.85
	heading	182.3	2.50	4.93
Blitox 0.3	boot	296.3	2.93	4.51
	heading	302.6	2.71	4.91
Kasu-B 0.1	boot	270.0	2.58	4.73
	heading	235.6	2.40	4.51
Control -	-	291.6	2.78	4.45
CD 5%		NS	NS	NS

** Mean of four replications*

Application of Tilt at 0.1%, during the boot stage, was the most effective treatment in reducing the number of smut balls in the harvested rice and improving grain quality and yield.However, this increase in grain yield may not be directly due to the false smut control but through the control of other diseases such as brown spot and sheath blight . In this study, the higher variability in disease incidence/severity and grain yields among the different replications and plots, prevented clear statistical separation of data and in most of the treatments ,differences were non- significant although there were distinct numerical differences.

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PATTERNS OF RESISTANCE TO ALS-INHIBITORS IN *CYPERUS DIFFORMIS* AND *SCHOENOPLECTUS MUCRONATUS* AT WHOLE-PLANT LEVEL

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Abstract

Populations of *Cyperus difformis* L. (smallflower umbrella sedge) and *Schoenoplectus mucronatus* (L.) Palla (rice bulrush) resistant to ALS-inhibitor herbicides have been reported in several rice areas of the world. To investigate the common traits of *C. difformis* and *S. mucronatus* resistant to ALS-inhibitors in Italy, California and Spain, a joined research program has been developed. This contribution reports the preliminary results of a study carried out in the greenhouse at the whole-plant level on 7 populations of *C. difformis* and 4 of *S. mucronatus* collected in Italian, Spanish and California rice fields. The following ALS-inhibitors were tested: bensulfuron-methyl, halosulfuron, cinosulfuron, imazamox, and bispyribac-sodium. The study indicated the presence of both simple and cross resistance in the two species. On the basis of GR50, GR90 and Resistance Index (RI) values, complex resistance patterns among the tested populations were observed. Further laboratory studies on enzymatic activity and molecular traits of ALS of tested populations have to be completed to explain the observed resistance patterns.

Keywords

Simple resistance; cross resistance; sulfonylurea herbicides; CYPDI; SCMPU; Resistance Index

Introduction

Cyperus difformis L. (smallflower umbrella sedge) and *Schoenoplectus mucronatus* (L.) Palla syn. *Scirpus mucronatus* L. (ricefield bulrush) are monocotyledonous species of the Cyperaceae family and are important weeds in several rice production areas of the world. The control of these weeds in irrigated, water-seeded, and continuously flooded rice production systems, has mainly relied on the use of herbicides. Since their introduction, ALS-inhibitors have been largely and repeatedly used to control sedges and other broad-leaf weeds. Cases of resistance to sulfonylureas (and to bensulfuron-methyl, in particular) in *C. difformis* and *S. mucronatus* have been reported within few years from their introduction in different countries in which similar rice production systems are developed. Simple of cross resistance occur when a weed population is not controlled by herbicides belonging to the same or different chemical classes, respectively. In order to investigate the common traits of *C. difformis* and *S. mucronatus* populations resistant to ALS-inhibitors, a joint research effort has been developed between the University of Torino (Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Italy), the University of California-Davis (Vegetable Crops Department, Davis, USA) and the University of Cordoba (Departamento de Bioquímica y Biología Molecular, Spain). The resistance has been studied both at the whole plant, enzymatic and biomolecular level, by adopting a common protocol for all the populations. This paper reports preliminary results of the studies carried out on the whole plants of both resistant and susceptible *C. difformis* and *S. mucronatus* populations collected in Italy, Spain and California rice fields.

Materials and methods

The experiments were conducted on several populations of *C. difformis* and *S. mucronatus* , which had been collected in paddies with a long history of use of bensulfuron-methyl. RCI and RSI indicate the two resistant populations of *C. difformis* and *S. mucronatus*, respectively collected in Italy; RCU1, RCU2 and RSU the accessions coming from California (USA) and RCS those from Spain. SCI and SSI are the abbreviations for Italian susceptible populations of *C. difformis* and *S. mucronatus*, respectively; SCU and SSU for California susceptible accessions and SCS for the susceptible Spanish *C. difformis* accession. All the resistant populations escaped the treatments with bensulfuron-methyl in the fields. For each species, a susceptible standard was collected in untreated areas in which bensulfuron-methyl showed a regular level of efficacy.

Plants were grown in the greenhouse and both resistant and susceptible populations were sprayed with one of the following ALS-inhibitors: bensulfuron-methyl (sulfonylurea), halosulfuron (sulfonylurea), cinosulfuron (sulfonylurea), imazamox (imidazolinone), and bispyribac-sodium (pyrimidinyl(thio)benzoate). The herbicides were applied at 6-7 rates ranging from 0 to 4 times the recommended field rate. Herbicide efficacy was measured by determining aboveground fresh weight per pot 20 days after spraying. The data were fitted to a log-logistic regression model (Brain and Cousens 1989; Seefeldt et al. 1995; Streibig et al. 1993; Schabenberger et al. 1999) to determine the herbicide dosages at which 50% of plant growth reduction occurred (GR50). The degree of resistance was quantified by Resistance Indexes (RI), expressed as the ratio between the GR50 of the resistant and the GR50 of the sensitive standard population (RI = GR50-resistant/GR50-susceptible). Simple resistance is defined as the ability to not be controlled by herbicides belonging to the same chemical class and cross resistance withstand herbicides from different chemical classes

Results

Italian populations

C. difformis

RCI population showed cross-resistance to bensulfuron-methyl, cinosulfuron, imazamox and bispyribac-sodium. The GR50 for these herbicides were 21.55, 12.05, 52.70, and 20.25 $g_{a.i.}ha^{-1}$, respectively. The calculated RIs were 125.73, 1097.91, and 8.66 for bensulfuron-methyl, imazamox and bispyribac-sodium, respectively. Halosulfuron controlled both RCI and SCI populations at the lowest rate (15 $g_{a.i.}ha^{-1}$) and no significant differences were observed between the dose-response curves of the two populations.

S. mucronatus

RSI population showed resistance to bensulfuron-methyl, halosulfuron, and cinosulfuron. The degree of resistance to cinosulfuron was lower than that observed for bensulfuron-methyl. The RSI population showed high susceptibility to imazamox and bispyribac-sodium. The calculated GR50 values were in general lower than those of the susceptible one. As consequence, the calculated RI values were 0.86 and 0.74 for imazamox and bispyribac-sodium, respectively.

USA populations

C. difformis

The accessions RCU1 and RCU2 of C. difformis collected in rice fields of the Sacramento valley (California) showed a similar pattern of resistance to bensulfuron-methyl. The accession RCU1 was resistant to bensulfuron-methyl, imazamox and bispyribac-sodium but effectively controlled by halosulfuron with plants killed at 0.5x field rate. An opposite resistance pattern was obtained with RCU2. This biotype showed resistance to halosulfuron but was completely controlled at 0.5x the field rate of imazamox. S. mucronatus

The rates required for 50% of fresh weight reduction of the resistant biotype (RSU) were 188 and 93 ga.i.ha-1 for bensulfuron-methyl and halosulfuron, respectively. For these two herbicides the calculated RI were similar (1372.3 and 1430.8, respectively). The responses to the other ALS-inhibitors imazamox and bispyribac-sodium were not substantially different. The GR50 values were lower than those obtained with the two sulfonylureas, although the biomass reduction recorded at the highest rates averaged around 80% and the plants were still alive.

Spanish populations

C. difformis

The RCS population showed simple resistance to the two sulfonylureas bensulfuron-methyl and halosulfuron. The calculated RI values for RCS accession were 239 and 136.1, respectively. In the case of imazamox RCS showed a slight tolerance to the herbicide, even though the plants were completely controlled at 0.5x the field rate. Both populations were susceptible to bispyribac-sodium and the RCS and SCS dose-response curves do not differ significantly.

Discussion

Simple and cross-resistance to ALS-inhibitors were recorded in the two species *C. difformis* and *S. mucronatus*. The study pointed out the ability of the sulfonylurea halosulfuron to control plants of *C. difformis* populations (RCI and RCU1) cross-resistant to the other ALS-inhibitors bensulfuron-methyl, cinosulfuron, imazamox and bispyribac-sodium.

This behavior can be theoretically attributable to structural dissimilarities between different sulfonylurea molecules or different structural elements of the same inhibitor-binding site (Schmitzer et al. 1993).

The high susceptibility of the standard reference populations of *C. difformis* (Italy, USA and Spain) and *S. mucronatus* (Italy and USA) often resulted in GR50 lover than 1 $g_{a.i.}$ ha^{-1}. As consequence very high values of RI were found even in populations not resistant to a certain herbicide. This result stresses the importance of a correct use of Resistance Index as discriminating criterion in herbicide resistance studies. The complex resistance pattern observed at the whole-plan level in the two species is being examined by further investigations at target-site and molecular level.

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BENSULFURON-METHYL RESISTANCE IN PORTUGUESE RICE PADDY FIELDS

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Abstract
One *Alisma plantago-aquatica* biotype resistant to bensulfuron-methyl was confirmed in rice paddy fields from south of Portugal (Sado river valley). The aim of this study was to determine the mechanism of resistance to bensulfuron-methyl. Whole-plant dose-response studies confirmed a high level of resistance to bensulfuron-methyl (RI, resistance index of 46). ALS activity *in vitro* was studied to investigate the mechanism conferring resistance to bensulfuron-methyl. Maximum ALS specific activity in the absence of herbicide was significantly less for susceptible biotype than for the resistant biotype. The resistant biotype showed a high level of resistance, with a resistance index (IC_{50} R/IC_{50}S) of 416 which represents a factor of 10 fold from the values of the RI at plant level. These data suggest that in the resistant biotype ALS enzyme was less sensitive to inhibition by the herbicide. This is the first study that confirms that in the species *Alisma plantago aquatica* the mechanism of resistance to the herbicide bensulfuron-methyl is target site based.

Keywords
bensulfuron-methyl, *Alisma plantago-aquatica*; sulfonylurea; mechanism resistance; herbicide resistance; acetolactate synthase (ALS); Portugal.

Introduction
Rice paddy fields in Portugal cover an area of about 30,000 ha (INE, 1993), located mainly in Tagus and Sorraia river valleys (10,000 ha), Mondego river valley (8,000 ha) and Sado river valley (7,000 ha). Nearly all rice fields are sprayed at least once with an herbicide and about 80% of total area is sprayed with multiple applications. As a result of spraying the same herbicides year after year in the same field causing high selection pressure over weed populations resistance problems arise. The first population of *Alisma plantago-aquatica* L. (Water-plantain) resistant (R) to bensulfuron-methyl was confirmed in a rice paddy field at Coruche (Sorraia river valley), which had been treated with this herbicide for six years (Calha *et al*.,1995).
Alisma plantago-aquatica belongs to the Alismataceae family (monocotyledonous). It is considered as a biannual plant (rosette-forming species), but is annual in seasonal water (Mabberley, 1996). Natural populations can be observed in Portuguese wetland areas (Moreira & Duarte, 2002) but it is also an important component of rice weed flora. Nowadays, *Echinochloa* spp. is the most important weed in Portuguese rice paddy fields followed by *Paspalum paspalodes* (Michx) Scriber and *A. plantago-aquatica*. (Vasconcelos et al., 1998)
From 289 worldwide known cases of herbicide weed resistance, affecting 172 different weed species, rice weeds represent 25%, with 30 different species with resistant biotypes. These include grass weeds resistant to propanil and quinclorac but most of them are cases of resistance to ALS-inhibiting herbicides (Heap, 2003). For example, the most affected weeds

with bensulfuron-methyl (sulfonylurea) resistance are Alismataceae [*Sagittaria montevidensis* Cham. & Schleschter (Hill et al., 1994), *Damanosium minus* R. Br. Buch. (Graham et al., 1996) and *Alisma plantago-aquatica* (Calha et al., 1995; De Prado et al., 1997; Sattin et al., 1998)] followed by Cyperaceae [*Cyperus difformis* L. and *Scirpus mucronatus* L. (Hill et al., 1994; Sattin et al., 1998)], Potendereacea [*Monochoria korsakowii* Regel & Maack (Wang et al., 1997; Park et al., 1999) and *M. vaginalis* Presl. (Kwon et al., 2000; Hwang et al., 2001)] , Scrophulariacea [*Lindernia microcantha* D. Don (Itoh et al., 1999), *L. dubia* (L.)Pennell, *L. dubia* ssp. *major L. pyxidaria* L. (Uchino et al., 2000), *Limnophila sessiliflora* Blume (Itoh & Wang, 1997)] and Litraceae [*Ammania auriculata* Willd. (Hill et al., 1994)].

Bensulfuron-methyl blocks the biosynthesis of the branched-chain amino acids valine, leucine and isoleucine through potent inhibition of acetolactate synthase (ALS; also known as acetohydroxiacid synthase E.C. 4.1.3.18). This enzyme along with others in this biosynthetic pathway participate in the biossynthsis of those amino acids (Ray, 1984).

The mechanism responsible for resistance to bensulfuron-methyl in *Cyperus difformis, Sagittaria montevidensis* (Saari et al., 1994); *Monochoria vaginalis* (Hwang et al., 2001) and *Lindernia* spp. (Tranel & Wright, 2002) is target site resistance, but until now it was not known for *Alisma plantago-aquatica.*

The aim of the study was to determine the mechanism of resistance to bensulfuron-methyl of *Alisma plantago-aquatica* resistant biotype from Sado river valley.

Material and Methods

Plant Material

Resistant biotypes of *A. plantago-aquatica* were collected from a field located in Alcácer do Sal (Sado river valley, south of Portugal), which have been submitted to applications of bensulfuron-methyl in mixture with molinate for 6 consecutive years. Susceptible biotype came from a rice field of Mondego river valley, which had been submited to low selection pressure from bensulfuron-methyl.

From those populations, seeds were picked up from mature inflorescences of 40 mature plants, before rice harvest (September 1999). After collection seeds were cleaned and stored at 4ºC until needed, in the dark inside beakers containing 300 ml KNO_3 (0,2%) (Munscher, 1936).

Herbicide

Commercially formulated bensulfuron-methyl (α-(4,6-dimetoxypyrimidin-2-ylcarbamoylsulfamoyl)-*o*-toluic acid) (Londax 60 DF, 60%, WDG, DUPONT) were supplied by Dupont Portugal (Lisboa,P). Reagents for ALS activity were obtained commercially from Sigma Chemical Co.

Greenhouse whole plant bioassays

Greenhouse studies were conducted to evaluate the level of resistance of one *Alisma plantago-aquatica* biotype to bensulfuron-methyl.

After the chilling treatment to break dormancy, seeds were germinated in KNO_3 solution (0,2%) in germination chambers (CASSEL CBT) at 15-25 ºC night/day with 16 h photoperiod, for 5 to 6 days, until the appearance of the cotyledon. Healthy seedlings, all at the same stage (2-3 linear phyllodium) were transplanted to plastic pots (10 cm height), six per pot, in shallow trays (20x26x5cm) –in a soil:peat (2:1) mixture (soil was a sandy loam pH: 7,5; K_2O: 188; P_2O_5: 100; o.m. 4,2 %, and peat - substrato Brill Tipo 3 especial 1500 PGMix). During the growing period, pots were continuously irrigated with a drip-feed system to simulate the conditions in rice paddy fields. A solution of copper sulphate (10-12 g

L^{-1} $CuSO_2$) was added weekly to shallow trays to avoid algae development (Graham *et al.*, 1996).

Herbicide dose ranged from 3.75 to 480 g ha^{-1} a.i. for resistant biotypes and from 1.875 to 60 g ha^{-1} a.i. for the S biotype. Control plants (no herbicide) were not sprayed.

Herbicide was applied to 6-leaf plants (45 weeks old) using a OPS (Oxford Spraying System) flat-fan noozle calibrated to deliver 256 L ha^{-1} under 275 kPa pressure. Shoots were harvest three weeks after treatment to assess fresh weight. The experiment was in a complete randomized design, with six replications and experiment was repeated at least twice.

ALS activity in vitro

Seedlings obtained from resistant and susceptible biotypes were grown in the greenhouse as described previously. Crude enzyme was extracted from 45 days old plant leaves. ALS activity was measured *in vitro* using a cholorometric assay based on the methodology described by Singh *et al.*, (1988). Acetolactate concentrations were determined by the method of Westerfeld (1945). A standard curve with commercial acetoin was used to quantify enzyme reaction products. Total protein content was measured using the Bradford method (Bradford, 1976). Maximum ALS specific activity (nmol acetoin mg $^{-1}$ protein h^{-1}) was determined in the absence of herbicide.

Stock solutions of herbicide were prepared with DMSO and technical grade bensulfuron-methyl, concentration range from 100 nM to 10 µM for resistant biotypes and from 1 to 100 nM for the S biotype. The herbicide concentration causing 50% inhibition of ALS activity (I_{50}) was established for each biotype.

Each biotype was assayed in triplicate and experiment was repeated at least twice.

Statistical analysis

Dose response curves for the whole plant bioassay and for ALS activity were expressed as a percentage of the control. The data were analysed by the non-linear regressing model proposed by Streibig (1988) and Seefeldt et al.*,* 1995):

$$y = c + \frac{d - c}{\left(1 + \exp\left[b(\log(x) - \log(I50))\right]\right)}$$

Where *y* predicted response, fresh weight or absorbancy (% control) at herbicide concentration *(x)*; *d* and *c* denote the upper and lower limits of plant response at zero and highest dose; I_{50} (inflexion point) is the dose giving 50% response between *d* and *c* and *b* is proportional to the slope around I_{50}.

Analysis of variance for the whole plant bioassays and ALS studies showed no significant interaction between treatment and experiment so data from the experiment were pooled and analysed. Data were fit to the model using PROC NLIN (SAS, 1987).

Results and Discussion

Whole plant bioassays

Bensulfuron-methyl resistance was established with dose-response experiments. The biomass for the untreated control was similar for both biotypes, fresh weight was 0.375 g and 0.659 g for R and S biotypes, respectively. ED_{50} values for R and S *Alisma plantago-aquatica* were 31.76 and 0.69 g $ha-^{1}$, respectively. The resistant biotype showed a high level of resistance to bensulfuron-methyl (R/S=46) compared to the susceptible biotype, expressing lack of efficacy at field level (table 1).

Table 1 Resistance indexes (RI) for *Alisma plantago-aquatica* biotypes resistant (R) and susceptible (S) to bensulfuron-methyl. Results from greenhouse assays (ED_{50}), ALS activity (IC_{50}) and protein.

Biotype	**ED_{50} (g ha^{-1})**	**RI**	**IC_{50} (ng)**	**RI**	**ALS activity (µM acetoine. mg $protein^{-1}$ h^{-1})**
R	31,76 (4,195)	46	15534 (5665)	416	62,91 (3.67) bc
S	0,69 (0,865)		37 (6,4)		21,77 (6,340)a

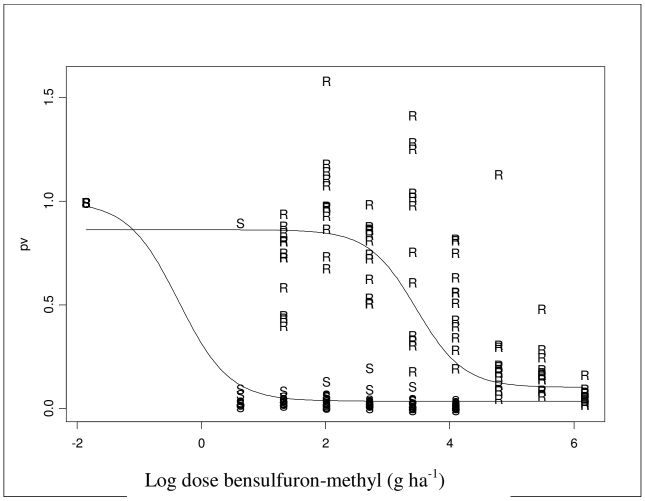
ALS activity in vivo

Maximum ALS specific activity in the absence of herbicide was significantly less (F=34.05; $p < 0.05$) for susceptible biotype (S) than for the resistant biotype (R) with values ranging from 22±6.34, and 63±3,67 µM acetoin mg $protein^{-1}$ h^{-1} for each biotype, respectively.

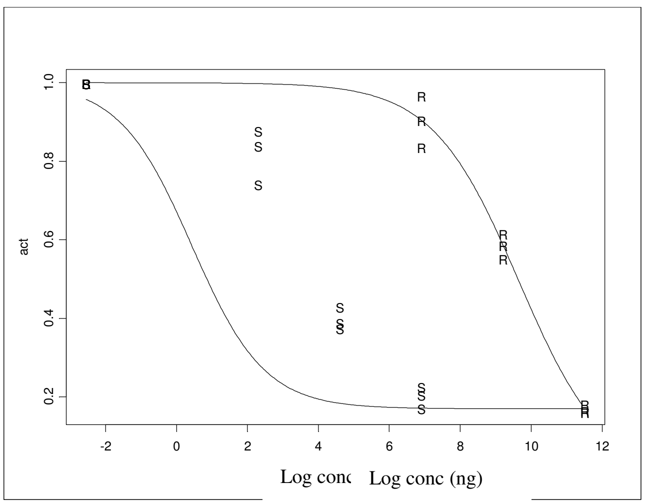
ALS activity results agree with results from whole plant assays. Resistance indexes (RI) of 416 at enzyme level, ten fold higher than RI at whole plant level (table 1), confirms that an ALS enzime less sensitive to bensulfuron-methyl was the responsible for resistance in *A. plantago-aquatica* Sado biotype. Similar results were obtained with other rice weed species from studies where the mechanism of resistance to bensulfuron-methyl involved reduction in the sensitivity of the ALS, with RI values ranging from 158 (Hwang *et al*., 2001) to 33 622.8 (Osuna *et al*., 2002).

This is the first study that confirms that in the species *Alisma plantago aquatica* the mechanism of resistance to the herbicide bensulfuron-methyl is target site based.

Finally it is reinforced the need to change weed control management in rice paddy fields in order to prevent or delay herbicide resistance and specific measures are recommended. As seeds of rice weeds are readily dispersed by water across irrigation channels, preventing the spread of resistant biotypes may be difficult. However resistant biotypes could be effectively controlled by herbicides with mode of action other than ALS-inhibition. Alternation of herbicides, applying mixtures and following integrated weed management strategies should result in sustainable *A. plantago-aquatica* management in rice reducing the risk of multiple resistance developing.



(a)



(b)

Figure 2 Dose response curves of bensulfuron-methyl for *A. plantago-aquatica* biotypes R-resistant biotype from Sado river valley and S, susceptible biotype from Mondego river valley. Fresh weight (a) and ALS activity (b) were expressed as percentage of control and data were analysed using the log-logistic model.

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EFFECT OF DIFFERENT N SOURCES IN IRRIGATED DIRECT-SEEDED RICE

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Abstract

In the Common Agricultural Policy of UE, incentives are offered to farmers who use farming practices compatible with the need to protect and improve the environment. So, it is important to search for N fertilizers which can reduce N losses, improving N efficiency. In this paper we present the results of an agronomic study conducted in Valencia (Spain) to investigate the effect of different N sources on agronomic performance and N responses for flooded rice during two consecutive crop seasons (2002-2003). Five N sources [urea (conventional in 2002 and super granules in 2003), polymer coated urea (PCU 40% N) and urea combined with three different contents of dimethyl pyrazole phosphate (DMPP at 0.5, 0.75 and 1% of the urea content)] were applied at 130 kg N ha^{-1}. The results showed that PCU 40% N applied basally before flooding was the best N source for grain yield and N recovery efficiency. The performance of urea combined with DMPP was better compared to conventional urea, whereas the behavior was similar when compared with urea super granules.

Keywords

Coated urea; DMPP; urea supergranule; flooded rice growth.

Introduction

In Valencian rice fields, constraints in the irrigation system have led to lack of control in water management, with difficulties in irrigating after incorporating N fertilizer. This delay in flooding result in elevated N losses (Fernández Valiente et al., 2000) and low N efficiency values (Carreres et al., 1996, 2000). In addition, rice growers usually apply excess N involving environmental pollution. The necessity of preserving natural resources makes essential to improve rice cropping system in terms of sustainability and limited emissions to the environment. Therefore, it is important to search for N fertilizers which can reduce N losses, improving N efficiency. The use of resin-coated urea (Inubushi et al., 2002; Carreres et al., 2003) has been reported to improve N-use efficiency in rice. The performance of DMPP, combined with ammonium sulphate nitrate, was slightly positive in comparison with conventional urea (Carreres et al., 2003). However, the behaviour of DMPP added at different contents to urea is not known in the rice field conditions of Valencia.

The aim of this study was to investigate the effect of different N sources on agronomic performance and N responses for flooded rice in Valencia.

Materials and methods

A field experiment was conducted during two consecutive crop seasons (2002-03) at the Rice Department (Valencian Institute for Agricultural Research), Sueca - Valencia, Spain.

The loamy clay soil had the following main characteristics: pH (1 soil: 2.5 KCl), 7.5; total N, 27.3 g kg^{-1}; organic C, 14.9 g kg^{-1}; cation exchange capacity, 20.3 cmol kg^{-1}; available P content, 42 $mgkg^{-1}$ and available K content, 250 $mgkg^{-1}$.
Rice was grown in flooded soil. The irrigation water, had an average NO_3^--N and NH_4^+-N content of 0.16 and 0.28 mg l^{-1} respectively and an electrical conductivity of 1.60 dS m^{-1}.
The field experiment layout was a randomized complete block design with four replicated plots per treatment. Experimental plot size was 7.0 x 15.0 m. Treatments were: urea (conventional in 2002 and supergranule in 2003), polymer-coated urea (PCU) 40% N, and urea with dimethyl pyrazole phosphate (DMPP) at the contents of about 0.5, 0.75 and 1% of the urea-N content. The PCU is a slow release fertilizer with a linear release pattern and the time of dissolution in water at 25 ºC to give 100% release of its total N content is 120 days. All N sources were applied at 130 kg N ha^{-1}. A control treatment with no N was also included. Experimental plots were laterally isolated in the field by using plastic sheets pushed into the soil. Nitrogen and P fertilizers (100 kg ha^{-1} P_2O_5 as superphosphate) were applied, 15 days before flooding, with a rotary harrow at 3 cm below the soil surface.
Every year all flooded plots were broadcast hand-sown with rice (*Oryza sativa*), *cv*. Ullal (semi-dwarf, medium grain) in 2002 and *cv* Senia (intermediate stature, medium grain) in 2003, at 240 kg ha^{-1} seed, pre-soaked in tap water. Weeds were controlled as required.
In each year, leaf N content was determined at mid-tillering (MT) (45-50 days after sowing), panicle initiation (PI) (60-65 days after sowing), and 50% heading (H) (75-80 days after sowing) stages. At the MT and PI stages, the N value was determined on the most recently matured leaf (Y leaf) and at H stage on the flag leaf of each plant. Samples were analysed for total N concentration by the Kjeldahl method (Bremner, 1965).
Data on growing duration were taken during the growth cycle and panicles m^{-2}, plant height and lodging, before harvesting. At maturity, ten stems from each plot were hand-harvested for both grain and straw N determinations.
At harvest, three 0.25 m^2 sample areas per plot were hand-harvested. Grain yield components and grain and straw dry masses were determined and averaged over the three sample areas. Individual whole plots were combine-harvested and grain yields were recorded. Moisture content of rice grain was determined in order to express the yield on 14% moisture basis. Total N uptake (kg ha^{-1}) and recovery efficiency (RE) of N fertilizer were evaluated.
Each year, traits of agronomic performance, yield components, grain yield and N responses, were subjected to a factorial analysis of variance for two factors (treatments and blocks). Mean comparisons were evaluated by the Duncan's multiple range test and orthogonal contrasts. N leaf content at different stages was subjected to a repeated measures analysis of variance with two between-factors (treatments and blocks) and one within-factor (growth stage) with the General Linear Models procedure in SAS (SAS Institute Inc., 1990). Blocks were included in the analysis but their interactions were not considered. Orthogonal contrasts were used to test differences in the analysis of the two between factors. Polynomial contrasts were analysed to determine differences over time in the analysis of the within factor and interactions. N leaf content showing a significant interaction for "stage x treatment" was subjected to a two-way analysis of variance (treatment and block) stage by stage.

Results

The content of leaf N decreased from mid-tillering (MT) until heading (H) in all N sources (Table 1). The predominantly quadratic decrease ($P<0.01$) in the level with time, had a similar trend in both years and among N sources (the interaction was not statistically significant).
In 2002 and at MT, there were not differences in N contents between PCU and any other N source treated plots. At H, the leaf N content was significantly higher ($P<0.05$) for PCU than

for any other N source. At any stage of the growth cycle in 2002, leaf N content tended to be lower in plots fertilized with conventional urea than with urea with the addition of DMPP. Moreover, this leaf content tended to increase linearly as the amount of DMPP in the fertilizer increased (this tendency was only statistically significant at H).

This trend was similar in 2003 but the higher N content in plots fertilized with PCU compared to those with conventional urea plus DMPP was already observed at MT, though was only statistically significant ($P<0.05$) at H.

In 2003, by contrast to that observed in 2002 when the applied urea fertilizer was the conventional type, the leaf N content averaged over growth stages and at any stage of the growth cycle, was not different between plots fertilized with supergranule urea form and those with conventional urea plus DMPP. Moreover, there was not any relationship between this trait and the amount of DMPP in the fertilizer.

Table 1 Effect of N sources on leaf N content during the growth cycle of rice[a]

	2002				2003			
N source	MT	PI	H	Mean	MT	PI	H	Mean
No N	3.48	2.51	2.48	2.83	2.62	2.42	2.51	2.52
Urea[b]	3.98	2.90	2.76	3.21	3.37	3.00	2.91	3.09
PCU	3.94	2.95	3.12	3.34	3.29	3.04	3.00	3.11
Urea + DMPP (1%)	4.27	3.08	2.90	3.41	3.16	2.97	2.90	3.01
Urea + DMPP (0.75%)	4.16	2.92	2.88	3.32	3.17	2.89	2.91	2.99
Urea + DMPP (0.5%)	4.03	2.90	2.87	3.27	3.10	2.93	2.83	2.96
Mean	3.98	2.88	2.84	-	3.12	2.88	2.84	-
5% LSD N source(15 D.F.)	0.37	0.31	0.17	0.19	0.24	0.19	0.15	0.17
" growth stage (36 D.F.)		0.16				0.05		
" N source x stage (36 D.F.)		0.40				0.12		

[a]Cv Ullal in 2002 and Senia in 2003. [b]Conventional urea in 2002 and supergranule in 2003
MT, mid tillering; PI, panicle initiation; H, heading; PCU, polymer coated urea; DMPP, dimethyl pyrazole phosphate; DF, degrees of freedom; LSD, least significant difference.

In 2003 and as shown by orthogonal contrasts, PCU application resulted in a higher growth duration than either the application of urea alone or with the addition of DMPP, regardless of its content. Though not statistically significant, plant height tended to be higher in plots fertilized with PCU than with any other N source. In 2002 as a result of growing a semidwarf cultivar, lodging was not observed. In 2003 with the cultivation of Senia, lodging was significantly higher for PCU application than for any other N source and there was not significant difference between urea alone and urea with the addition of DMPP (Table 2).

Table 2 Effect of nitrogen fertilizer source on agronomic performance, grain yield and N responses of rice[a]

N source	Days to maturity		Plant heigh (cm)		Lodging[c] (%)	Grain yield (t/ha)		Panicles (/m^2)		N uptake (kg/ha)		Recovery efficiency (%)	
	2002	2003	2002	2003	2003	2002	2003	2002	2003	2002	2003	2002	2003
No N	118	113	66.0	74.0	0.5	3.61	4.07	274	462	44.6	68.2	-	-
Urea[b]	121	118	72.5	95.7	23.0	6.73	7.43	385	433	82.7	116.4	29.3	39.2
PCU	123	121	75.5	98.2	72.2	8.00	7.73	453	460	105.2	125.9	46.6	46.5
Urea+DMPP 1%	122	120	75.2	96.2	34.8	7.08	7.24	388	444	78.4	113.3	26.0	36.8
Urea+DMPP 0.75%	124	119	75.0	96.2	26.0	7.14	7.13	399	447	87.1	110.1	32.6	34.4
Urea+DMPP 0.5%	122	119	74.7	95.7	21.2	7.24	7.00	403	415	86.3	106.1	32.1	31.3
5% LSD (15 DF)	2.4	2.1	3.7	3.6	15.7	0.66	0.96	46	60	9.4	17.2	7.0[d]	14.7[d]

[a]Cv Ullal in 2002 and Senia in 2003. [b]Conventional urea in 2002 and supergranule in 2003

[c]Analysis of variance conducted on data transformed to $\sqrt{x+0.5}$*. The table gives backtransformed values. In 2002 there wasn't lodging. [d]DF = 12*

PCU, polymer coated urea; DMPP, dimethyl pyrazole phosphate; DF, degrees of freedom; LSD, least significant difference.

Plots fertilized with PCU tended to have a higher number of panicles m^{-2} than uncoated-fertilizer treated plots (only significant in 2002). During the two crop seasons, no statistical differences were detected for grain weight and total and unfilled spikelets per panicle (data not shown).

As shown in Table 2, PCU application resulted in a higher grain yield ($P< 0.01$ in 2002 and not significant in 2003) than any other N source application. Though differences were not statistically significant, grain yield tended to be different between plots fertilized with urea alone and urea with DMPP. Thus, this trait was lower when urea conventional was applied in

2002 and higher with the application of urea supergranule in 2003, compared to urea with DMPP. Grain yield was not significantly affected by the content of DMPP in the fertilizer.
When conventional urea was applied in 2002, N responses increased quadratically ($P<0.05$) as the content of DMPP in the fertilizer increased up to 0.75%. By contrast, with application of urea supergranule in 2003, there were not significant differences between it and conventional urea with DMPP. Though without statistical signification, a slight relationship between the content of DMPP in the fertilizer and N responses was observed when the urea supergranule treatment was not considered in the statistical analysis.

Discussion

Differences in some plant growth traits between N sources were probably related to differences in leaf N content (Hasegawa et al., 1994; Carreres et al., 2000) throughout the phases of the growth. During the two seasons, the amount of N in the plants at heading was higher in the PCU application than in other treatment. The differences in leaf N content were high enough to result in differences of growth characters. Accordingly, the application of PCU resulted in increasing growth duration, plant height (Carreres et al., 2003) and lodging.
The delay between application of N fertilizer and flooding resulted in elevated N losses (Fernández Valiente et al., 2000). The nitrogen from PCU application is released gradually and at a slow rate throughout the growing season; for this reason very low concentrations of mineral N were maintained in the soil and N losses were reduced compared to those with conventional urea alone (Lee et al., 1999) or with the addition of DMPP. Obviously, due to the reduction in N losses and to the N release in synchrony with plant requirements, the amount of available N was higher in the PCU-treated soil with stimulation of effective plant tillers development without reduction of plant N concentration, in comparison to application of any other N fertilizer. Moreover, at end tillering, N from PCU was totally released and plant N concentration was higher in plots fertilized with PCU, unless its higher number of panicles m^{-2}, than with conventional urea with or without the addition of DMPP. Consequently, the rest of yield components, as total and unfilled number of grains per panicle and grain weight, were similar for all N sources. In accordance with the yield components behavior, PCU was the best N source for grain yield (Lee et al., 1999), increased plant N uptake and improved N recovery efficiency (Carreres et al., 2003). The good performance of this fertilizer is probably due to its ability to slow down N release (Inubushi et al., 2002), minimize N losses (Ueno and Yamamuro, 1996) and increase the persistence of available N in soil with consequent improved plant growth.
The permanence of NH_4^+ in soil for a longer period when urea was applied in combination with DMPP, reduced N losses (Lee et al., 1999) and, therefore, the leaf N contents throughout the growth stages were higher in comparison to those with conventional urea alone. However, the differences were not high enough to result in significant differences of growth characters, yield components or grain yield. Nevertheless, according to Carreres et al. (2003), a slight good performance was confirmed as the effectiveness of DMPP, in terms of plant N uptake and N-recovery efficiency, was quadratically related to the amount of DMPP in the fertilizer. The response to amount of DMPP was significant up to 0.75%.
Ammonia volatilization losses are very small if little fertilizer N is dissolved in the floodwater after fertilizer placement (Mikkelsen et al., 1978). The application of urea supergranules slow down of the process of dissolution, decrease the ammonium volatilization and results in high availability of N to the crop (Sahu and Mitra, 1989). Consequently, the observed inferior performance of normal prilled urea in comparisson with urea + DMPP in 2002, is in marked contrast to the similarity between the last source and urea supergranule in 2003. The observed

superiority of urea supergranule over normal urea is in accord with the observations, in terms of grain yield, N uptake and recovery, of Mishra et al. (1999).

Conclusions

Our results indicate that PCU applied basally before flooding was the best N source for grain yield and improved the recovery efficiency. The nitrification inhibitor DMPP showed a superior performance compared to normal prilled urea. The application of urea supergranule has shown an encouraging behaviour.

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CLIMATE INFLUENCE OVER AIR CONTENT OF *PYRICULARIA ORYZAE* CONIDIA AND SYMPTOMATOLOGY OF RICE CROP IN SOUTHERN SPAIN

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Abstract
A close relation between the climatological data and the number of *Pyricularia oryzae* conidiae trapped in the air was detected under the trials conditions. Conidiae were trapped from middle July until harvest, in middle October. Thus, it is confirmed that this technique for trapping spores, together with the use of thermohigrographers, are useful tools for carrying out epidemiological studies. In 2002 and 2003 campaigns two major moments of conidiae presence in the air were observed. These phenomena were preceded by an increase of the relative humidity, reaching values over 95%, and average temperatures over 26ºC.
First symptoms in leaf appeared at the end of July, when the crop was in the phenologycal stage of middle tillering. Injuries on the leaf and the nodule zone provided with conidia to infect the panicle during the ripening phase (first two weeks of October). The relation between the percentage of leaf viable injuries (conidia producers) and the total number of lesions detected in the plant fits to an exponential decreasing regression. The relation between the percentage of node and panicle damages fits to an exponential increasing regression.

Keywords
Rice; blast; weather effects; spore catch.

Introduction
Blast disease of rice, caused by the fungus *Pyricularia oryzae* Cav. (teleomorph. = *Magnaporthe grisea*) is endemic to all European rice growing areas. The majority of these areas have Mediterranean climate, characterised by hot and dry weather conditions for most part of growing season, and unfavourable for blast disease development (Ciheam, 1996). The ability of the pathogen to infect rice at different stage of growth, and its adaptation to any rice ecosystems, are indications of the plasticity of *P. oryzae* to changing environments (Bonman et al., 1992; Teng, 1994).
On the other hand, forecasting technique for rice blast disease progress usually emphasize the importance of aerial spore density as an essential predictor because the number of infected sites on plants in the field is closely related to the aerial spore density (Asai et al., 1976; Kim 1982; Kim et al., 1989; Ono 1965; Ou 1985). Therefore, accurately measuring the amount of airborne inoculum is crucial for developing and applying blast forecasting systems, in the future and under our environmental conditions.
The objectives of these experiments were studying the air content of spores and the climatological conditions that are implied in the infection process, as well as monitoring the evolution of the symptoms caused by *Pyricularia oryzae* in the rice crop.

Materials and methods

During 2002 and 2003 campaigns the trials were established in the farm *Sartenejales* (located at La Puebla del Río, Sevilla) on clayey soil. The appropriated tillages for inducing the infection on the rice crop were carried out: late seeding date, high plant density and high N-fertiliser rate.

Puntal cultivar seeding was carried out in a seedbed between the 4th and 10th of June. Seedbeds were placed near the trial plot, and 20 days later the plantlets were transplanted into it. The trial plot consisted of a randomised block with three replications. Elemental plots were constituted by three furrows of 6 m, separated by 25 cm., and the distance between plants was 20 cm. Due to their high susceptibility to the pathogen, on each replication and campaign 4 lines of cultivar *Baixet* and 2 of cultivar *Maratelly* were included as inoculum source. *Baixet* shows a high susceptibility under the conditions of *Las Marismas del Guadalquivir*, and *Maratelli* is internationally known for its high susceptibility to *Pyricularia oryzae* (Roume et al., 1997). The whole trial was surrounded by an edge of 5 furrows of cultivar *Baixet*, in order to perform as an additional inoculum source.

N-Fertilisation was carried out twice. First application was carried out by engine the 10th of May as basal fertilisation. 100 Kg ha^{-1} of nitrogen (urea 46%) were applied. The second one was carried out by hand the 1st of August (ammonium sulfate, 21%) and completed the intended 250 Kg. ha^{-1}.

The air content of spores of *Pyricularia oryzae* was detected by collectors with adherence tramps placed at two levels (20 and 70 cm over the ground). Tramps consisted of microscope slides covered with glicerine (Ono, 1963). Spores recount started two days after transplanting the plantlets, and was maintained with a frequency of 2 days. The weather data were continuously registered by digital thermohygrographers placed 90 cm over the ground, which registered data of moisture, relative humidity, and maximum and minimum temperatures.

Bearing in mind that in this area the first spots, as it was observed in precedent campaigns, appeared about middle July, from the 15^{th} of July samples begun to be taken every 10 days. 60 plants per replication were taken, which were packed individually and classified by sampling date. Once in the lab, small pieces of the affected tissues (leaf, node, panicle neck and ligule) were surface disinfected in a 1:9 (v/v) solution of household bleach (5.25% sodium hypochlorite) and plated onto media. Disinfected pieces of the affected tissues were placed in moist chambers and incubated at 25 ± 1ºC and alternating 12 h periods of fluorescent and NUV light of about 2.500 lux for two days. To obtain monoconidial cultures of *P. oryzae*, individual spore from a single conidiospore were identified with a stereomicroscope on sporulating spots in moist chamber and aseptically streaked onto a thin layer of water agar (WA). After six days under the same growth condition as previously described, germinated conidias were cultured on rice-polish agar (RPA) medium. Growth and sporulation of the pathogen was obtained by placing the Petri dishes under incubation conditions for seven days.

Finally, the percentage of viable lesions (sporulated) among the sampled lesions of each part of the plant was calculated for each sampling date. Node zone includes ligule and node; panicle zone includes panicle neck, raquis, pedicels and grains. With these data we can describe the symptomathology tendence of *Pyricularia oryzae* in *Las Marismas del Guadalquivir*.

Results and discussion

First symptoms of *Pyricularia oryzae* appeared in the leafs, when the crop was in middle tillering stage. Spots used to appear in whitish or green-greyish colour, surrounded by darker

greenish edges. As the damage developed, the spots turned gradually into whitish-greenish colour, with brown-reddish necrotic edges.
Infections at the panicle base are usually the most harmful ones, as the may cause, as well as those infections in the nodes do, a decrease in the grain weight or, even, the apparition of blank and erected panicles with empty grains in case of early and severe attacks.

Air conidiae content
Conidia of *P. oryzae* were trapped from middle of July until the end of crop season. The mean temperature, relative humidity and mean number of conidia trapped per cm^2 are represented in Figure 1, 2. In the mentioned figures two rises of relative humidity were registered along the crop cycle due to cold and humid winds of atlantic precedence from south-west direction. These increases of relative humidity, together with the registered decrease of temperature in middle August, allowed the apparition of dew and fog, which provide with optimum conditions for the pathogen infection. Keeping in mind that first leaf symptoms appeared at the end of July, when the crop was at middle tillering phenological stage, we can consider the mentioned primary infection and the favourable climatic conditions as responsible of the rise of the air content of spores and its propagation in time.
In Figure 1a and Figure 1b an outstanding period (03/08/02 – 10/08/02) characterised by a rise of relative humidity, reaching levels over 95%, can be observed. The average temperature ranged around 27ºC in both locations during the mentioned period. These relative humidity and temperature values have favoured substantially the expulsion of conidiae, as described by Leach, CM 1980. Thus, values of trapped conidiae in air over 0,7 conidiae/cm^2 were registered in this period. The number of spores decreased as the season developed, reaching a second maximum of about 0,3 conidiae/cm^2, in the last two weeks of September. In this time, rice was at 80-90% of heading, under the conditions of the trial.
In 2003 campaign (Figure 2a and Figure 2b) the maximum level of trapped air conidiae was 0,8 conidiae/cm^2 recorded between 20 and 25 of August. Before this period an increase of relative humidity to 95% and an average temperature around 26ºC took place. As for 2002 campaign, the number of spores decreased reaching a second maximum of 0,5 conidiae/cm^2, between 25 and 30 of September. This period was preceded by optimum conditions to cause infection. These spore levels, together with favourable climatic conditions, were responsible for reaching the maximum symptomathology due to *Pyricularia oryzae* in early October, during the ripening phase, causing damages in different air parts of the plant.

Lesions evolution
The evolution of the percentage of infected sites by *Pyricularia oryzae* during the crop cycle showed similar tendencies in both campaigns (Figure3). Thus, it was observed a 72 and a 39% of leaf lesions in the first samples of 2002 and 2003 campaigns, values that decreased in a decreasing exponential regression as the plant growing developed. Infections in node zone begun in late August reaching values of 7 and 3 % in the first samples of both 2002 and 2003 campaigns, and developed in an increasing exponential regression until middle October. Panicle zone infections begun in late August with first values that did not reach 2% in both years, and increased exponentially to values over 45-50 %.

Conclusions
A close relation between the climatological conditions and the number of *Pyricularia oryzae* conidiae trapped in the air was demonstrated, as well as the relation between the air conidiae content and the development of the symptomatology caused by *Pyricularia oryzae*. This fact shows that, under the trial conditions, the use of spores traps with horizontally exposed glass

slides to sample conidiae can be efficient for carrying studies on epidemiology. By this trap the conidial deposition take place by sedimentation and impactation (Pinnschmidt *et al.* 1993).

Lesions on leafs, auricles and ligules provided with conidiae to infect the panicle, a fact that confirms the references given by Hori, M. 1963; Ono, K., & H. Suzuki,1960 and Thomas, K.M.1930. On the other hand, it was observed a fast increase of conidiae during the second half of August (heading stage), as Kato, et al. 1970 indicated.

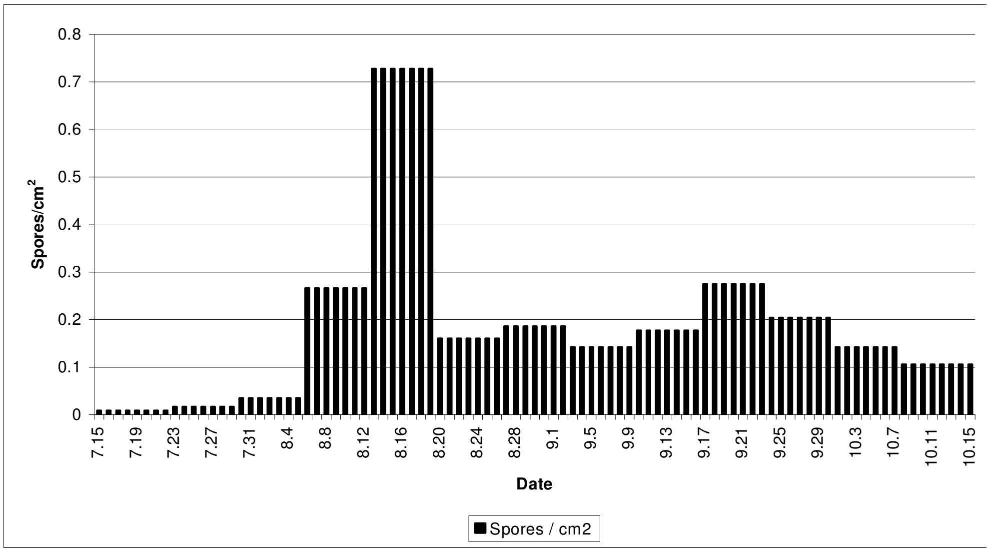

Figure 1a Evolution of the number of trapped *Pyricularia oryzae* conidiae per cm^2 (Sartenejales, 2002).

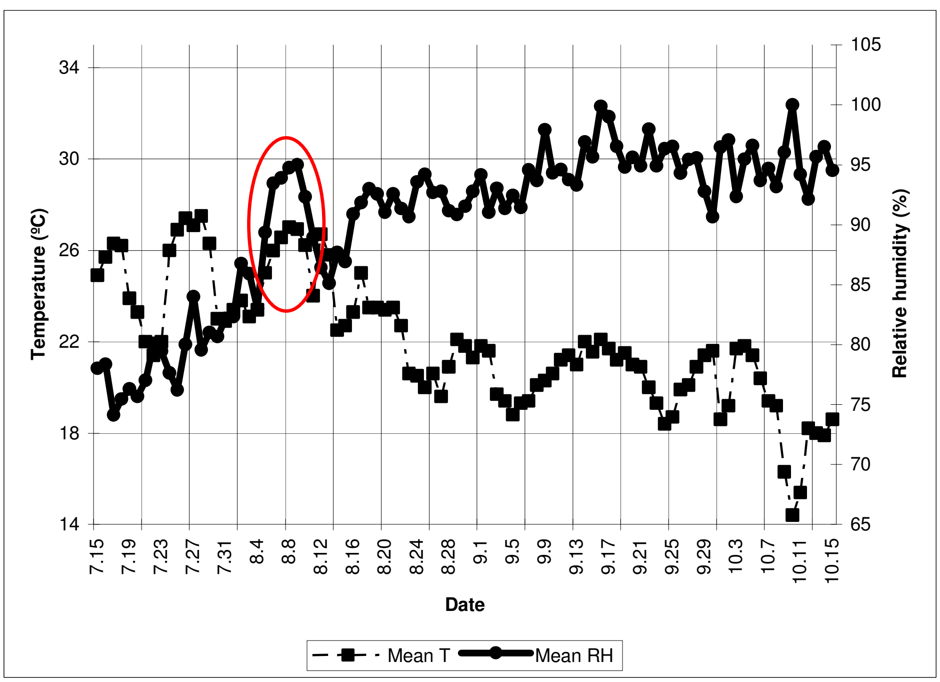

Figure 1b Average temperature and relative humidity evolution (Sartenejales, 2002).

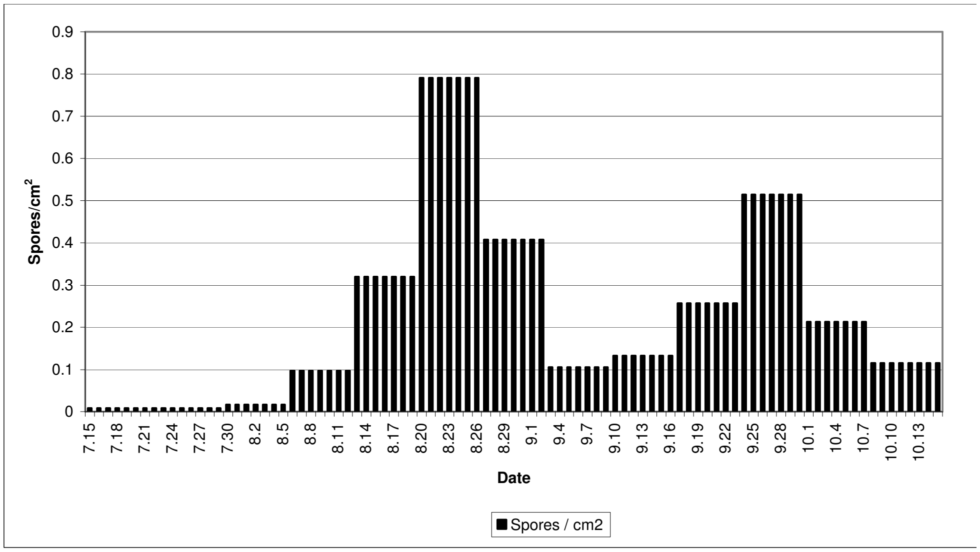

Figure 2a Evolution of the number of trapped *Pyricularia oryzae* conidiae per cm^2 (Sartenejales, 2003).

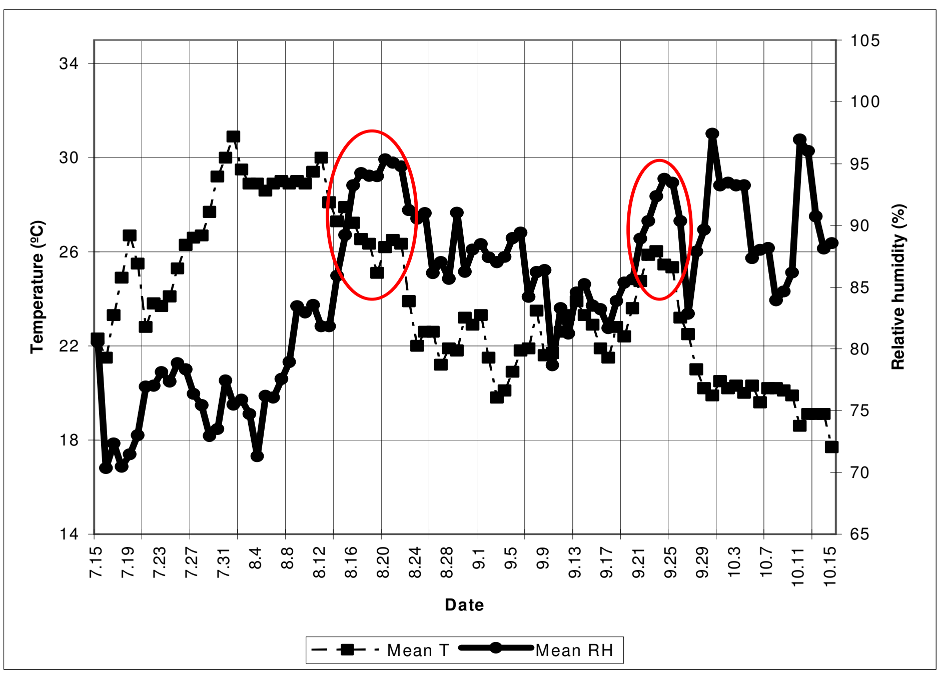

Figure 2b Average temperature and relative humidity evolution (Sartenejales, 2003).

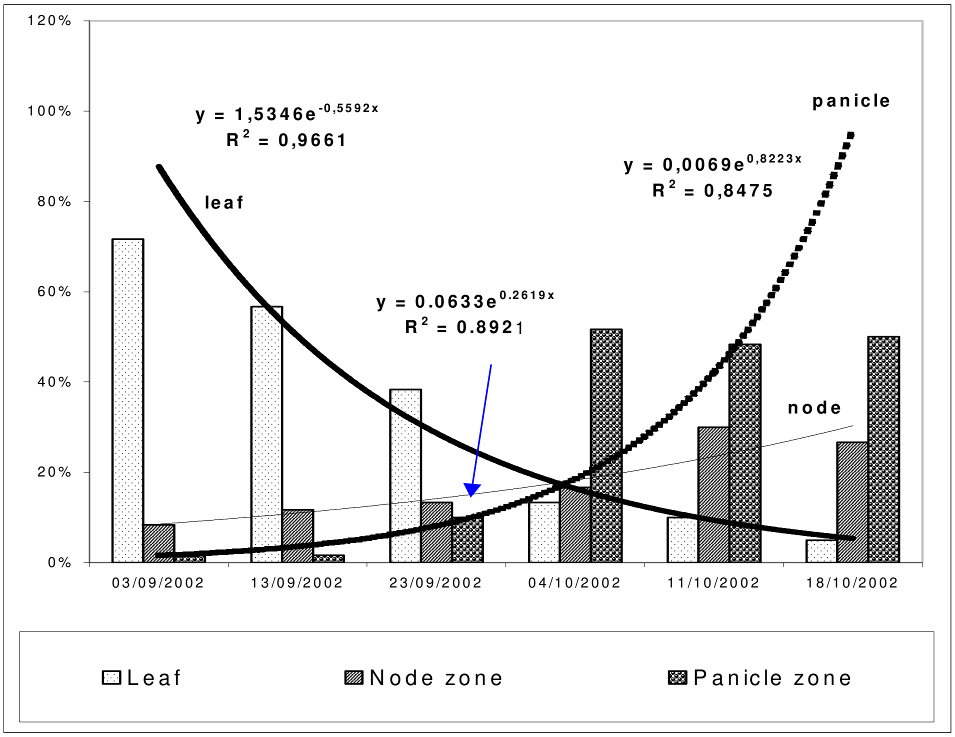

Figure 3a Lesions percentage evolution per infected sites. (Sarternejales 2002).

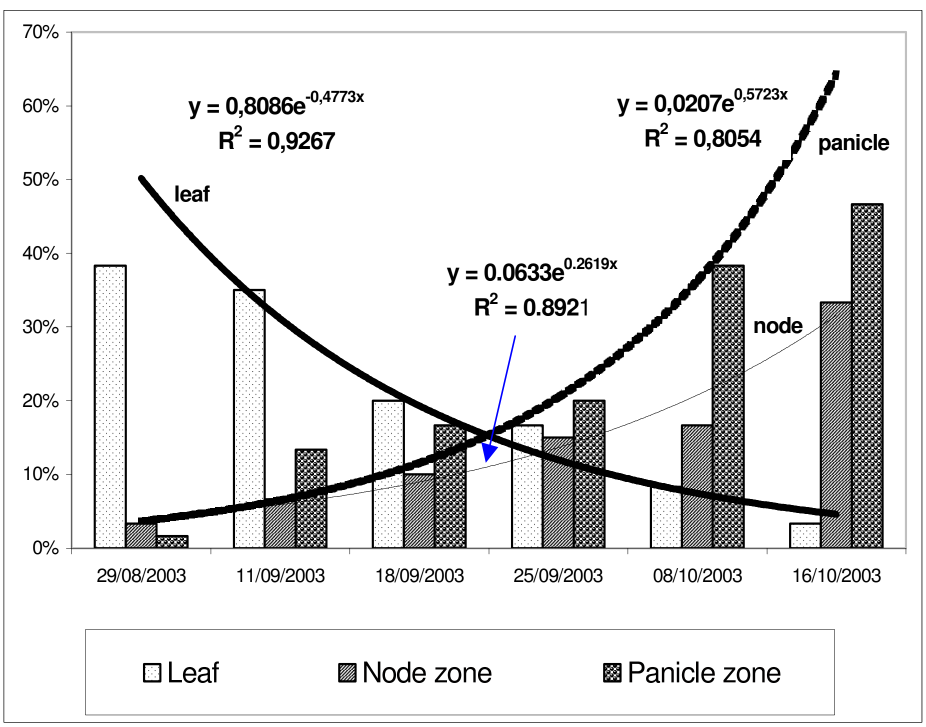

Figure 3b Lesions percentage evolution per infected sites. (Sarternejales 2003).

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HOW TO INCREASE N-USED EFFICIENCY ON FLOODED RICE

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Abstract

A nitrogen trial with four strategies has been developed in two Ebro delta rice fields with different soil textures: sandy and clayish soil. The tested strategies were: 1) standard fertilitzation: the most widely used fertilizer 175 and 150 Kg N/ha for sandy and clayish soil respectively; 2) 150 KgN/ha with 50 Kg N/ha at panicle initiation (PI), 3) 120 kg N/ha with urea as basal and ammonium sulfate as split, and 4) 120 UF N/ha with a nitrification inhibitor (Entec46). In sandy soil, and Bahia cultivar, even without significant differences, 150 UFN/ha with 50 KgN/ha at tillering and 50 KgN/ha at PI showed the best strategy. Reducing N input lower than 150 KgN/ha led an economical reduction of 122.62 €/ha. In clayish soil and Bahia cultivar, 120 kgN/ha can be enough with two top dressing: at tillering and PI. The Minolta clorophylle meter (model SPAD 502), became a profitable tool; especially remarkable has been the significative correlations between yield and SPAD level during reproductive period.

Introduction

Nitrogen (N) is the highest determinant macronutrient of the yield. Fertilizer-N used efficiency of irrigated rice is relatively low, due to rapid losses of applied N through volatilization (De Datta, S.K. and Buresh, R.J. 1989; IRRI, 1997; Murayama, 1978) and denitrification (R.J. Buresh ans D. K. De Datta, 1990; ENR, 1999) in soil-flooded water systems. The used methodology to estimate nitrogen losses are costly from the economic and technical point of view (De Datta, S.K. and Buresh, R.J. 1989). Fertilizer N is relatively inexpensive and deficiencies can result in substantial yield reductions. Producers are inclined to manage fertilizer N to minimize the risk of deficiency, which can lead to excessive fertilizer applications; on the other hand fertilizer nitrogen is increasingly recognized as the source of contamination of groundwater. An appropiate N management must be able to maximize the N absortion in critical developing stages and assure that N applied is used to produce grain, and hence to minimize the N losses.

Rice cultivation in Catalonia is located in the Ebro delta (North-East Spain) inside the Natural Park, occupies 20749 ha, with an average yield 6200 Kg/ha (DARP, 1999). From 2003 important restrictions in N aportations (120 KgN/ha for medium grain and 130 KgN/ha for long grain), must be adapted by rice farmers in order to get economical subsidies (BOE, 2001). Soil textures in Ebro delta are widely differents, from sandy to clayish soils; rice farmers usually apply around 140-180 KgN/ha (Catala, MM and Reguant, F, 1994); and N contributions of 200 Kg/ha in sandy conditions often used (2/3 at basal application + 1/3 at midtillering). In clayish conditions farmers usually applied 150 KgN/ha before seeding.

No previous work has been done in our conditions and with our cultivars to increase nitrogen efficiency.

The objectives of our study were: i) to evaluate the economical impact of the restriction in the N input and ii) to study alternative N management in order to increase productivity.

Material and methods

Field experiments were conducted in farmers fields located in Ebro delta from 2001 to 2002 in two locations (Table 1), location 1 with sandy soil and location 2 with clayish soil. A medium grain japonica cultivar Bahia was used.

Table 1 Summary of soil propierties and the most relevant growing data.

		Soil properties							Growing data				
Location		Sand (%)	Clay (%)	CEC	EC	OM (%)	P_2O_5 (ppm)	K_2O (ppm)	BF KgP_2O_5/ha	BF KgK_2O/ha	S-IP (days)	IP-M (days)	Total cycle (days)
1	2001	98.6	5.4	1.6	1.1	1.2	13.5	31.3	75	150	63	84	147
	2002										73	59	132
2	2001	4.7	21.1	9.1	2.6	3.1	31.5	141.1	69	91	64	79	143
	2002										68	78	146

CEC: cation exchange capacity (meq/100 gr); EC: electric conductivity 1:5 dS/m; OM: organic matter; BF: Basal fertilizer; S: Seeding; M: maturity

Nitrogen management treatments were arranged in a completely randomized block design with three replications. Plot size was 800 m^2. Plots were separated by levees and canals to provide appropiate and independent water management. Soil analysis was determined by plots separately. Treatments description are presented in Table 2. Treament 1 was the standard fertilizer management used by rice farmers according to soil conditions. Treatment 2 is the alternative option, with N restriction for sandy soil and an appropiate N management (two split applications). Both treatments 3 and 4 applied 120 Kg N/ha, but urea and amonium sulfate were used to treatment 3 and an nitrification inhihitor (Entec 46) to treatment 4.

Table 2 Description of the treatments according to the location.

	Location 1				Location 2			
Treatment	Total N applied (Kg/ha)	BS	MT	PI	Total N applied (Kg/ha)	BS	MT	PI
1	175	117	58		150	135	15	
2	150	50	50	50	150	100		50
3	120	40	40	40	120	40	40	40
4	120	40	80		120	40	80	

N aplication as basal (BS), at midtillering (MT) and panicle initiation (PI). Fertilizer N was applied as basal as urea and for top dressing as ammonium sulfate for the treatments 1,2 and 3. For the treatment 4 we used an nitrification inhihitor (Entec 46)

Fields were flooded 4 days after fertilizer incorporation, and the soil was then kept submerged with deep of 5-10 cm until 15 days before maturity when fields were drained. Weeds, insect and diseases were controlled to avoid yield losses.

Plant density and panicle density were detemined by throwing 15 times a 50x50 cm^2 per plot during a zig-zag transect. Diseases incidence and severity was monitored at milky stage by throwing 15 times a 50x50 cm^2 per plot during a zig-zag transect.

Foliar content of macro and micro nutrients was determined at tillering stage; at the same stage the biomass was determined. The content of macro and micro nutrient of the harvested grain was also determined.

In 2002 the Minolta chlorophyll meter (model SPAD-502) was used for measurements on the 30 topmost fully expanded leaves per plot. Initial measurements were made at early tillering stage and continued at 7 days intervals until flowering. Grain yield was determined by harvesting individual whole plots at field maturity. After harvest an economical study was done.

Results and discussion

When non statistical differences were found between treatments, data were not presented: nutrient foliar content at tillering, nutrient grain content at maturity, diseases severity (*helminthosporium*, *fusarium*...).

The most relevant results are presented in Tables 3 (location 1) and 4 (location 2).

Table 3 The most relevants results of the location 1 (sandy soil)

Treatment	Total N applied (Kg/ha)	Plant density (n/m^2)	Panicle density (n/m^2)	Plant height (cm)	Biomass fresh weight (gr/m^2)	Biomass dry weight (gr/m^2)	N foliar content	Pyricularia incidence (%)	% whole grains	Yield (Kg/ha)	N grain content
1	175	289.8	357.0	98.6 a	2299.3 a	344.7 a	3.5 ab	0.0	60.7	6637.3	1.2 b
2	150	304.6	345.0	94.3 bc	1955.6 a	297.7 ab	3.4 ab	0.0	60.3	6852.6	1.3 a
3	120	284.1	313.2	92.4 c	1608.2 b	259.0 b	3.1 b	0.0	58.5	6371.9	1.3 a
4	120	313.2	342.8	96.0 ab	2090.0 a	331.7 a	3.5 a	0.0	59.9	6436.1	1.2 b
sl	ns	ns	ns	0.007	0.004	0.03	0.10	ns	ns	0.07	0.0003

sl: significance level; ns: no significant.

Table 4 The most relevants results of the location 2 (clayish soil)

Treatment	Total N applied (Kg/ha)	Plant density (n/m^2)	Panicle density (n/m^2)	Plant height (cm)	Biomass fresh weight (gr/m^2)	Biomass Dry weight (gr/m^2)	N foliar content (%)	*Pyricularia* incidence (%)	% whole grains	Yield (Kg/ha)	N grain content (%)
1	150	270.2 ab	341.6	100.0	3640.4 a	508.5 a	3.3 a	9.2	56.4	5646.3 b	1.2
2	150	285.2 a	369.3	100.8	2432.4 b	387.4 b	3.0 ab	1.7	56.8	6339.7 a	1.1
3	120	265.9 ab	349.4	98.3	2455.6 b	423.6 ab	2.8 b	2.8	56.1	6736.2 a	1.2
4	120	232.4 b	359.5	102.4	2527.5 b	401.8 b	3.3 a	3.7	56.7	6389.2 a	1.2
sl		0.05	ns	ns	0.002	0.06	0.02	ns	ns	0.001	ns

sl: significance level; ns: no significant.

Non statisitical differences appeared in terms of plant and panicle density between treatments in sandy conditions. In location 2 (clayish soil) statistical differences were found for plant density, although treatment 1 (standard) did no differ from the rest of treatments. Bahia cultivar has an important tillering ability, so the plants are able to compensate a different plant density, therefore no differences in terms of panicle density were showed.

Plant height did no differ significantly in location 2, but shorter plants appeared in location 1 for the treatments 2 and 3.

In terms of biomass (fresh and dry weight) at maximum tillering, treatment 1 offered the highest weight in both location, but only location 2 presented statistical differences.

In location 1, lower biomass appeared when the N aportation was 120 KgN/ha (treatment 3), but no differences appeared when the same N was applied with an nitrification inhihitor (Entec 46).

No differences appeared in N foliar content between treatment 1 (standard) and the rest of treatments in location 1; but in location 2, treatment 1 only differed from treatment 3.

Even with fungicides treatments, in location 2 highest severity was found in standard treatment although without significant differences.

No differences between treatments for the milling grain in both locations were found. In location 1 (sandy conditions) the yield for standard N aportation did not differ from the rest of the treatments but highest yield was obtained by treatment 2, however in location 2 (clayish soil) the lowest yield was obtained for the standard N aportation. N content in the grain was very similar between treatments.

In 2002 an important relationship was found between SPAD values and yield at the reproductive stage (Table 5); while no relationship was found between the level of SPAD and yield at the vegetative stage.

Table 5 Correlation between SPAD values and yield

Location	Panicle initiation	Booting stage	Heading
		Correlation R^2	
1	Y=241.99x –1706.7	Y=45.23x+5032	Y=85.73x+3208.9
	0.71	0.65	0.73
2	Y=57.70x+4303.7	Y=51.33x +4470.8	Y=77.50x+3245.8
	0.63	0.66	0.84

According to economical results (Table 6), the cheaper fertilizer option (cost of product + application) was treatment 1 in the location 2, and treatment 3 in location 1 althought with slightly difference. But the best option for rice farmer was treatment 2 in sandy soils and treatment 3 in clayish soil.

Table 6 Economical results. Location 1 and 2

Treatment	Locations	Cost of the treatment (fertilizer+aplication)	Benefit (Kg/ha x €/kg)	Benefit-Cost
1	1	111.03	1915.75	1804.72
	2	96.33	1628.87	1532.54
2	1	125.45	1976.88	1851.43
	2	105.59	1828.90	1723.31
3	1	109.38	1838.19	1728.81
	2	109.39	1943.31	1833.92
4	1	133.56	1856.71	1723.15
	2	133.57	1843.18	1709.61

Conclusion

In sandy soils (location 1), rice farmers standard N application was around 175 Kg N/ha (75% as basal application and 25% at tillering), new reglamentation force rice farmers to reduce this apportations to a maximum of 120 Kg N/ha. With this trial we could demonstrate that 120 Kg N/ha are enough to achieve the same yield; but with 150 Kg N/ha (1/3 as basal, 1/3 at tillering and 1/3 at PI) the obtained yield was higher than with 120 Kg/ha and as well as income.

In sandy conditions the contribution of Entec46 increases lightly the yield, but the highest cost did not compensate yield. The use of nitrification inhibitors, in general, did not increase yield when water management was appropiate. By reducing N input lower than 150 KgN/ha led to an economical reduction of 122.62 €/ha.

In clayish soil (location 2), rice farmers standard N application was around 150 Kg N/ha (90% as basal application and 10% at tillering), 120 Kg N/ha were enough to get the same yield with Bahia cultivar, but it is very important to split the N dose (1/3 as basal, 1/3 at tillering and 1/3 at PI).

Any rice grower in Ebro Delta split nitrogen at panicle initiation moment, treatments 1 and 2 received the same N apportations (150 Kg/ha), but yield obtained when N was applied at PI moment increased in 12 %.

The nutrient status during reproductive stage has been correlated with yield, new research must be done to deep in this point.

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STRATEGIC CONCEPTS FOR HERBICIDE RESISTANCE MANAGEMENT IN CALIFORNIA RICE

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Abstract

Weeds adapted to the water-seeded and continuously flooded system of California have now become the most serious production problem. Although flooding provides considerable weed suppression, some problematic weeds such as the watergrasses (*Echinochloa oryzoides* (Ard.) Fritsch and *Echinochloa phyllopogon* (Stapf) Koss.), sedges (particularly *Schoenoplectus mucronatus* (L.) Palla syn. *Scirpus mucronatus* L. and *Cyperus difformis* L.), and broadleaf weeds (*Ammannia auriculata* Wild. and *A. coccinnea* Rottb.) are only partially controlled by flooding and thus weed control is strongly herbicide-dependent. Herbicide resistance, resulting from the continuous use of a few available herbicides, is threatening the viability of chemical control, and restrictions to herbicide registration limit the availability of new compounds. Innovative concepts for integrated weed management in California rice will rely heavily upon breaking weed cycles through rotation of stand establishment methods, alternating herbicides modes of action, as well as effective crop interference to reduce herbicide use.

Introduction

The *Echinochloa* complex, which includes the most serious weeds of California rice, has evolved resistance to all available grass herbicides (molinate, thiobencarb, fenoxaprop-ethyl, cyhalofop-butyl, bispyribac-sodium, bensulfuron-methyl, and clomazone) except one (propanil) (Fischer et al., 2000a). Resistance in *Echinochloa* spp. is widely distributed throughout the California rice growing area. Gene flow studies with *E. phyllopogon* have demonstrated that resistant seeds have spread from a single source and that seed dispersal is a relevant source of field reinfestation (Tsuji et al., 2003).

Long-term field experiments have shown that watergrass has a dynamic seedbank susceptible of being quickly reduced by mixtures and alternation of herbicides representing different chemistries and modes of action, particularly when a second application was made to prevent seed production by escapes and propanil was included in the program (Fischer & Hair, 2002). Glufosinate, used in conjunction with glufosinate-resistant rice, can also be a useful tool in a diversified herbicide use program in red-rice-free CA. Herbicide synergism is a powerful tool for herbicide-resistance management. The best herbicide treatment to control herbicide-resistant *Echinochloa* spp. is a synergistic combination of bispyribac-sodium and thiobencarb, which allows using bispyribac-sodium at 1/3 its normal rate, thus greatly reducing selection pressure for this herbicide (Fischer et. al, 2004). Thiobencarb on its own has no effect on these grasses (susceptible biotypes) at this rate and timing.

Using cytochrome P-450 monoxidase inhibitors, herbicide metabolism has been detected as a mechanism of resistance in certain cases (Fischer et al., 2000b). Target-site and metabolism-based resistance has resulted in complex cross-resistance patterns involving different herbicides and species (Osuna et al., 2002). Given this complexity, it is clear that the sole reliance on the use of alternative selective herbicides to manage herbicide-resistant weeds in CA rice would be a risky strategy that could lead to the further evolution of resistant biotypes. Complex resistance patterns and non-specific mechanisms that confer resistance to herbicides even before weeds are exposed to them, together with recurrent immigration to fields prompt for integrated long-term solutions involving non-chemical options.

Crop-based strategies for weed suppression are viable options. The water-seeded and continuously flooded rice in CA is a competitive crop capable of preventing the establishment of weeds if their emergence is briefly delayed (Gibson et al., 2002). There is variability for rice competitiveness with *Echinochloa* spp. and traits for competitiveness have been identified using field experiments and path analysis approach (Gibson et al., 2003). Currently, genetic correlations between traits and competitiveness and the genetic bases of those traits are being studied in order to assist in the breeding of more weed suppressive and weed tolerant rice cultivars.

Rice in CA is usually, water seeded, continuously flooded, and not-rotated. Alternating the way rice is established, such as when rice is seeded on a dry seedbed, modifies the weed recruitment environment and causes dramatic shifts in weed populations. Understanding the critical environmental determinants of seed germination and seedling growth of common weed species in rice cropping systems and identifying processes associated with weed species recruitment among rice establishment systems will enhance the ability of growers to manipulate weed species frequencies for managing herbicide-resistance.

A modeling exercise integrating this knowledge with a quantification of the relative competitive ability of weed communities associated with different rice establishment methods can be used to identify cost benefits associated with herbicide applications among rice establishment methods.

Procedure

A large field experiment was established at the Rice Experiment Station near Biggs, California, to test the following alternative establishment techniques to water-seeded rice:

1. drill seeding
2. stale seedbed
3. zero tillage prior to rice planting.

To quantify:

- weed species shifts
- competitive abilities of various weed communities
- the effect of no soil removal coupled to a stale-seedbed technique to deplete weed seed from surface soil, and
- the efficacy of herbicide options unique to each rice establishment system.

Rice Establishment Systems

System	Crop establishment	Herbicides
Conventional water-seeded	Weed emergence flush: May 14 and 26 Permanent flood: June 2 Water-seeded: June 4	Pre-plant: glyphosate 1.3 kg/a.e./ha
Conventional drill seeded	Drill-seeded: May 12 Flushed: May 13 and 24 Permanent flood: June 5 (rice height 5 in.)	clomazone: 0.672 kg/a.i./ha, ½ rice leaf growth stage propanil: 6.72 kg/a.i./ha, 3-4 rice tiller growth stage
Water-seeded following a stale seedbed	Weed emergence flush: May 14 and 26 Permanent flood: June 2 Water-seeded: June 4	Pre-plant: glyphosate 1.3 kg/a.e./ha Post-emergence: propanil: 6.72 kg/a.i./ha, 3-4 rice tiller growth stage
No-till water-seeded following a stale seedbed	Weed emergence flush: May 14 and 26 Permanent flood: June 2 Water-seeded: June 4	Pre-plant: glyphosate 1.3 kg/a.e./ha
No-till drill-seeded following a stale seedbed	Weed emergence flush: May 14 and 26 Drill-seeded: June 3 Permanent flood: June 23	Pre-plant: glyphosate 1.3 kg/a.e./ha Post-emergence: pendimethalin/cyhalofop-butyl: 1.12 kg/a.i./ha / 0.27 kg/a.i./ha Application date: June 15 (6 d after rice emergence)

Note: *Crop oil concentrate (1.25% by volume) was added to applications of cyhalofop-butyl and propanil. Ammonium sulfate (2% by volume) was added to applications of glyphosate*

Results summary

Weed recruitment and control

Weed species recruitment differed greatly between the water-seeded and drill-seeded systems. Weed communities in the water-seeded treatments were dominated by sedge (*Scirpus mucronatus* and *Cyperus difformis*) and broadleaf (*Ammannia* spp., *Bacopa* spp., *Heteranthera limosa*, and *Sagittaria montevidensis*) weed species. Alternatively, the weed communities in the drill-seeded treatments were dominated by grass (*Echinochloa* spp. and *Leptochloa fascicularis*) weed species. However, pendimethalin/cyhalofop provided very good control of these grass species. Pendimethalin is a mitotic inhibitor, which is a different mechanism of action (MOA) compared to other rice herbicides commonly used in California. Therefore, periodic use of drill seeding and applications of herbicides with alternative MOA, such as pendimethalin, may provide a means to periodically reduce the seed bank of grass species that may be resistant to other commonly used herbicides.

Alternative rice establishment techniques may also be used to decrease weed populations in rice communities. On average, weed populations were reduced by approximately 50% when the stale seedbed technique was used prior to water seeding. Eliminating spring tillage in a water-seeded stale seedbed system reduced weed populations by an additional 75%. Application of the stale seedbed and no till techniques in drill seeded rice also reduced grass weed populations, but low densities of *Leptochloa fascicularis* (0.5 plants m^{-2}) still emerged after rice emergence. Nevertheless, an application of pendimethalin/cyhalofop provided very good control of these grass populations. These results suggest that utilization of the stale seedbed and no till techniques may reduce weed populations that must be controlled with post-emergence herbicides. In addition, application of a non-selective herbicide in the stale seedbed systems facilitates the use of an alternative herbicide MOA for controlling herbicide resistant weed populations.

Management considerations

The stale seedbed systems were most effective at reducing populations of the *Echinochloa* spp., slightly less effective at reducing *Leptochloa fascicularis* populations, and even less effective at reducing populations of *Cyperus difformis*. In each of the stale seedbed treatments, nearly all of the *Echinochloa* spp. that emerged in this season emerged prior to rice planting. A similar response also occurred for *Leptochloa fascicularis* in the water-seeded treatments, but nearly 20% of *Leptochloa fascicularis* emerged after rice emergence in the drill-seeded treatment. The greater suppression of the grass species in the water-seeded treatments may be partially due to the effects of anaerobic soil conditions caused by flooding. Approximately 60% of the *Cyperus difformis* population was eliminated with the stale seedbed system in water seeded rice but only 20% of the population was eliminated in the drill-seeded system. Therefore, alterations in the stale seedbed system may be needed to more effectively reduce populations of *Leptochloa fascicularis* and *Cyperus difformis*.

The efficacy of the stale seedbed system may depend on the weed species targeted for control and the type of seeding technique that will be used (i.e. water seeding or drill seeding). Preliminary results suggest that emergence rates of weed species in rice differ greatly. In optimal conditions, *Echinochloa* spp. germination can approach 100% in approximately 5 d (Figure 1). However, *Cyperus difformis* may require approximately 12 d and *Scirpus mucronatus* 20 d to approach 100% emergence. The rapid germination rate of *Echinochloa* spp. partially explains the success of controlling this species in the stale seedbed treatments used in this study. *Leptochloa fascicularis* germination data is now needed to determine the length of time required to maximize its germination in a stale seedbed used prior to drill seeding. However, the amount of time available for the stale seedbed is limited in drill-seeded rice as 10-14 d may be required to adequately dry the field for planting. For water-seeded systems, it may be beneficial to keep the soil partially flooded (<1 in. water) for approximately 2 wk prior to planting to eliminate many of the sedge weed species prior to rice planting.

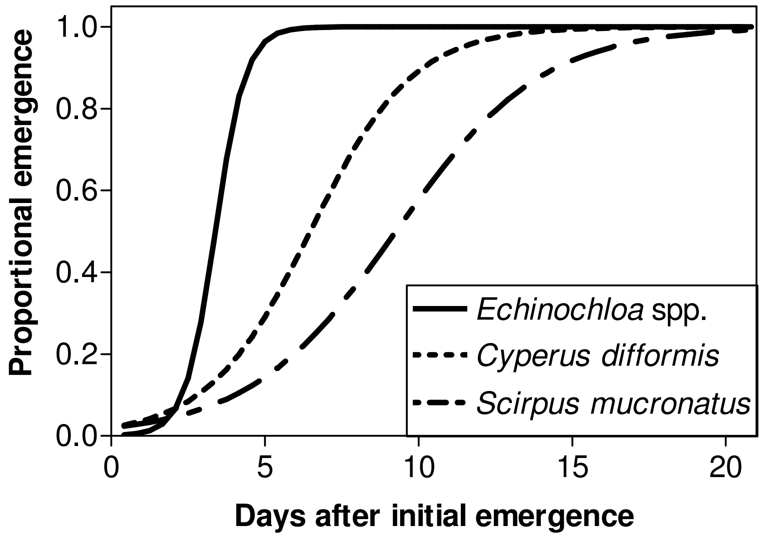

Figure 1 Proportional emergence as a function of time for *Echinochloa* spp., *Cyperus difformis*, or *Scirpus mucronatus*.

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OSTRINIA NUBILALIS (HÜBNER) STEM BORER OF RICE IN ITALY

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Abstract

In the last 2-3 years, particularly in the 2003 summer, white heads has increased in the Italian rice-fields: plants bearing discoloured panicles with empty or partially filled grains showed a hole in the culm, disintegrated tissues and frass due to boring and feeding larvae. The plant examination and sampling let to classify the stem borer as *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), the European corn borer. This species is polyphagous but it is the first time it is recorded on rice in Italy, whereas in the U.S.A. the first severe infestation in rice occurred in 2003. Probably in the Italian rice growing areas, *O. nubilalis* migrated, not at damaging levels, from the less attractive early maturing corn to the more succulent rice plants owing to the unusual summer high temperatures and dry weather. Preliminary observations on the symptoms and on the cycle of *O. nubilalis* on rice are here reported but the relation between this moth and rice has to be more probed. It is interesting to point out that egg masses were often found parasitised by tiny wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae).

Keywords

Ostrinia nubilalis; European corn borer; *Trichogramma*; white head; rice; Italy.

The presence of discoloured panicles just after flowering was sporadic without causing yield loss in Italian rice-fields in the last thirty years, whereas it is increased in the last two-three years. Therefore a survey starts in the Italian rice cultivated area in order to improve our knowledge on this phenomenon, observing and collecting a great number of plants to study symptoms, to identify the causal agent and to understand its relations with rice.

The distribution of discoloured panicles in the rice-field is random and they are less or more frequent according to the seriousness of the attack.

Affected plants bear white and dried panicles with unfilled spikelets and their panicles come out easily, if gently pulled out from the sheath of the flag leaf, showing the brownish, bored and rotting stem: this phenomenon is due to insect larvae which, feeding on culm tissues, interfere with the translocation of water and nutrients. This symptom is called "white head" and is the typical one resulting from the attack of stem borers after panicle initiation, whereas when rice is attacked during the vegetative phase, the central leaf does not unfold, turns brownish and dries off and symptom is called "dead heart" (Pathak, 1977). Stem borers are the most serious pest of rice in many rice growing areas in the world (Centre For Overseas Pest Research, 1976; Pathak, 1977; Boyd, 2004; Castro, 2004), but they have never been a problem for Italian rice-fields.

The recently observation of white heads, but not of dead hearths, with increasing frequency led us to fear that the stem borers *Chilo suppressalis* Walk., which is present in Portugal (Lima, 1997), Spain (Herruzo and Morote, 1996) and France (Goarant *et al.*, 1996) with yield losses up to 25%, or *Sesamia nonagrioides* Lef., reported on rice in Sardinia in 1959 (Boselli, 1959) and now the insect causing the most damage in Greece (Ntanos, 2001), had reached and infested Italian rice-fields.

But the only stem borer found during our survey is the European corn borer, *Ostrinia nubilalis* Hb. (Lepidoptera: Crambidae), well known and dreaded by corn growers. Although species is polyphagous (Süss, 1983; Tremblay, 1986), it is the first time it is reported on rice in Italy, whereas in the U.S.A. the first severe infestation in rice occurred in 2003 (-, 2004; Castro, 2004).

This insect was present in the districts of Pavia, Milan, Vercelli and Ferrara and it was mostly noticed where plants were more vigorous in rice-fields that had received high rates of nitrogen fertilisers. It infested both old and new variety, such as Titanio, Torio, Rubino, Baldo, Volano, Ariete, Cripto, Loto, Rodeo, etc., and, in great number, also the vegetation on field borders.

Ostrinia nubilalis was found in rice-field at all the instars of its life cycle: egg, larva, pupa or crysalid, and adult. To further confirm the classification, larvae of different instars were collected from infested plants and reared at room temperature on healthy plants, in pots, in laboratory until the adult emerged.

In this paper our preliminary observations are referred, but they have to be confirmed and integrated pursuing this research.

In the summer 2003, yellow-brown to medium-brown adults, of both the sexes, were first noticed in rice-field in mid-Jun, but a far greater number was observed at late July–early August.

Flat, translucent, white, eggs overlapped like fish scales in two-three regular and parallel rows and were laid preferably on the underside of the leaf blade of the first and second internode below the flag leaf. The day before hatching they appear to have a black centre corresponding to the head capsule of the developing larva.

The white newly hatched larvae are long about 2 mm and become creamy white to grey reaching the length of 25 mm. Very seldom more than one larva per culm was found. Each stem showed a only hole, always on the upper internodes, and when frass was extruded the larva had left the culm to look for another one: therefore it seems that the caterpillar go through the same hole both to enter and to exit.

On the contrary the adult exit hole, which usually rice stem borers cut for the moth emergence (Centre For Overseas Pest Research, 1976; Pathak, 1977), seems not exist. In fact the brown pupa has always been found in the sheath of the flag leaf, few centimetres below the panicle, which sometimes was chewed by the larva. The crysalid, with the exuvia still on its caudal end, was almost always protected by a very light silken web woven by the caterpillar before pupation. The pupa position in the flag leaf sheath and the fact that its head was put always towards the plant apex suggest that moth should exit going up and getting the same natural opening through which the panicle emerges.

In some cases the panicle spikelets were not empty but partially filled because the stem had been bored after the grain formation was started. The larval feeding on the leaf sheath inside surface made the sheath itself turn yellow-brownish, often in the areas corresponding to the hole and to the chewed parts of the culm, or of the panicle when it was gnawed before rice heading.

It is interesting to point out that egg masses were often parasitised by tiny wasps of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae). Parasitised eggs are easily single out thanks to their black colour and these parasitoids are natural enemies, which are studied for biological control of the European corn borer (Maini and Burgio, 1990; 1991; Hoffmann, 1999).

In the Italian environment *Ostrinia nubilalis* usually goes through one or two generations a year and overwinters as mature larva within corn or other plant stubble (Süss, 1983); Tremblay, 1986): what occurs on rice has still to be investigated.

The recent increasing rice infestation, till now fortunately not at damaging levels, may be due to several factors, but climatic changes are certainly very important. In the last two-three years, particularly in 2003, summers were warmer than usual and probably high temperatures shortened the life cycle of the European corn borer and increased its population. Moreover in 2003 the unusual drought made corn dry early, leading moths to migrate from the less attractive maturing corn to lay eggs on younger and succulent crops, like rice, or on other normally less attractive hosts, like flowers (Colombo *et al.*, 2003) and grapevines (Cravero and Bosio, 2004). The new details and knowledge we are now acquiring on the behaviour of the European corn borer in rice-field may be also influenced by the current summer weather which is rather different from that of the last years.
The here reported preliminary observations on the symptoms and on the cycle of *O. nubilalis* on rice are only the first step and the relation between this moth and rice has to be more probed.

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THE ARMYWORM *MYTHIMNA UNIPUNCTA* (HAWORTH) FOUND ON RICE IN ITALY

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Abstract

An unusual and continuous flight of birds (Common Tern, *Sterna hirundo* L.) over a rice-field close to the Po delta called the farmer's attention at the beginning of August 2002. The rice plants were carefully observed and a great number of caterpillars were found feeding on leaf margins, sometimes leaving only the midribs. In order to classify those insects, some larvae were collected and reared on rice plants in laboratory until the adults emerged. The pest was the armyworm *Mythimna* (*Pseudaletia*) *unipuncta* (Haworth), (Lepidoptera: Noctuidae, subfamily Hadeninae). It attacks mainly wild and cultivated grasses, including forage, corn, oat, wheat, barley, rye and rice, in North and South America and in West Africa but it has been reported for damage in Europe only from 1950. This is the first report on rice in Italy. These armyworms are not usually noticed until severe damage occurs, because they feed at night and hide during the day. Often the infestation outbreaks from the field borders from which caterpillars march in great number to find a new food source. Probably also in this case, *Mythimna unipuncta* infested rice when weeds were eliminated from the field borders. Fortunately birds controlled the pest population before larvae reached the most dangerous instars cutting off rice panicle.

Keywords

Mythimna unipuncta; armyworm; rice; Italy.

The number of rice pests in Italy is rather low (Moletti, 1980) and insects do not usually seriously affect the rice cultivation. However sometimes rice can be infested and damaged also by pests moving from another crop, as in the case of the European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae) (Giudici and Villa, 2003; 2004), or by insects attacking it accidentally, as in the case of the ground bug *Ischnodemus quadratus* Fieber (Rhyncota: Lygaeidae) (Bianchi, 2001).
At the beginning of August 2002, at Ca' Bonelli, situated in the Po delta area, an unusual and continuous flight of birds over a rice-field of about 20 hectares called the farmer's attention. Only a high bank, which was recently mowed, separated that rice-field from the sea from which birds, the Common tern (*Sterna hirundo*), came hovering and diving down into the mass of rice plants. Though Common terns feed mostly on small fishes, shrimp and insects are also taken regularly.
The rice variety grown was Volano and it was at the end flowering stage when the infestation was noticed. Rice plants were carefully observed and several caterpillars were found feeding on leaf blade margins and chewing angular pieces off leaves, sometimes leaving only the midribs. The larvae were greenish-brown or greenish-grey with a narrow broken stripe on the back and with two orange white bordered stripes separated by a dark stripe on each body side, all of them running along the body. They showed also a black band on the prolegs.
In order to classify those insects, some larvae of different instars were collected and reared at room temperature on rice plants, in pots, in laboratory until the adults emerged. About a month later, only three tan to grey-brown moths emerged with a characteristic white dot on

the forewings. Both larvae and moths correspond to the description of the armyworm *Mythimna* (*Pseudaletia*) *unipuncta* (Haworth), *Lepidoptera Noctuidae* (subfamily *Hadeninae*). This pest attacks mainly wild and cultivated grasses, including forage, corn, oat, wheat, barley, rye and rice, in North and South America and in West Africa (Grist, 1969; Centre For Overseas Pest Research, 1976; Quisenberry, 1999) but it has been reported for damage in Europe only from 1950 (Tremblay, 1986). This is the first report on rice in Italy.
According to literature (Maund, 2002; Godfrey, 2004), these armyworms (so defined because they travel in great multitudes destroying grass) defoliate plants, but they may also feed on the panicle near the developing kernels with the result that kernels dry before filling and all or parts of the panicle turn white. The seriousness of armyworm injury depends on the maturity of the plant and on the amount of tissue consumed. Defoliation greater than 25% at 2 to 3 weeks before heading can cause significant yield reduction. Caterpillars are not usually noticed until severe damage occurs, because they feed predominantly at night and hide during the day. When larvae are numerous and the food supply become depleted, they may also be seen feeding during the day. Usually the first generations develop on other plants and food shortage induce larvae to migrate or moths, which fly at night, to move into rice-field to lay eggs. In flooded rice-fields most mature larvae drown before reaching a suitable pupation site because armyworms normally pupate 3-5 cm below the soil surface or in debris.
Often the infestation outbreaks from the field borders from which caterpillars march in great number to find a new food source. The presence of large numbers of birds in a field may indicate that large numbers of armyworms are present. Probably also at Ca' Bonelli, *Mythimna unipuncta* infested rice when weeds were eliminated from the levee banks. Fortunately birds controlled the pest population before larvae reached the most dangerous instars cutting off rice panicle and the rice yield was not reduced. On the other hand in the following summer, 2003, *Mythimna unipuncta* infested the same rice field to a lesser extent and it was noticed again thanks to the presence of birds. The infestation of this pest for two consecutive years suggests to keep a careful watch both on that rice-field and on the surrounding area in order to know whether the *Mythimna unipuncta*'s presence is going to become frequent and/or to cause more significant damage.

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THE CALIFORNIA RICE CROPPING SYSTEM: AGRONOMIC AND NATURAL RESOURCE ISSUES FOR LONG-TERM SUSTAINABILITY

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Abstract

California rice is produced on approximately 200,000 ha mostly in the Sacramento Valley. The crop is planted in April/May and harvested in September/October. The growing season is characterized by a Mediterranean climate with negligible rainfall, high solar radiation and relatively cold nighttime temperatures, thus yields may exceed 9 t ha^{-1}, 20% above the US average. California is a highly urbanized State with an affluent population demanding agricultural practices to be environmentally benign and food products to be safe for human health. This has contributed to a rigorous regulatory climate for plant protection chemicals thus increasing the cost of production. Likewise, the resource base is being challenged. Increased demand for clean potable water for urban expansion and the demand for environmental water compete with rice for limited supplies while raising cost. Production problems, such as straw management for cleaner air, weed resistance to herbicides and the introduction of exotic pests also contribute to higher costs. The California rice industry is challenged by the increasing complexity of the rice production system to meet both the off-farm public demands and the on-farm need for higher productivity.

Keywords

Rice; Sustainability; California; Production.

Overview of California Rice

The agronomic sustainability of rice farming in California is broadly related to the preservation of natural resources, efficiency of the production system and maintaining and improving yields. Most US rice is produced in semi-humid to humid environments in five contiguous southern states. California is unique among the US rice producing states in its geography and climate; and in its political, social and environmental views. Because of its dry Mediterranean climate and northern latitude of 38 to 40^{o} (McLean et al., 2002), California varieties and many of the agronomic practices are quite different from those in the southern states. Additionally, California's urbanized population, largely unengaged in agriculture, demand, sometimes unreasonably, that rice (and other crops) be produced with environmentally benign methods with no off-farm impacts. Since agriculture represents much of the landscape in general, environmental and conservation organizations push hard for conjunctive use of farmland for habitat and other purposes. These groups have focused on rice in particular because it is representative of the vast wetlands that were once the major ecosystem of California's Central Valley. The industry has been highly successful in developing partnerships with urban and conservation groups, but these compromises have confounded agronomic practices from once relatively simple to complicated management-intensive crop production systems. About 96% of California rice is grown in the Sacramento Valley, and the balance in a few counties of the San Joaquin Valley (Figure 1).

Climate, Soils and Water

The rice growing season is relatively rain free and characterized by high daytime temperature, high solar radiation, low humidity, and relatively cool nights. Cloud cover during the growing season is limited such that radiation remains high while cool nighttime temperatures minimize respiration losses. The soils on which rice is grown were formed from sediments carried by two major rivers and several tributaries to produce clay and silty-clay soils. Basin soils have clay content from 40 to 60% while the older terrace soils frequently are loam in the topsoil with dense clay in the subsoil overlying a cemented layer.

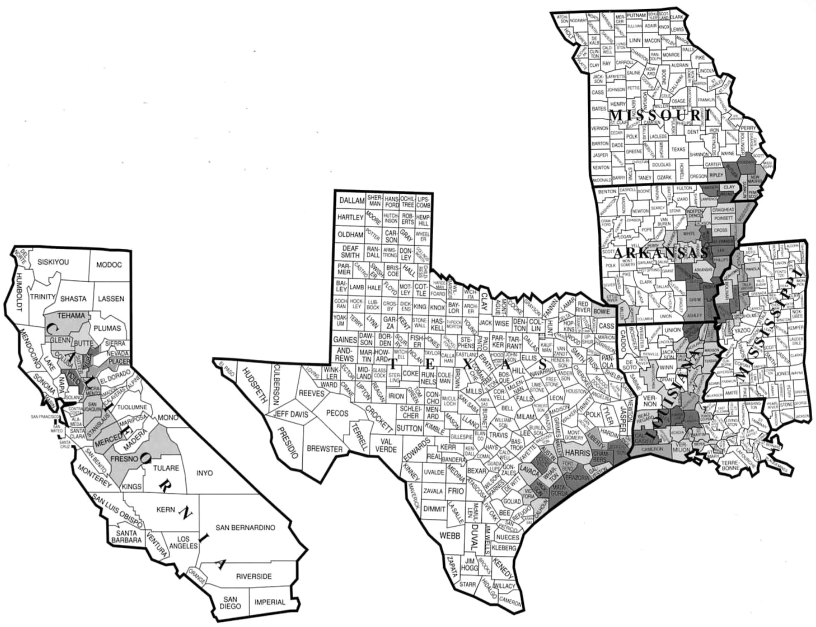

Figure 1 Contiguous southern US rice producing states and California. Darker shading represents greater planted counties. Source: Specialized Agricultural Publications, Inc. Raleigh, NC, USA.

Seasonal infiltration rate of typical rice soils is 1 to 5 mm day^{-1}. Soil reaction ranges from very strongly acid to moderately alkaline, in the range of pH 4.5 to 8.0. About 300,000 ha of land in the Sacramento Valley are suited to rice production because of impeded drainage. Somewhat more than half of this land is only grown to rice. The remaining area varies in suitability for rotation crops (Carter et al., 1994). Approximately 80% of rice irrigation water is supplied from reservoirs captured as snowmelt from the Sierra Nevada Mountains to the east. Goundwater is also pumped from 15 to 50 m depth. Farmers who are some distance from primary water sources, often use drainage water as a primary source of irrigation. Water is distributed through water companies, irrigation districts and drainage districts, although some growers have riparian rights and lift water directly from rivers and streams.

California Cropping System

All California rice is direct seeded; most is pre-germinated and aerially seeded into standing water under a continuous flood. Increasingly rice is drill- or dry-seeded and permanently flooded after stand establishment. The water-seeded system was established to suppress barnyardgrass (*Echinochloa crus-galli*) and subsequent research proved that N could be used efficiently and that overall rice productivity was high. The California rice growing system is highly mechanized. A typical California rice farmer has an equipment investment of about US$ 1250 ha^{-1} of rice. Airplanes are typically contracted for seeding, fertilizer top-dressing and the application of certain pesticides. Ground applicators are increasingly used for herbicide application to minimize drift to sensitive crops. Seeding rates average 150 kg ha^{-1}. Seed is pre-germinated by soaking in water for 24 h and draining for 24 h. Over 90% of the area is planted to medium grain rice with limited sowing of short and long grain varieties. Seasonal length varies from 130 to 165 d, depending on variety, with the majority in early maturing varieties of 140 to 145 d duration. The irrigation season is about 120 d. Harvest takes place when grain moisture content is about 20 to 22% for short and medium grain and 16 to 18% for long grain. Post harvest activities include management of straw residue, primarily by soil incorporation, but with limited burning for disease control and minimal removal for use in straw based products.

Sustainablility of the Natural Resource Base

The foundation of the California rice industry includes the availability of clean, abundant and low cost water and land well suited to rice production. In California's urban environment, farmers must also be concerned about the off-site impacts of rice farming so as not to degrade air and water, to share water resources and to maintain or improve environmental services. These complex issues are a challenge to the California rice industry and have dramatically changed the production system. These issues will be discussed in the following sections.

Water Supply

Rapidly increasing demand for water by municipal and environmental users threatens the supply and cost of rice irrigation water**.** California's water supply varies geographically and seasonally. About 71% of the supply originates in the north of the State but 75% of the urban and agricultural demand is south of the Sacramento Valley. Since most of the runoff occurs from November to March, facilities to store and convey water from one area to another (California Department of Water Resources, 1998) are required. No major new facilities have been developed since 1968, but the Central Valley is the fastest growing component of a rapidly growing California population, with the Valley population expected to triple by 2040 (American Farmland Trust, 1995). Additional issues around competition for water are loss of substantial amounts of Colorado River water in Southern California and the need to flush the Sacramento-San Joaquin Delta of salt. In 1999, an additional one million megaliters was diverted from the Sacramento River for environmental services including maintenance of low temperature for migrating salmon and habitat for waterfowl. This change effectively reduced the supply for agriculture.

Among the irrigated California crops rice is a major water user, behind only alfalfa and pasture based on application rate ha^{-1}. It is similar to several crops based on evapotranspiration (California Department of Water Resources, 1998). Seasonal water delivery for rice varies a great deal depending on soil type, management and seasonal length (Table 1). The average delivered use is approximately 22.5 to 24.4 ML ha^{-1}, but varies from about 15.5 to 31.7 ML ha^{-1}, depending on the source, delivery system and how it is calculated. Extensive laser leveling and conversion of fields from contour levees to parallel levees has improved water use efficiency (WUE) so that drainage has been considerably reduced. Additionally, regulations restricting drainage to keep rice herbicides from entering public waterways has improved WUE as well. Extensive recirculation of drain water and some use of a 'static water irrigation system' in which water does not leave the field has also reduced total water requirements. Deep percolation is related to soil characteristics and few rice fields have high infiltration rates. Evapotranspiration has also been reduced by about 16% by planting shorter season varieties. Currently, about 88% of California rice has a seasonal length of 145 d or less, compared to the period prior to 1980 when over 50% had a season length of 165 d.

Table 1 Approximate seasonal water use by rice in California.

Seasonal Water Use	**ML ha^{-1}**
Evapotraspiration (Et)	9.7 – 13.4
Percolation/seepage	1.8 – 7.6
Drainage	0 – 7.6
Delivery at field inlet	15.5 – 31.7

Water prices have risen quickly in response to greater demand. Water costs in 2001 were about US$ 133 ha^{-1} compared to US$ 111 ha^{-1} in 1998. Water transfers from the north to the south for agriculture and urban uses once unthinkable, is now more acceptable. Issues arising from water transfers include preservation of water rights, conserving local supplies, environmental affects and third party impacts on agricultural support infrastructure and tenant farmers who don't gain from the sale but may lose their water. Powerful urban political forces are gradually changing California water supply, cost and rights, and along with it water for rice will likely diminish in the future. Water promises to be a primary sustainability issues for the foreseeable future.

Water Quality

California has strict laws governing agricultural pollution of surface and groundwater pertaining to sediment, nutrients, pesticides and other "constituents of concern." Because flooded rice fields dominate the landscape of the Sacramento Valley, rice has always been under the microscope for it's potential degradation of water quality. In the late 1970s, fish kills were attributed to the herbicide molinate and later a metabolite of thiobencarb was found to be the cause of off-tastes in the Municipal drinking water of the City of Sacramento. The industry responded with a highly successful program to reduce off-site water degradation and became a recognized leader long before most other commodities had to address the issue.

A combination of research, education and regulation largely solved the water quality problem from rice field tailwaters. Through publications, meetings and demonstration projects, growers adopted practices to mitigate water runoff. The field demonstrations compared conventional flow-through irrigation with a recirculation system and a novel static water irrigation system for utility in holding water and growing rice (Hill et al, 1991). From 1990 to 1993, 50 static systems and 41 recirculation systems were installed; several irrigation districts also began to operate as closed systems. Careful monitoring of these novel systems demonstrated they were effective in reducing pesticide residues in rice drain water. Most growers have now learned, however, to hold water in conventional flow through fields for up to thirty days without drainage. Regulation of drainage from treated rice fields was implemented by adopting "performance goals", residue levels considered safe for people and the environment. For example, the performance goal was set at 10 ppb for molinate and 1.5 ppb for thiobencarb. Holding periods were then determined to allow sufficient degradation to occur before drainage to meet the performance goals. Typical holding periods after application are 30 days for granular thiobencarb and 28 days for molinate. This program continues today and, together with adoption of improved irrigation practices, has reduced mass flow of pesticides in the Sacramento River by 97%.

However, the issue is not entirely resolved. The legal framework has changed and public resolve for zero degradation has increased. Currently, agriculture has a two-year extension of a waiver from non-point source pollution regulations. Within two years, each agricultural industry must file an irrigation management plan that addresses their particular water degradation issues. The rice industry is attempting to have its current pesticide program adopted as sufficient to meet the new regulations. If unsuccessful, the most severe consequence may be to require each grower to file a separate plan, including highly expensive water sampling to prove compliance. Increasing demands for clean water will likely add new water quality issues, for example from organic carbon as the result of the decay of straw, incorporated ironically to protect air quality from smoke.

Air Quality

Alternative straw management practices to replace burning for residue disposal are creating fundamental changes in production practices, long term yield potential and production costs. Burning rice straw came to an end in 2001 after years of public debate about the contributions of smoke to air pollution and related human health problems.. . Regulations enacted in 1991 (Table 2) phased out burning while simultaneously promoting off field uses of rice straw. The net result is that 25% of planted acres may be burned for disease control and the balance must be disposed by soil incorporation or removal. Until 1991, nearly all the rice straw was burned after harvest. By 2000, 25% of the acreage was burned, 1.6% baled and 73.2% soil incorporated (Williams et al., 2002). However, it is likely that growers will be able to burn far less than permitted because of tightening burn regulations that are related to declining air quality. There are a decreasing number of 'good' burn days in the confined air space of the Sacramento Valley. An inversion layer persists from July through December, during much of the burn season, greatly lowering the ventilating properties of the valley, trapping all forms of air pollutants. Hence, only 7% of the 2002 (10) and 13% of the 2003 straw residue had been burned before winter rains arrived.

Table 2 Rice straw burning phasedown schedule. Amending legislation in 1998 changed the original schedule.

Year	Actual schedule	Original schedule
1992	90%	90%
1993	80%	80%
1994	70%	70%
1995	60%	60%
1996	50%	50%
1997	38%	38%
1998	200,000 ac	25%
1999	200,000 ac	25%
2000	200,000 ac	Conditional burn
2001	Conditional burn	

Growers use two primary methods to incorporate rice straw including no winter flooding and winter flooding. Straw is usually chopped and/or disked directly into the soil after harvest. About 80% of soil incorporated rice straw was winter flooded in 2000 (Williams et al., 2002). Winter flooding is a grower-developed practice which combines straw disposal benefits with creation of additional waterfowl habitat for winter migrations of ducks, geese and swans. The rice industry has gained wide public recognition for this practice, although there has also been criticism regarding the additional use of water during migrations of anadromous fish that spawn in the Sacramento River and its tributaries, and potential organic carbon loading of streams from straw decomposition and subsequent drainage.

Transition from burning has brought a mix of benefits and problems. Burning provided a measure of disease control and easy disposal at low cost, less than US$7 ha^{-1}. Many studies have demonstrated an increase of stem diseases when straw is incorporated. But in a recent multiyear California study (Webster and Cintas, 2001), winter flooding rice straw tended to suppress stemrot disease and increase yield compared to incorporation without winter flooding and may be a viable management strategy for stem rot. Disease levels appear to have increased slightly as sclerotia levels rose to the point where their number was no longer limiting to disease incidence or severity. The weed seed bank also increased when practices included burial of the straw (Hair, 2001). Compared to burning or removing, soil incorporation has beneficial impacts on soil fertility (particularly N and K), allowing a reduction of fertilizer inputs after several years of incorporation, and on carbon sequestration that may reduce greenhouse gas emissions. For example, applied N can be reduced 28 kg ha^{-1} after 5 years of incorporation. However, yield potential of incorporated fields may be limited compared to burned fields because of increases in disease and weed pressure, and possibly unidentified causes. Nitrogen rate experiments comparing incorporated vs. burned straw showed that incorporated yields were higher at zero N and peaked at lower N than burned yields. However, maximum yield was consistently higher in burned plots suggesting there are non-N mechanisms affected. Reduced weed control and higher disease levels are suggested as the primary causes of lower yield in incorporated plots (Bird et al., 2002).

Fall straw management has also increased the post harvest workload, and greatly increased cost of rice production without tangible increase in returns. In a study reported in 1998 (Williams and Gold-Smith, 1998) average cost of soil incorporation, across all methods, was US$ 89.72 ha^{-1}. A 2001 update of this study (Najita, 2001) increased the cost to US$ 106.25 ha^{-1}.

Rice straw utilization has developed very slowly because of economic and technical constraints, yet a commercial market could relieve the financial burden and agronomic problems associated with soil incorporation. Many products including paper pulp, livestock feed and bedding, compressed panels for interior walls, medium density fiber board (MDF), straw bale houses, erosion and sediment control devices, ethanol, methane, electricity, etc. have been successfully manufactured, but each product has its own set of constraints. The cost of acquiring and delivering rice straw is often the primary deterrent. Straw baling, storage and transportation costs range from US $27 t^{-1} to over US $100 t^{-1}, depending on the method of baling, storage conditions and distance transported (Jenkins et al., 2001). Today, rice straw usage remains very low and growers are dependent on soil incorporation of straw. While the industry continues to work diligently toward economic straw uses, the long term prospects for significant use is not clear.

Land

Land is a limited resource and its availability for rice is challenged by rice farming economics, competing agricultural and urban uses, and potential degradation issues. Most California rice land has impeded drainage and over half is poorly suited to most upland crops. Many efforts have been made to grow summer and winter crops on "rice-only" land, and have failed due to poor yields and high input cost. For this reason, many rice fields, in addition to having poor internal drainage, are designed to optimize rice production, frequently with permanent levees and zero grade slopes, further restricting their utility for rotation crops. While rice yields remain reasonably strong in a rice only production system, rotation to alternative crops would help improve weed and disease management and improve soil fertility. Research on rotation crops for heavy rice lands is needed as expenses rise and the need for cropping diversity increases.

Rice fields close to urban areas are subject to conversion to commercial or residential uses, thus reducing the total supply of suitable rice land. Urban sprawl is estimated to consume over 400,000 ha of farmland in California by 2040 at the present growth rate coupled to low-density housing developments (American Farmland Trust, 1995). Substantial development is underway on the north side of the city of Sacramento and south of Marysville consuming former rice fields. Many conflicts with agricultural operations exist at the interface between town and country, including dust, noise, pesticide drift, mosquitoes, drainage, aircraft operations, etc. As population increases, this loss of agricultural land will continue and these problems will increase. These problems can only be solved by policy to reduce the rate of urban expansion through land use designations, tax incentives and purchase of development rights. Problems at the interface must be addressed with effective buffers and logical land use planning.

Two in-field land degradation issues require discussion. First, salinity is a current problem for some growers on the west side of the Sacramento Valley particularly for those farmers who depend on drain water, but also for some areas when water is held without spillage. Salinity is also a problem with some well water on the east side of the Sacramento Valley. Recent salinity studies have shown that rice is highly salt sensitive in the seedling and flowering stages of growth. Threshold values of average seasonal salinity for no yield loss have been lowered from 3.0 to >1.9 dS m^{-1} (Grattan et al., 2002). Long term holding periods mandated for pesticide residue degradation, and tightening water supplies will increase salinity problems. Second, approximately one million kg of copper sulfate is applied annually to California rice fields for tadpole shrimp and algae control. Very little copper in organically bound forms leaves the field. Nearly all remains behind as low solubility salts. While no copper toxicity problem in rice or rice rotation crops has been identified, continued use of copper eventually will cause toxicity problems. Research is needed to provide effective alternatives to copper use.

Sustainability of the Production System

Herbicide Resistance

Resistance to sulfonylurea herbicides among four broadleaf and sedge species, and multiple and cross-resistance to several grass herbicides has increased the complexity and cost of rice weed control. Beginning in 1989, the use of bensulfuron (Londax) an extremely effective, broad-spectrum broadleaf herbicide, rapidly increased to almost 100% of the acreage. In 1992, after only three years of use, resistance was confirmed, traced to a single gene difference in susceptible populations of four weed species. The number of fields with bensulfuron resistant weeds increased rapidly from four in 1999 to most fields in 1995. Growers substituted less effective and more injurious alternatives, primarily the phenoxy herbicides. As a result, there was a marked increase in the average number of spray applications, greater cost, less effective weed control, greater incidence of crop injury and more drift damage to neighboring crops, primarily cotton.

In 1996, thirty years after its introduction, the first confirmed resistance to molinate was found in watergrass (*Echinochloa phyllopogon*). By 2001, approximately 400 fields had confirmed resistance, including three species of *Echinochloa* and several herbicides. Cross resistance (resistance to the same mode of action in different herbicides) and multiple resistance (resistant to multiple modes of action) have been identified. A typical weed control program in 1991 included molinate or thiobencarb as the primary grass material, usually without additional grass herbicide application. By 2001, the program consisted of a thiocarbamate herbicide followed by propanil, and more recently, cyhalofop and clomazone have become major players. In difficult cases, mixtures of two or three products, or up to three applications of grass herbicides may be used. From 1996 to 2000, the number of grass herbicide applications rose from 1.03 to 1.59 per field (California Department of Pesticide Regulation, 1996-2000).

Agronomic research has focused on the evaluation of candidate herbicides with new modes of action, characterization and confirmation of resistance, and programs to manage resistance. Herbicides can be degraded by metabolic activity, for example oxidation by cytochrome P_{450} enzymes or conjugation to sugars, amino acids or peptides. A primary issue is understanding whether our observation of multiple resistance is resistance to more than one site of action or is metabolic resistance Metabolic resistance would suggest that some weed populations have the ability to detoxify a broad range of herbicides, including those not currently in use. Of the confirmed resistance, the greatest number is in *E. phyllopogon*, and it appears that much of it arises from a pre-existing mutant population in the Sacramento Valley, based on morphological traits and AFLP fingerprints. This implies that control of resistant seed dispersal and elimination of survivors after herbicide treatment are required for integrated management of resistance (Ryouichirou et al., 2003) which means an increase rather than a decrease in herbicide use.

<u>*Alternative systems:*</u> Increasingly, growers with severe weed resistance problems are seeking innovative methods to grow rice. These provide opportunities to use new mode-of-action chemistry. For example, a primary reason some growers drill seed is for access to glyphosate and pendimethalin as both are unsafe in water seeded rice. Other innovative methods are also under evaluation, including “false” drill seeding which employs dry broadcast seed lightly covered and irrigated. “Stale seedbed” systems encourage weed growth in the unseeded, prepared seedbed either with winter rain or spring irrigation, which are then sprayed with nonselective herbicides. The seedbed is not disturbed and either drill seeded or flooded and water seeded. Research is just beginning to evaluate the utility of these novel systems.

Drift

Periodic episodes of rice herbicide drift triggered regulatory activity that has dramatically changed application procedures. Propanil drift to prune trees in 1968, phenoxy drift to cotton in the mid-1990s, carfentrazone drift to prunes in 2000, and cyhalofop drift on cling peaches in 2001increased public scrutiny of rice production activities. Propanil was banned from use except in designated areas; most registrations were withdrawn for phenoxy products; carfethrazone was restricted to ground and specialized aerial application; and cyhalofop was confined to ground application in most areas. The primary method of managing products prone to drift is to limit their use to ground applicators equipped with low drift nozzles that produce spray droplet sizes above allowable limits. In addition, buffer zones near sensitive crops are created for each product. Typically, a ground buffer zone is 0.8 km, and an aerial buffer is 8 km. Given multiple products, methods of application and numerous crops to protect, a complex network of no-spray zones has been created that requires significant management by regulatory officials and difficulty for growers. Many producers are denied access to effective chemicals and must use less effective substitutes. Resistance management is particularly impacted when growers can’t use effective products. New granular products that can be applied directly into the water are urgently needed to reduce drift problems, lower the regulatory burden, and decrease the high water management requirements for the use of foliar applied herbicides.

Exotic Pests

Exotic pest introductions jeopardize production and markets. To maintain California's position as a supplier of high quality rice and to develop specific market niches, germplasm and cultivars have been freely exchanged. But exotic pests and diseases on seed, processed rice and equipment have been introduced where appropriate procedures were not followed. For example, rice blast (*Pyricularia grisea*) was first identified in California in 1996 and bakanae (*Gibberella fujikuroi*) in 1999. Both are thought to have entered the state on seed that did not go through quarantine procedures. Blast caused significant yield loss in 1996 and 1997 but has since been at a very low level. Bakanae rapidly spread in the seed and by 2002, an estimated 80% of fields had disease present. Yield loss associated with bakanae was as high as 30% although most fields experienced no yield loss. Existing laws governing importation of crop seeds are sufficient, but have been willingly violated. Interstate shipment of rice seed and equipment is another avenue of introduction. The Rice Certification Act of 2000 is a state law intended, in part, to supplement existing regulations by creating a program that approves research protocols to prevent introduction of exotic pests.

Yield

Rice yields before 1950 were a function of improvements in water management and use of certified seed. In 1947, 2,4-D was introduced followed by MCPA in 1952, and synthetic fertilizer nitrogen in 1945. Yields responded and received another boost in 1962 by introduction of propanil and molinate in 1966. The first semidwarf cultivar was introduced in 1976 and by 1982-5 most acreage was in semidwarf cultivars. Yields rose dramatically in response to improved photosynthate partitioning and better agronomic characteristics that allowed higher application of fertilizer nitrogen. From 1976 to 2002, 33 semidwarf cultivars have been released from the public breeding program providing the industry with improved yields, higher quality, and better agronomic characteristics in a range of market types. Yields peaked in 1991,1992 and 1994, among the highest in the world. Yield gains through plant breeding and production practices have been in small increments, and since 1994, have trended downward (Figure 2).

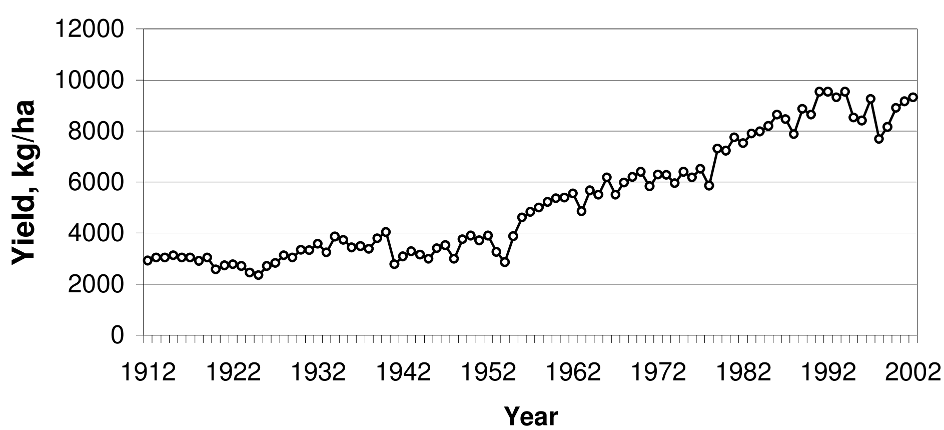

Figure 2 California rice yield as a three-year moving average.

Inclement weather has played a role in perceived yield decline, and is probably the biggest cause. For example, heavy rain from El Nino in 1998 caused delayed planting and low temperature in 1999 caused widespread panicle sterility. Rain also played a role in 1995-6. However, other factors are involved. For example, straw incorporation was widely used concurrent with declining yields in the 1990s, thus weed and disease interactions resulting from that new practice may be partly responsible. In addition, weed resistance became widespread during this same period and likely contributed to reduced yields. Yields for the past three years, however, have rebounded to near the 1991-4 record. The cultivars in use do not appear to be limiting, with maximum yields in plots of 14.8 t ha^{-1} compared to a record average yield of 9.5 t ha^{-1}. Some producers routinely average over 11.2 t ha^{-1}. The component of yield decline not related to weather is most likely caused by weed and disease effects related to resistance and straw management, and research in these areas must be emphasized.

Conclusions

The current natural resource and production issues facing California rice farmers differ from those in the past in that they must be cast in a changing context of a rapidly growing population, tightening regulations and an enhanced regard for environmental services. The industry is highly organized, willing and able to fund research, promotion and political activities. Furthermore, rice farmers have a high level of technical skills to increase the rapid adoption of practices as well as the political sophistication and understanding of issues to communicate with policy makers. And, through their various leadership organizations, usually speak with a united voice that is very powerful in gaining recognition and favorable policies. Competition for and degradation of the resource base and threats to the production system are real and substantial. The strength of the industry is in its talent for understanding and working to affect the outcome rather than "stone walling" their importance. How well they resolve these issues will depend on success of future research and conservation of resources as well as continued involvement in the policy process.

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WATER REQUIREMENTS AND WATER PRODUCTIVITY OF IRRIGATED LOWLAND RICE (*ORYZA SATIVA* L.) UNDER MOROCCAN CONDITIONS

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Abstract

Rice in Morocco is located in the west region of the country on flat heavy soils with an area of about 12000 ha. This crop is conducted under soil submersion. A tremendous amount of water, between 1500 and 2500 mm, is then used in the rice field by cropping season.

Cultivation of rice under submerged conditions is currently facing several problems due to water shortage. In this order, researches on evapotranspiration and irrigation methods had been conducted in order to use other irrigation alternative than submersion.

The results obtained showed that seasonal average water consumption of rice was 6.8 mm/day with a maximum value of 8.3 mm/day during panicle enlargement stage (R2).

A new irrigation method (T5) among nine tested, had shown high water productivity compared to the submersion (0.80 Kg /m^3 against 0.50 kg/m^3 for the soil submersion) with 40% of water saving and a rice yield significantly equal to the rice yield conducted under soil submersion. The basic feature of this method is that rice is conducted under shallow soil submersion (2 to 5 cm of pounding water depth) only during vegetative phase. There is no water layer above the soil during the reproductive and the mature phases of rice. During those phases, rice is carried out under periodic irrigation with an irrigation interval of one week.

Keywords

lowland irrigated Rice (*Oryza sativa* L.); evapotranspiration; water productivity.

Introduction

Water requirement of rice is very high because it is a semi-aquatic culture which requires more water than other crops. It grows under submerged conditions. The main reason for submerging a rice field is that most rice varieties maintain better growth and produce higher grain yields when grown in a flooded soil than when grown in a nonflooded soil (DeDatta, 1976). This type of irrigation does not help save water and may cause abundant water loss (Shah and Edling, 2000). Rice water requirement ranges between 750 and 2500 mm, with an average value of 1250 mm (Mikkelsen and DeDatta, 1991).

Rice in Morocco occupies less than 0.24 % of the cereal total area. Nearly all the rice crop area is located in the west region of the country on flat heavy soils with an area of about 12000 ha, which it represents about 5 % of irrigated area. Actually there is an expending rice area of more than 1300 ha in the north of Morocco. Rice crop induces a significant development of the industrial activity related on the rice postproduction technology. This sector contributes largely to the rice national consumption and generates regional employment upstream and downstream, with approximately 2.3 million working days by year. Rice cultivation fluctuates vastly. On a potential of 25000 ha in the Gharb plain, the sowed area varies from 500 ha to 9000 ha depending on climatic conditions. This crop is conducted under submerged conditions. A tremendous amount of water is used for the rice fields under this conventional irrigation method. It requires between 1500 and 2500 mm of water for a cropping season.

Cultivation of rice under submerged conditions in Morocco is currently facing several problems due to water shortage. Intensifying rice cropping or increasing its area without proper water management could increase the incidence of water logging, salinity and contamination of underground water by pesticide and nitrogen residues and also increase water loss (Odhiambo and Murty, 1996).

The research currently undertaken concerning rice culture is directed towards the conservation of the environment while testing the increase in the rice water productivity. Among these researches, the effective use of water, take a significant place considering the decrease of water availability (MADRPM, 1999). In this order, researches on evapotranspiration and irrigation methods had been conducted in order to use other irrigation alternative than submersion.

Estimation of evapotranspiration (ET) is an important factor in irrigation management for efficient water use. In fact, good estimates of rice ET provides a basic tool for computing water balance and predicting water availability and requirements (Humphreys et al., 1994; Pereira et al., 1999). The evapotranspiration depends upon the evaporative demand of the atmosphere and on the transport processes of heat and water from soils and plants through the sublayers which are next to the evaporative surfaces and through plant canopies to the outer atmosphere (Kutilek and Nielsen, 1994). This phenomenon is controlled by physiological functions of rice under submerged conditions (Li and Cui, 1994).

Two experimentations were conducted in the main rice region in morocco in order to:

- review field measurements of evapotranspiration from paddy rice field ;
- test alternative irrigation methods to submersion. The aim of this study is to find ways to increase water productivity (WP) in rice field.

Material and methods

The experimental work was conducted in the experiment station of the National Institute of Agronomic Research (INRA) in Morocco, during the summer seasons of 1995, 1996 and 1997 on heavy levelled soils. The experiment site was located in the main rice region (Gharb region) of the country, with a latitude of 34°31'21", a longitude of 6°21'40" and an altitude of 10.5 m.

The field layout of the experiment consisted of four replicated plots with nine irrigation modes in comparison with a control, submersion(T1) (Figure 1). One rice cultivar was used, 'Hayat', a lowland cultivar developed in Morocco and normally grown in submerged paddies.

The evapotranspiration of rice was measured using a microlysimeter designed by (Tomar and O'Toole, 1980) with slight modifications. It was measured only for the submerged treatment (T1).

Total water consumption for each treatment was computed by using a parshall flume, installed in a ditch at the entrance of the experimental site, to record the flow rate.

The carry out of rice from the sowing to 5 leaves stage was the same for all treatments. From 5 leaves stage, each treatment is conducted in a different way (Figure 1).

Crop water productivity is defined by Kassam and Smith (2001) at FAO as "Crop yield/Water consumptively used in ET" and by Moulden (1997) "the physical mass of production or the economic value of production measured against gross inflows, net inflow, depleted water, process depleted water, or available water. Water consumed is essentially evapotranspiration. In this study it is calculated as the grain yield against the total water consumption recorded at the entrance of each treatment. The yield was determined by weighing the grains, obtained in each treatment, at the complete maturity of rice. Harvest was done with grain moisture of 20%. The yield was calculated with 14% of grain water content.

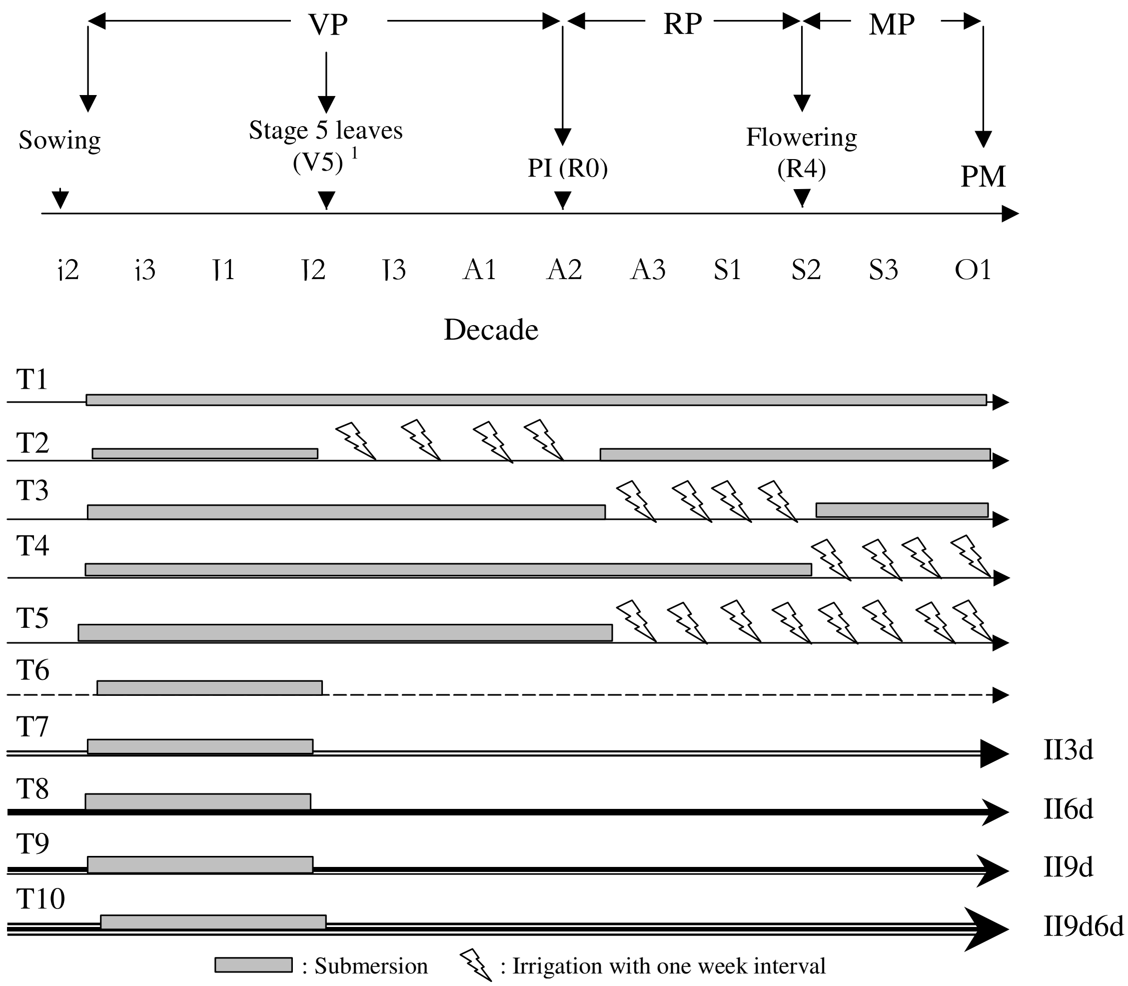

Figure 1 Graphical description of irrigation treatments tested. II3d : Irrigation with 3 days interval during all the growing season; II6d : Irrigation with 6 days intervalle; II9d : Irrigation with 9 days interval; II9d6d : Irrigation with days interval à intervalle during vegetative growth and 6 days interval during the reproductive and mature stages
VP= Vegetative Phase; RP= Reproductive Phase; MP=Mature Phase; PI=Panicle Initiation ; PM= Physiological maturity. Abbreviations: V5: Five leaf stage; R0: Panicle Initiation stage, R4: Flowering stage. [1]: an adaptive system for rice development by Counce et al. (2000)

Agro-morpholgical traits were recorded for each treatment, as plant height, growing cycle length from sowing to flowering, dry matter, leaf area, yield components, industrial yield and technology analysis, but are not presented in this paper.
The statistical analyses of the data were done by using the software SAS based on LSD (5%) for the comparison between treatments and Dunnett (5%) for comparing treatments with the control.

Results and discussion

Rice evapotranspiration

The average values of ET by decade from sowing to physiological maturity of rice are presented in figure 2. For the whole rice growing season, ET was 665 ± 5 mm with a daily average value of 6.7 mm/day. World-wide estimates of rice ET ranged between 450 and 700 mm/season depending on the climate and growing season (Dorrenbos and Kassam, 1980). The mean value of ET represented then 32-47% of rice water requirement under Moroccan conditions (total water consumption varies between 1700 and 2500 mm). The mean seasonal value of ET obtained was in the range of the major areas of rice in Asia and America. In fact ET falls within the range given by many authors, which varies, between 4 and 9 mm/day (Shah and Edling, 2000; Sugimoto, 1976; Wickham and Sen,1978; Yoshida, 1979). In south and Southeast Asia, ET varies from 4.4 to 14.3 mm/day (Tomar and O'Toole, 1979).

ET during the growing season was influenced by climatic conditions when computed over each growing phase. For the vegetative, reproductive and ripening stages in 1996, ET was 8.0, 7.2 and 5.5 mm/day, respectively. The respective data of relative humidity, net radiation and temperatures were RH= 65 , 73 and 74%; Rn=17 , 14.08 and 11.5 Mj/m² and T°C =25.4 , 22.8 and 20.6°c. The average values of rice ET measured by decade, during the two years, showed two picks, where ET reached its maximum value. The first one occurred around 36 days after watering (DAW), on July, before tillering stage where the value of ET was 9.3 mm/day (Figure 2). The Rn and temperatures reached also their maximum values during that period and the relative humidity reached its minimum value (respectively 17.3 Mj/m²; 26.9 °C; 56.8%). The field was not covered yet by rice crop and it was influenced more by climatic conditions. The second pick was shown during the reproductive stage. There was an increase of the mean value of ET during the panicle enlargement (stage R2), before heading stage, between the second and the third decade of August (Figure 2).

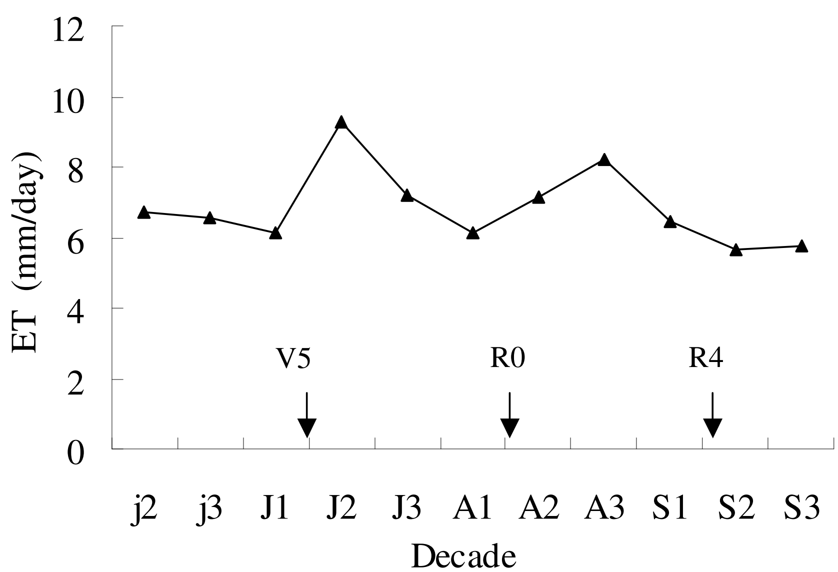

Figure 2 Evolution of rice evapotranspiration (ET) by decade during rice growing season (average of 1995 and 1996).

It reaches a mean value of 8.3 mm/day. However Rn and temperatures were in their decreasing phases. This tendency of ET perhaps might be explained by the fact that some significant regional advection activated by strong hot winds called "Chergui" which occurred usually in the region during the rice growing season and supplied energy for evaporation. This wind occurred frequently between July and August in the Gharb region. As reported by some authors, rice ET might be influenced more by climatic conditions than by crop growing stage (Naito, 1969; Nakagwa, 1976), or it appeared to vary more with crop growth stage (Kutilek and Nielsen, 1994) or it is dependant to the water status in a paddy field (Li and Cui, 1994). Some other authors, found that the evapotranspiration from flooded-irrigated rice in a semi-arid region is 1.7 times that which could be caused by radiation because of a large-scale advection which was present to a marked degree (Evans, 1971).

Rice water consumption and water productivity under different irrigation modes

Treatments tested showed a significant reduction in their average water consumption compared to the control. This reduction allowed an economy of the water of more than 45% for the treatments conducted under intermittent irrigation (T7, T8, T9 and T10) (Table 1). For the treatments where rice is submerged with one or two phases of its development, only the T5 treatment could show an economy of the water consumption of approximately 40% compared to the control. The other remaining treatments, showed only low water saving compared to the control (T1) (around 20 %). The treatment under continuous saturation (T6) allowed a saving in water of 23 % compared to the control. Some studies showed that rice under saturation allows a water saving between 24 % and 58 % compared to the submersion (Soriano and Bhuiyan, 1989; Badawi and Ghanem, 1999), whereas the irrigation with 6 days interval allows only a water saving of 7 to 8 % (Badawi and Ghanem, 1999). Under our conditions, the intermittent irrigation with a 6 days interval (T8) allowed a water economy of 56 % compared to the control.

Table 1 Water consumption (m^3/ha) at the field level for the treatments tested

Treatment	Year			Average	ES	LSD 5%
	1995	1996	1997			
T1	15711 - (b)[1]	12291 - (a)	13714 - (a)	13905-	1962	3138.2
T2	15616[2] ns (b)	10789 * (a)	10835 ns (a)	12413*	486.5	778.2
T3	14969 ns (b)	9640 * (a)	9205 * (a)	11271*	567.0	907.5
T4	15671 ns (c)	11297 * (a)	12570 ns (b)	13179*	449.6	719.2
T5	8812 * (a)	8279 * (a)	8425 * (a)	8505*	459.6	ns
T6		10881 *	10629 *	10755*	247.7	ns
T7		7588 * (b)	6636 * (a)	7112*	70.1	121.3
T8		6563 * (b)	5682 * (a)	6122*	228.3	395.0
T9		6369 * (b)	5405 * (a)	5887*	79.9	137.9
T10		6813 * (b)	5132 * (a)	5972*	120.4	207.8
Dunnet 5%	2222	443.5	4583	613.8		

*Values followed by the same letter are note significantly different (LSD, 5%); ns = no significant, * significant at 5%. [1]Differences into the same year between treatments (Dunnett test) (Vertically). [2]Difference between years for the same treatment (LSD test) (Horizontally).*

Among all the treatments tested, the T5, T8, T9 and T10 showed a WP significantly higher than the control (Table 2). However we can state that when rice is not conducted under soil submersion during its two last growing phases (T5), or during all its growing cycle (T7, T8, T9, T10), a significant increase in WP compared to the control is observed (Table 2). On the other hand, when it is conducted under saturation during all its growing cycle, or under soil submersion during two of its growing phases, the WP is not significantly affected (T2, T3, T4 and T6).

It is publicized that rice conducted under soil saturation gives a WP higher than the soil submersion (0.77-0.93 kg of grain/m3) (Borrell, 1991; Tabbal et al., 1993; Nour and Mahrous, 1994, Badawi and Ghanem, 1999), and the more the irrigation interval is increased, the more the WP decrease because of the yield reduction. This WP varies from 0.71 water kg/m^3 when the interval of irrigation is 6 days to 0.64 water kg/m^3 when the interval of irrigation is 9 days (Nour et al., 1994). The irrigation interval of one week was recommended as the better irrigation interval for rice (Hassan and Sarkar, 1993; Nour et al., 1994). In the present study, the WP was 0.66 kg/m^3 for 3 days irrigation interval (T7) and around 0.80kg/m^3 when the interval of irrigation increases (T8, T9 and T10). Also saturation did not give a high WP because of the low yield obtained.

Table 2. Water productivity of rice (kg /m^3) conducted under different irrigation treatments at the field level.

Treatment	Year			Average	ES	LSD 5%
	1995	1996	1997			
T1	0.38 - (b)[1]	0.75 - (a)	0.41 - (b)	0.50-	0.06	0.09
T2	0.27 * (b)	0.60 ns (a)	0.47 ns (a)	0.42 ns	0.08	0.13
T3	0.37 ns (c)	0.80ns (a)	0.59 ns (b)	0.55 ns	0.06	0.10
T4	0.37 ns (b)	0.70ns (a)	0.45 ns (b)	0.49 ns	0.11	0.18
T5	0.71 * (ab)	0.89ns (a)	0.74 * (ab)	0.80 *	0.18	0.29
T6		0.64ns	0.52 ns	0.58 ns	0.10	ns
T7		0.63ns	0.69 *	0.66 *	0.12	ns
T8		0.64ns	0.93 *	0.77 *	0.21	ns
T9		0.69ns	0.91 *	0.79 *	0.17	ns
T10		0.63ns	0.98 *	0.78 *	0.80	ns
Dunnet 5%	0.12	0.22	0.22	0.13		

*Values followed by the same letter are note significantly different (LSD, 5%); ns = no significant, * significant at 5%. [1]Differences into the same year between treatments (Dunnett test) (Vertically). [2]Difference between years for the same treatment (LSD test) (Horizontally).*

Results of the average water productivity (WP) obtained over three years of study, showed a significant superiority of T5 treatment compared to the control (T1), because the yield was not significantly different from the control (2%) and the water consumption was lowered by about 40 % (Figure 3).

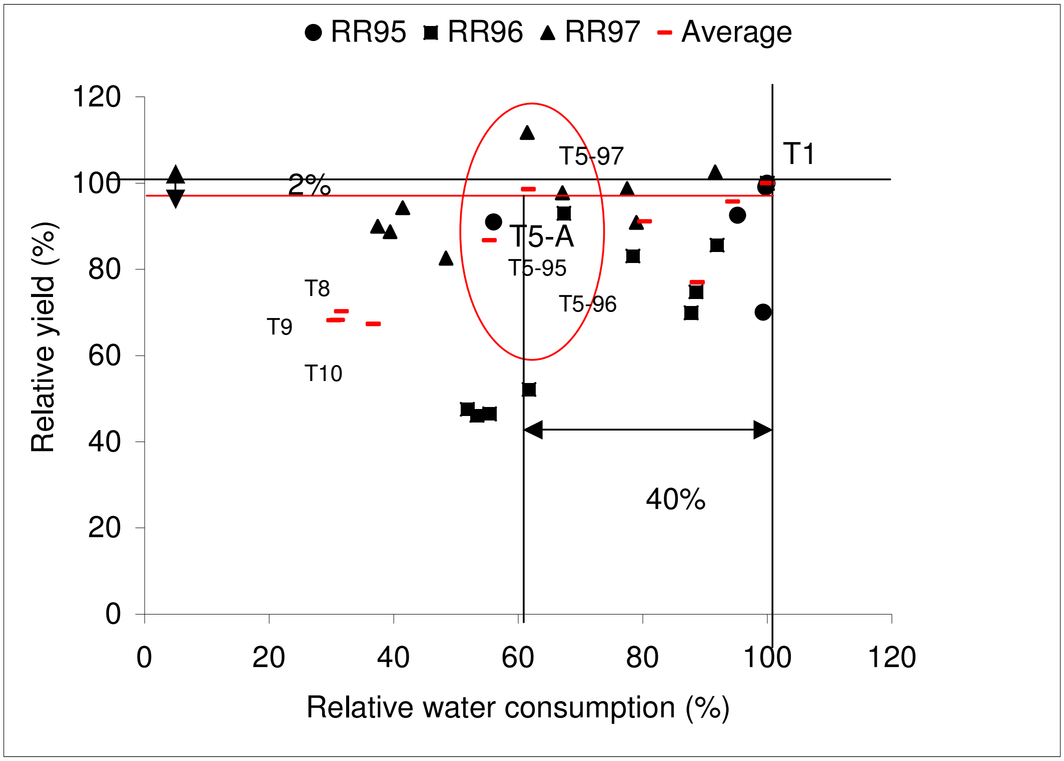

Figure 3. Relative yield (% of the control) versus relative water consumption (% of the control) for all the treatments tested.

Conclusion

Results showed an irrigation mode (T5) among the nine tested which has shown higher water productivity (0.80 Kg of grain/m^3 of water of irrigation against 0.50 kg/m^3 for the submersion). The basic feature of this method is that rice is conducted under shallow submersion (2 to 5 cm of pounding water depth) only during vegetative phase. There is no standing water layer above the soil during the reproductive and the mature phases of rice. During those phases, rice is carried out under periodic irrigation with an irrigation interval of one week. This technique not only saves water (40 %) and keeps up the yield as the rice conducted under soil submersion, but also it keeps down weeds. If other weed control techniques, either by cultural, mechanical or chemical means, are used, the field does not have to be conducted under submersion continuously during the vegetative stage.

If the irrigation mode T5 showed an optimal result to save water without significant reduction in the yield, other modes among those tested, could be interesting if the climatic conditions are worse. Thus, it would be more acceptable to lose 30% on the production for saving 50% of water as shown by T8 irrigation treatment.

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RICE PEST CONTROL WITH MICROWAVE ENERGY AND RICE QUALITY

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Abstract
Alternatives to milled rice disinfestation with methyl bromide have to be found due to 2005 using prohibition of the mentioned chemical. The Polytechnic University of Valencia (UPV), has studied the possibility of microwave energy as an alternative method to control insects in stored rice. Previous works showed 100% insect mortality, evaluating afterwards the possible influence of this type of energy in rice quality. Results showed only minimum quality modifications that could be tolerated, as microwave energy is cleaner than methyl bromide applications. As a consequence, an industrial prototype was developed and experimented during one year in which no insects were found when 170 $J.g^{-1}$ were applied to milled rice. In conclusion, microwave energy is an effective and clean alternative to rice grain chemical disinfestation.

Keywords
Rice; disinfestations; microwave.

Introduction
Methyl bromide using ban in year 2005 (in developed countries) according to the Montreal Protocol, has supposed a big effort in searching more respectable alternatives to the environment. Application of methyl bromide to disinfest rice grain is the most used method around the world, as it is very fast and effective, although it is recently being displaced by phosphine treatments due to 2005 ban. One of the disadvantages of this last chemical could be resistance apparitions in treated insects (Mabbet, 1983). The possibility of using microwave energy to disinfest stored grains was already studied by Hamid, M.A.K. in 1969. In this study, a commercial microwave oven (1.2 kW at 2,45 Ghz) was used and 100% mortality was achieved with temperatures ranging 55–65 ºC in infested wheat samples with *Tribolium confusum*. In all cases hot wheat samples remained in containers at least 30 minutes. In a following work a microwave rice disinfestation method was developed but heating uniformity could not be obtained and rice disinfestation temperature was 80 ºC (Locatelli and Traversa, 1989). Preliminary experiments at UPV demonstrated that total disinfestation of rice was possible without surpassing 60 ºC and using an energy of 100 $J.g^{-1}$ (Rubio *et al*, 1998). After the preliminary assays, four rice varieties widely cultivated in Spain were irradiated with a laboratory prototype at two microwave energy levels (70 and 100 $J.g^{-1}$) and effects on different rice quality attributes were studied. Using microwave energy to disinfest milled rice, slightly modifies rice quality (depending on the variety) although this changes can be assumed as this method is clean, safe and easy to handle.

After the conclusion of the related experiments and the establishing of 100 $J.g^{-1}$ as the suitable energy level to obtain 100% insect mortality, next assay was to test an industrial prototype in order to evaluate if disinfestation efficacy could maintain the same level.

Materials and methods

The experimented rice variety was Senia (*Japonica* type, medium length grain) as it is widely cultivated and consumed in Spain. Grain was already milled and no disinfestation treatments were done, before the microwave one.

Insects of the species *Rhizoperta dominica* were bred in laboratory during two months as it was noticed that this species was the most resistant to irradiation in preliminary assays. Approximately 200 adult insects were placed in each container with 5 kg of rice during two months at room temperature. After this period of time the necessary sample weight of 20 kg for each assay was achieved with four containers (5 kg each). Another common insect in our rice producing area (*Sithophilus oryzae*) was evaluated, because it was thought that there were eggs of these insects in the rice samples previous to the *Rhizoperta dominica* introduction that emerged during the experiment.

Simulation tests (3D electromagnetic suite MAFIA-Maxewell Finite Integration Algorithm) were done to design the industrial prototype, developing a cylindrical oven based on heating uniformity, mutual coupling and distribution mode principles. The developed prototype has a grain bin that feeds uniformly the treatment conduct, designed to perform a continuous treatment of 250 $kg.h^{-1}$ and provided with eight 850 W magnetrons. In order to work properly it requires an electricity supply of 15 $kW.h^{-1}$. The prototype dimensions are 3.8 m height and 1.1 m wide (Díaz-Morcillo *et al*, 2000).

Different 100 kg sets of rice were used in each assay. Several preventive measures were taken in order to guarantee that irradiation dose was the correct one. Therefore it was necessary to wait until the whole rice sample placed in the prototype (before the switch on or before any flow change) came out and continuous regime conditions were achieved. Although the prototype capacity was 20 kg of rice, each assay required a few more quantity. Four treated rice subsamples were taken from the 20 kg sample in each assay and were stored to be evaluated, checking the disinfestation percentage obtained. Some control samples were taken as well before treatment.

Milled rice with the insects was placed into the upper bin of the industrial prototype and when it was full, irradiation started. Different microwave energy flow rates were tested, from 60 to 250 $J.g^{-1}$.

A thermocouple situated inside the exit conduct was used to measure temperature and another device (pirometer) to measure grain temperature was placed at the industrial prototype exit. Different quality attributes were evaluated, like broken grain percentage, number of fissured grains and gelatinization time.

After irradiation rice treated samples were taken and stored in non hermetic plastic containers to ensure insect survival during storing time (1 year) at room temperature. Every 15 days, evaluations of the stored samples were done, rejecting those samples with living insects.

Results and Discussion

Insect inventories showed a total mortality since flow regulator was in the 45-40% position. It can be assured a complete disinfestation with a 40% flow rate (122.5 $kg.h^{-1}$), that is equivalent to an applied microwave energy of 170 $J.g^{-1}$.

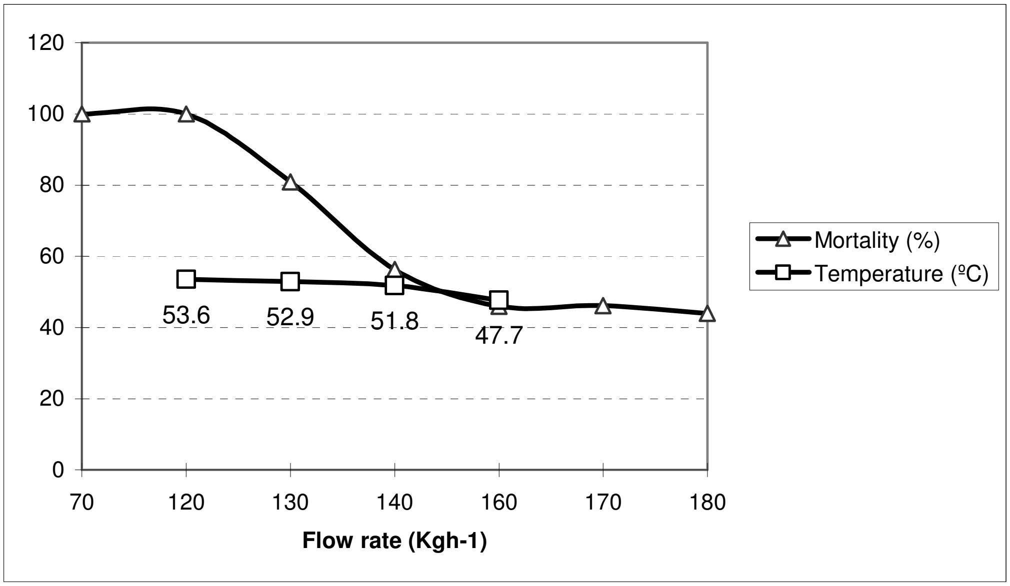

Figure 1 Mortality of detected insects during 12 months storage and rice achieved temperature vs. flow rate of the industrial prototype.

As it can be seen in Figure 1, with low flow rates like 70 kg.h^{-1} total insect mortality was achieved, however flow rates increases denote a decrease in insect mortality, because smaller flow rates result in greater doses of applied microwave energy. The applied microwave energy in the industrial prototype is higher than the laboratory prototype energy, because of the big energy wastes produced when rice flows under continuous regime through the prototype, being the achieved rice temperature less than 60 ºC.
Between both temperature measuring devices (thermocouple and pyrometer) the grain cool down, what implies 2-3 ºC of difference. The energy flow rate that gave a total disinfestation caused a 53.5 ºC rice temperature. It was also evaluated presence of broken and fissured grains, not finding any relation between the achieved grain temperature after treatment and the broken grain increasing, however there was an slightly significant increasing in the percentage of fissured grains (Díaz-Morcillo, 2002). Those samples showing total disinfestation were evaluated as well, in terms of gelatinization time; it was observed a 1 minute decrease of the gelatinization time vs. control rice without treatment.

Conclusions

170 J.g^{-1} is the necessary microwave energy to have a total disinfestation of rice with the industrial prototype, achieving rice grain at temperature of 53.6 ºC approximately. Quality changes in rice produced by irradiation are acceptable; taking into account that this type of energy is easy to handle, respectful with the environment and does not generate any type of residues in rice. Economic viability depends on the costs of changing methyl bromide tanks by the microwave energy radiation device.

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AN INTEGRATED STUDY OF THE DEVELOPMENT OF ORGANIC RICE CULTIVATION IN THE CAMARGUE (FRANCE)

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Abstract

In the Camargue, rice and durum wheat are associated in rotations that have an ambivalent ecological impact: on the one hand, these two crops contribute to the preservation of the surrounding ecosystem, while on the other hand, when cropped intensively, they can threaten the ecological equilibrium of this protected area. In this context, organic agriculture would seem to be an alternative adopted by a certain number of producers and processors. However, the pioneers of this practice encounter numerous problems, both agronomic and economic. The study presented here aims: to construct a pluridisciplinary approach to analyse the conditions of the development of organic cereal cultivation in the Camargue: to identify the principal factors that limit the development of this new practice: to produce knowledge useful in helping ricegrowers put into practice organic cropping systems.

Keywords

Organic rice; Camargue; interdisciplinary approach; agronomy; ecology; sociology; weed science.

Introduction

In the two regions Languedoc-Roussillon and Provence-Alpes-Côtes d'Azur (P.A.C.A.), durum wheat and rice accounts for nearly 160,000 hectares (20,000 ha of rice, 140,000 ha of durum wheat). In the Camargue, these two crops are associated in crop rotations, wifh an ambivalent ecological impact. On the one hand they contribute to the maintenance of the surrounding ecosystem (soil desalinisation due to the flooding of ricefields, weed control and soil structuring due to wheat cropping) and on the other hand, when managed intensively, present a risk to the ecological equilibrium of a conservation area (National Regional Park and National Reserve).

Today questions are raised concerning these two crops as seen within their mass production logic (conventional agriculture, undifferentiated markets) because of fierce competition from other Mediterranean production areas, the threat to regulatory protection (the Common Agricultural policy, P.A.C., subsidy) and agronomic difficulties related to climatic constraints (temperature for rice, hydraulics for durum wheat).

In this context, organic agriculture appears as an alternative adopted by a certain number of producers and processors. Beyond responding to an ever-growing demand for organic rice, semolina and pasta, the development of these networks could be the opportunity (i) on a regional level, to create a commercial advantage by combining the Mediterranean identity with the credibility of a national certificate, (ii) at the Camargue level, to fully assure the durability of a protected sensitive ecosystem.

However, the first producers to adopt this practice encounter numerous problems both agronomic and economic:

- the lack of technical data (weeds, fertilisation, varieties),
- the frequency and management of the durum wheat/rice rotation
- the economic viability of switching to organic farming.

What's more, other professionals (breeders, collectors, processors) question how to adapt to or anticipate the development of this new agricultural prototype.

Interdisciplinarity, the first scientific challenge.
This was conceived following the hypothesis that a new agricultural prototype should be approached in an interdisciplinary way, to both find practical solutions and help advance disciplinary investigations. The main agricultural organisations and participants involved in the development of organic cereal networks throughout the Languedoc and P.A.C.A. regions were contacted and accepted to participate in a common project.
The aim is thus to work towards a pluridisciplinary and reflexive analysis of the building of collective innovation processes associating producers, collect/process organisations and researchers of different disciplines to resolve jointly and progressively agronomic and economic problems.
Interdisciplinarity is defined as the joint research of several disciplines concerned by a common object, here being the cereal production in Camargue soil conditions in the framework of organic farming specifications. The aim is to be able to cross-examine the questions that led to and that are tackled by the project, the methods and the results of each participating discipline; agronomy, ecology, genetics, weed science and sociology. The need for such a questioning concerning organic farming is all the greater as this prototype profoundly implicates the experimental sciences. It is indeed based on the principle of environmental artificialisation which entails the taking into account of a number of elements that don't respect disciplinary borders and highlights the diversity of choices for applied sciences. While shaking up disciplinary attitudes this prototype is in harmony with a social movement to which researchers must respond, as must decision-makers of the research institutions who support their work.
In the construction of the project, an emphasis was placed on the organisation of centres of activities based on different themes « associating a limited number of disciplines so as to initiate a number of various collective training processes that contribute to a progression towards a pluridisciplinary approach associating all partners ».
In practice, the limited participation of partners to the project as well as the number and the availability of scientists prevented a formalised multi-centred functioning, and the activity centres are mainly reduced to just one researcher from the relevant core discipline. In reality, the organisation of the interdisciplinary approach was developed by actual events; either by the crop cycle imposing a schedule on the different disciplines involved (agronomy, ecology, genetics, weed science, sociology), or by production deadlines to which the collection was subjected.

Activities
Activities retained as contributing to interdisciplinarity are those that generate interactions, one to one or public, between researchers of different disciplines concerning common elementary objectives. Their cognitive content is that of the explanation of the manner that each one has to qualify these objectives, of the contribution to the group production of mutual knowledge, and of the establishment of rules and of creating confidence. They thus help define the role of each implicated scientist, regarding the type of question to address as much

as the participation within the interdisciplinary work group. They also help to understand the institutional conditions within which each person situates his activity in the group and to evaluate available resources.

Activities centred on these interactions are extremely varied according to the actors implicated and action developed. In addition, they are by definition, complex in the sense that they associate, to different degrees, exchange and co-construction on the one hand, and on the other simple presentation of facts and results.

Nevertheless, for clarity, they can be classed into two groups. In the first activity class are retained:

- in situ visits, field tours, interviews with actors,
- joint participation in meetings initiated by members of the project, open to partners and other colleagues. They can also result following invitation from outside organisations
- the diffusion of various papers from the different disciplines
- the setting-up of training programmes on a subject when there is parallel intervention

In the second group, activities are presented whose aim is to encourage exchange and debate between concerned researchers leading to objectivisation and formalisation of actual knowledge and of the project situation:

- joint drafting of replies to tenders – programming and conception of various interventions (holding a meeting of organic farmers)
- joint supervision of student work
- the setting up of training programmes on subjects jointly constructed and with a common objective for associated scientists.

The establishment of a set of references of situations encountered

These different activities nourish and instigate dialogue between scientists. They are the basis for the production of a common bank of knowledge which consists of situation or case references. These are intermediary objects in that they are the result of the acceptation of the work definition of objects of common interest. Everyone sees them from their viewpoint but responds to a common designation, a qualification richer than a single disciplinary viewpoint, and an awareness relatively alert to all the elements that influence the studied object. These work definitions, established by practice rather than formal procedure, position the aforementioned, stimulating the contextualisation of disciplinary contributions. However they remain sufficiently vague so as to let each person identify, behind these denominations, that which is relevant to his discipline.

The research objects cover the entire range of components of an « agricultural prototype ». They permit disciplines to come together, for the most part two by two, but sometimes as well in a bigger group. They involve:

- field parcels and their biophysic characteristics, soil and populations
- technical objects : seeds, inputs, crop management sequence
- systems of activity
- farmers and their relationships
- production and processing network personnel, and local network types
- counsellors (organic and conventional)

This list is far from complete and reveals gaps concerning the natural environment (irrigation water, lagoons…), biological life in the soil and micro-economic aspects. Contacts between agronomy and sociology are by far the most frequent. This is of course mainly due to the physical and institutional proximity at the Innovation Mixed Research Unit (U.M.R.). It is though also due to the fact that these disciplines have a great number of research objects in common through which they embrace their own questioning of the studied situations and, in particular, agricultural practices. By consequence numerous activities are jointly led which multiplies even more their contact with the aim of stimulating cognitive exchanges.

The building of partnerships

Beyond reference sets, research training also embraces the manner actors function together, formally or informally, as well as the resources, particularly cognitive, available to the project.

The programme was build on the presence in the field for the last fifteen years of agronomists (led by J.C. Mouret) from the U.M.R. Innovation and on the realisation of a survey design concerning conventional field parcels as well as more and more organic situations (Dreyfus, Mouret, 2002)

Since 1998, researchers of other disciplines (weed science, ecology) and of other institutions (C.I.R.A.D.), Tour de Valat Organic station) interested by organic development have already been in contact with the agronomists.

This beginning partly accounts for the gaps and weaknesses in the project. The former are related to the non-consideration during drafting of certain fundamental aspects of the new area of study. This concerns hydrology and questions related to the quality of irrigation and lagoon water. Also the ecology of the microbe population and the functioning of the rhizosphere are essential elements for a study of soil fertility, yet are not included in the project. The latter are related to a lack of preparatory work which prevented a clear appraisal of commitments taken and an evaluation of the contribution reasonably expected of everyone concerned. Indeed, the various institutions employing researchers place different degrees of importance on the question of organic agriculture. Also, researchers not in contact with members of the work group never significantly participated in prior work. Thus can be found an important weakness concerning rice genetics, or again, ecophysiology.

Besides unequal scientific relations, the partnership with professional organisations could not fully operate. Up to the present these institutions worked within the dominant framework of conventional agriculture. Organic questions represent a minor part of their activities.

Concerning producers themselves, one of the programme objectives is to facilitate their contribution to the construction of a system of collective action aimed at producing knowledge relevant to their action. Because of their small number, their diversity and the absence of representative organisations, agronomists and sociologists endeavoured to contact individually each producer.

In any case, professional partners are more and more interested by the subject of organic farming. The scientific community is organising itself within transversal structures dealing with this question. The work undertaken will thus rapidly be taken into account, and the confidence built within the group will help to tackle more directly the necessary construction of formalised scientific and professional partnership.

Nevertheless, beyond these advantages, it is the disciplinary interest that there is to work together and to have available an enriched set of references enabling the production of quality work that bodes well for the future of this research action.

For the sociologist, the weed scientist, the geneticist, the ecologist, the work of the agronomist (rooted for years in the dynamics of local apprenticeship) is the indispensable key to understand the real environment. When experimental sciences come up against a new paradigm, they are then an obligatory « pass point ». The results presented below cannot be overloaded by methodology. However it is only after detailed discussion with them that the different protocols of all the other disciplines are drawn up.

A presentation of the results of different disciplines

Agronomy aspects

Within the framework of an integrated research approach involving sociology, economy, ecology and genetics, the agronomy programme realised four specific research actions:

- an analysis of the functioning of organic crop cultivation systems
- a spatio-temporal field survey of organic and conventional ricefields
- experiments aimed at testing the effects of mechanical weeding in organic ricegrowing
- a survey of the weed population in Camargue ricefields

An analysis of the functioning of organic crop cultivation systems in the Camargue

In-depth interviews of a directive or semi-directive nature dealing with the manner in which farmers reason and put into practice their organic crop cultivation systems have helped to identify and analyse

- the different types of farms, totally or partially converted to organic practices
- the technical aspects of the conversion period
- crop cultivation systems employed and their agronomic logic
- relationships between rotation and crop planning
- the consequence of these agricultural activities on the spreading of the work load throughout the year

Crop cultivation systems were analysed from three angles:

- the identification of rules of decision that govern the installation of rotations and crop planning
- associated crop management sequences
- the overall coherence

Rice management within an organic crop cultivation system cannot just be limited to practices operated during a given season. It must take into account all practices realised during the management of the rotation.

Strategies of conversion to organic farming and the management of the organic rice-based cultivation systems that result are strongly correlated to farmer motivation. Two distinct groups have been identified:

- pioneers, motivated for ethical reasons and converted to organic farming around twenty years ago when the first commercial niches for organic products were established. Within this group, crop rotations include diverse species (pulses for example) and crop cultivation practices (hand weeding) are relatively stabilised. On these farms, most, if not the whole of the Utilisable Agricultural Land Area (S.A.U.) is organically managed
- the newly converted, for whom recent conversion can be seen as a timely strategy to counter difficulties in the conventional sector. The sought objective is to ensure the viability of the farm by seeking the most economic production combinations. These farmers choose mixed cropping systems (organic and conventional) in order to limit risks related to a technical and/or economic failure of the organic production system. On these farms, crop cultivation systems are not stabilised, crop rotations little established and cultivation practices, while respecting organic specifications, refer to conventional practices.

Our work was essentially centred on the functioning of present-day organic crop cultivation systems. Thus overall perspectives of these farms could not be established, so no prevision is possible of the future functioning of these crop cultivation systems that takes into account the evolution of technico-economic factors. It would therefore be relevant to use simulations by means of an appropriate tool to assess the reproducibility and the durability of these systems in various scenarios with the objective of helping farmers in their decision making.

Spatio-temporal ricefield surveys

This research action had three objectives:

- to realise an agronomic diagnostic based on a study of the relationship between the effects of crop management on the environment along with the climate and the consequences on yield variability
- to constitute a suitable observatory for the gathering of information specific to each discipline involved in the project
- to create conditions favourable for the putting into practice the interdisciplinary approach

These last two objectives are dealt with elsewhere in the chapters on disciplinary and interdisciplinary advances.

The agronomic diagnostic underlines a very important variability in paddy rice yield. For the year 2002 the average yield was 3,5 t/ha with a minimum of 0,5 t/ha and a maximum of 7,0 t/ha. Seventy five per cent of this variability is explained by the weed aerial biomass at harvest. In spite of a wide range of weed species, two groups are the most frequent and the most abundant (Echinchloa and Cyperaceae). Weed infestation is related to the nature of the preceding crop and the time lapse between two rice years. Fields where the preceding « crop » was « new land » comprising of wasteland or old prairies are only lightly infested by weeds. Opposed to this fields parcels with a second or third successive rice crop are very infested except for parcels surveyed in 2003 which were manually weeded and where at the same time a complex management of weed control was imposed throughout crop cultivation and between cropping. With durum wheat or alpha-alpha as a preceding crop, weed infestation was always important but variable according to intercrop management (false seeding for example).

The effects of mechanical weeding in organic ricegrowing

An experiment carried out « in the field » in 2002 demonstrated that it was possible to realise a mechanical weeding operation in a flooded ricefield. In the Camargue, rice is traditionally seeded directly into water. In order to test the effect of mechanical weeding it was necessary to adapt a crop management system so as to sow in lines to enable the passage of a tractor drawn cultivator. The experiment results show a positive effect of the weeding on paddy grain yield. The weeding resulted in a significant reduction of weed infestation between the lines and by consequence competition with the rice was diminished. However, this weeding method, or at least the cultivator used in the experiment, did not eliminate weeds in the row and consequently, although yield was significantly higher than in the control, it was unsatisfactory.

Thus, within the conditions of the experiment (field parcel with a second successive rice crop, and the instigation of an operation entailing a modification to the cropping sequence), the result did not incite the farmer to develop this technique.

Mechanical weeding must be seen as an operation that is complementary to other priority interventions that aim to significantly reduce the weed grain stock before crop installation. Nevertheless it would be perhaps beneficial to conceive a weeding tool that diminished weed competition on the row, and this in relation to the development stage of the rice crop. Thoughts turn for example to a form of weeding tool, which would slightly turn over the soil, burying weed seedlings without derooting the rice plants. Apart from the effects of weeding, perhaps it would be useful to analyse the effect of working the soil on re-oxygenating the flooded environment and the consequences on the evolutionary dynamics of nutritive elements; mineral nitrogen in particular.

A weed survey of Camargue ricefields

Weed control in conventional ricegrowing is essentially centred upon the use of herbicides. Their suppression in organic systems has not, in the majority of cases, been replaced by other control techniques. In 2002, a weed survey of Camargue ricefields covered 218 parcels, of which 29 were organically cultivated. The analysis of floristic notations illustrates the important weed population in organic managed fields. In these fields 61% are very infested and only 13% could be considered sufficiently weed-free, whereas these proportions for fields conventionally managed are 5% and 56% respectively.
The main species are Poaceae, as *Echinochloa crus-galli* (barnyard grass) or *Oryza sativa* (red rice), Cyperaceae (« triangles ») mainly *Scirpus maritimus*, *Scirpus mucronatus* and *Cyperus difformis*, and Dicotyledones such as *Lindernia dubia* or *Ammania coccinea*.
During the 2003 season, floristic notations carried out in organically managed fields chosen for the agronomic survey confirmed these results. Weed control begins with the ability to identify different weeds and by a better knowledge of damage caused by the principal species: a manual presenting the flora of the Camargue ricegrowing environment is being prepared and a assessment of weed competition with the rice crop has begun (with *Echinochloa crus-galli* in 2003). An inventory of control methods used by ricegrowers will equally be established by analysing the agronomic field survey and will be published in a booklet to be widely diffused.
Crop management sequence experiments are necessary to establish the worth of false-seeding operations, of increasing plant density and of mechanical weeding, as well as water management, especially between main crops.

Ecology aspects

The purpose of our research action was to analyse cultural practices (conventional as opposed to organic) on invertebrate and vertebrate populations in Camargue ricefields. Places designated for the gathering of information were within the framework of field parcels used for the agronomic survey.
Populations of epibenthic macroinvertebrates (invertebrates whose size is equal to or superior to 1mm present on the surface, in the water column or in the first few centimetres of sediment) constitute an important source of food for water birds (of potential importance in preservation terms, the Camargue being classified as an important international site for water birds, a migratory halt of primary importance). Our results show that certain groups of invertebrates are particularly affected by conventional practices. This is true for coleopteran, heteropteres in June and odonates during August. Contrarily molluscs prosper much more with conventional cultivation. The absence of species or groups of species and the dominance (abundance) of certain groups such as molluscs would seem to reflect the type of agriculture. From this point of view, results obtained on several of the ricegrowers farms of the agronomic survey network, would cast a doubt on cultivation practices announced (the non-use of insecticides for example).
The analysis (G.L.M.) illustrates the major role of pesticide (Fipronil) of which the sole purpose is to control chironomidae grubs. The low number of predators in conventionally managed fields (2 to 12% as opposed to 18 to 40% in organic conditions throughout the growing period), indirectly leads to an absence of effect of the treatment on Chironomidea (a phenomenon already observed in the case of other insects). If, from an agronomic point of view, the insecticide application doesn't achieve its aim, two of the invertebrate groups heavily affected (coleopteran, Odonates) are among the favourite prey of an emblematic group of Mediterranean wetland and ricefield birds: the heron.

As regards amphibian populations, two taxons, mainly *Hyla meridionalis* and more rarely *Rana sp.* have been catalogued. The abundance of the two species varies throughout the season. Tree frog tadpoles have been observed from the very first sampling period, whereas green frog tadpoles appeared later. This first study concerning amphibians in Camargue ricefields would indicate that amphibians are more abundant in organic systems than in conventional. In addition, organic fields seem more favourable for the reproduction of both species. Old ricefields attract a larger number of individuals than those more recently flooded due to a fidelity to the laying site from one year to another, as seen concerning frogs. The situation of the field parcel (the presence of a water supply near the ricefield, a cover of vegetation around the borders), as well as other cropping practices such as water management (the role of draining) are equally important factors concerning the presence of amphibians in ricefields.
During this study only the most visible anomalies were sought, the real impact of pesticides used in ricefields on amphibian development could not be clearly illustrated. Essentially future experiments ought to concern amphibians (a group threatened on a worldwide scale). The objective would then be to test the effect of different pesticides individually and in combination on the reproduction and development of grubs.

Genetic aspects
During this first phase of the study, an analysis was undertaken concerning the variety selection criteria adapted to the organic cultivation of durum wheat. Rice variety selection will be studied during the second phase to start in 2005.

Sociology aspects
The principal objective of the sociologist is to study, at the moment of the development of a new agricultural prototype:

- the conditions of the restructuring of agronomic research on conventional ricegrowing practices based on a network of referential field parcels
- local dynamics of knowledge nourished by the cognitive strategies of actors concerned

From an operational point of view, the sociologist intervenes, interacts with his study object by attempting to help in an evolution towards a collective system of action destined to jointly build up practical knowledge for organic ricegrowing.
Work is centred around three programmes:

- to characterise and to categorise the various training strategies relating to underlying technical questions in the aim of assuring their diversity but also their similarities and complementarities. Data gathering over, they are now being treated. However the drawing up of a typology has already been possible. This distinguishes six cognitive blocks more or less engaged in innovation processes either active or temporarily stabilised. Reflecting the agronomic survey, cropping systems are again an important explanatory variable of this typology. The importance of cereals in the crop rotation or the presence of forage and the availability of organic matter are discriminatory. The methods and the motivations for conversion account for a lot in the difference between blocks. This typology has been completed by a first contact with the technical advice network. Generally interrelationships are few and infrequent and co-operative technicians and input supply agents represent the most important source of apprenticeship, but not for every block.
- to employ a research/intervention type of approach destined to create a structure for, and to stimulate, dialogue between producers and between producers and researchers. A first meeting was organised, each and every organic ricegrower being invited. 30% attended

and met for the first time their colleagues. The different cognitive blocks, previously identified during the programme, were represented. Themes of interest shared by the participants in all their diversity were brought to the fore. They rejoin previous work as well as the agronomic investigations. Already the production of a pamphlet on weed control practices is programmed for the end of the year. Other meetings are being prepared.

- to construct the reflexivity of the group of researchers so as to analyse the emergence conditions (i) of the interdisciplinary group to which they belong and (ii) of the professional and scientific partnerships in which they engage. This research activity is based on data gathered by the observations of the participating sociologist. At the same time, the agronomist, having the most direct contact in the field, keeps a diary to register events, activities, interviews which feed a database of paramount importance for enriching his own discipline, interdisciplinarity and collective partnership; the group only treated this theme during a meeting concerning the writing of the present paper. An outside viewpoint could prove necessary to develop this analysis.

Conclusion

The recent development of organic ricegrowing in the Camargue has been accompanied by the emergence, for farmers committed to this production system, of numerous technical, economical and social questions. To attempt overall reply to these questions, an interdisciplinary and interinstitutional approach has been envisaged which associates agronomists, ecologists, sociologists, genetic scientists and economists. Interdisciplinarity became a scientific challenge centred on common activities necessitated by the building-up of sets of situation references and by the establishment of a researcher/actor relationship. The first results presented in this communication bring partial discipline replies to the aforementioned questions. A global analysis brings to light transversal questions, in particular:

- nitrogen dynamics related to organic fertilisation practices, crop precedents and genotypes
- the collective analysis of organic ricegrowing practices and the production of agronomic knowledge concerning the new agricultural prototype in the Camargue
- the prospective development of organic crop rotations and their impact on the natural fauna

These questions aren't the only ones raised by the development of organic agriculture in the Camargue. However, these are the ones that stimulate, at the present stage of the development programme, the interactions between members of the work group. They incite however a reinforcement and an enlargement of scientific and professional partnerships.

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MEETING THE CHALLENGES OF GLOBAL RICE PRODUCTION

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Abstract

Rice is the second most widely grown cereal crop and the staple food for more than half the world's population. More than 3 billion people consume more than 100 kg of rice per year. Rice is cultivated on 155.5 million ha with an average growth rate of 0.39% a year, in the last 30 years. In the near future, the possibility for expanding area under rice-based systems will remain very limited because of the scarcity of water resources for agriculture all over the world, the expansion of urban and industrial sectors in Asia, the high costs of developing new lands that are suited for rice production in Sub-Saharan Africa and Latin America. The average growth rate of rice yield, that was 3.68% per year in the early 1980s decreased to 0.74% per year in the late 1990s. Several factors may contribute to the decline of the areas of cultivation and yield growth. The most important of these fators are: stagnation of the yielding potential of the high yileding varieties, declining productivity in intensive rice production systems, pressures from abiotic and biotic stresses, low returns in develping countries, increasing production costs in industrialized countries and increasing public concern for the protection of the environmental resources.

One of the most effective means of addressing the issues in rice cultivation and raising the average yields at the farm level is through research and subsequent dissemination of the resulting data. Rice science has made some considerable progress. In the area of rice varietal improvement, recent advances in hybrid rice and the new rice for Africa (NERICA) are just two examples of the successful contributions of science to the development of rice.

Research could also reduce the gap between the potential yiel obtained in the experimental stations and the actual yield obtained in the fields. This could be possible by promoting and developing rice integrated crop management (RICM) systems for improving productivity and reducing the production cost per unit of output.

The need for a sustainable increase in rice production affects everyone. The International Year of Rice provide us with a chance to improve food security, alleviate poverty and preserve the environment for the billions of people for whom *Rice is Life.*

Introduction

In December 2002, the General Assembly of the United Nations declared 2004 the International Year of Rice. The dedication of an International Year after a single crop is unprecedented in the history of the United Nations. It reflects the fact that rice is not only the primary food source for over three billion people, but also a focus within complex rice-based production ecosystems that influences issues of global concern, such as food security, poverty alleviation, preservation of cultural heritage and sustainable development. The declaration of the International Year of Rice was also a recognition of the serious constraints that the rice-based systems are facing today and the need for concerted efforts to overcome them. The world's population is still growing, many rice farmers and their families are still living in poverty and there are still about 800 million people in the world today who are suffering from malnutrition; while rice production is facing declining yield growth rates, soil fertility depletion, negative environmental effects, falling rice prices, and increased production costs.

Fortunately, there are emerging technologies and innovations in the field of technology transfer, which are promising for enhancing the productivity and efficiency of rice-based systems to safeguard the food security and to reduce the rural poverty. The implementation of the International Year of Rice provides an opportunity for all concerned stakeholders to meet the challenges of rice production.

Major Challenges Of Rice Production

Global rice production, so far, has been able to meet popular demand. However, its ability to continue this performance is in question unless appropriate action will be taken in the near future. The global rice production is faced with numerous issues, but in the immediate future, the main challenges of rice production include the increasing demand for rice by the population growth, the limited possibility for expanding the rice harvested area, the declining rice yield growth, and the low return from rice production.

The Increasing Demand for Rice

In 2001, the world's population consumed, as food, more rice than wheat and/or maize, the other two major cereals. In the same year, more than 3.1 billion people consumed over 100 kg of rice (Table 1). Worldwide, rice provides 27% of dietary energy supply, and 20% of dietary protein. Rice production nearly doubled during the period from 1970 (316 M tonnes) to 2001 (592.8 M tonnes). The average growth rate of rice production during this period was 2.29% per year (Table 2). However, since 2000, the world's rice production has declined and it has been less than the world rice consumption (Figure 1). Against this background, the world's population was projected to increase from 6.13 billion in 2001 to 8.27 billion in 2030. Rice demand is projected to increase from 571.9 million tons in 2001 to 771.1 million tons in 2030. The food security of the growing population in the next 30 years requires a reversion of the decline in rice production growth that has been observed since 2000.

Table 1. Maize, Rice and Wheat: Production, Consumption as Food and Number of People with more than 100 kg supply in 2001 (Source of data: FAOSTAT)

	Maize	Rice, Paddy	Wheat
Food (Mt)	112,573,807	517,969,638	419,090,760
Population with > 100 kg supply/Cap (1000)	185,895	3,143,875	991,557

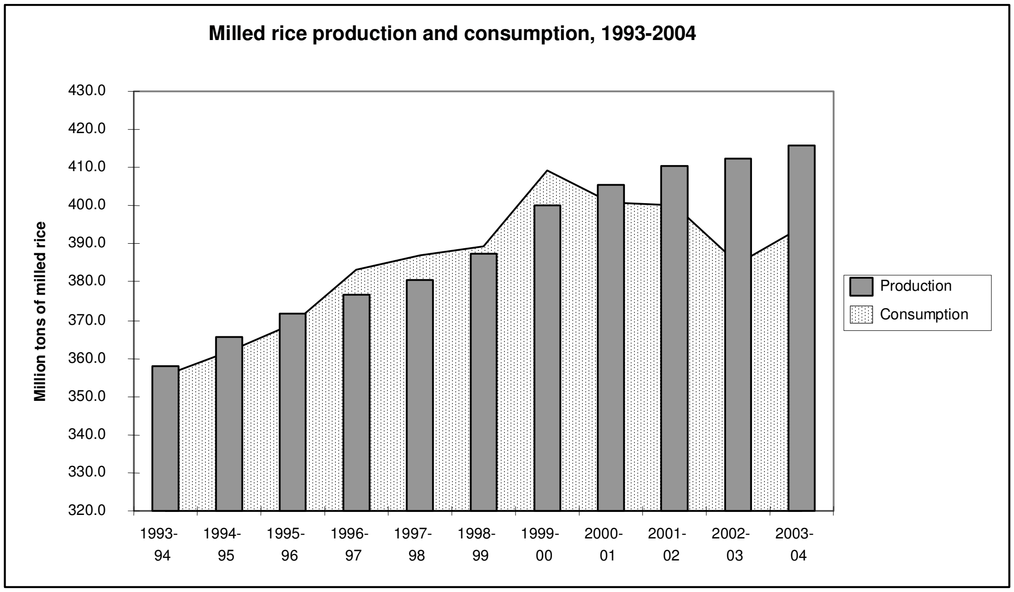

Figure 1 World's rice production and consumption, 1993 to 2003 (Source of data: Calpe, personal communication).

The Limited Growth in Rice Harvested Area

During the 1970-2001 period, the rice harvested area grew only about 0.39% per year, which resulted in an increase from 133 M ha in 1970 to 155.5 M ha in 2001 (Table 2). This increase in the harvested area was due mainly to the increase in cropping intensification. In tropical climate areas, with favorable temperature regimes, two or more rice crops can be grown on the same land in a year. Rice-rice and rice-other crop-rice systems are very popular with farmers in Bangladesh, soutnern China, southern India, Indonesia, Myanmar, Philippines and Vietnam. Rice-rice systems are also widely practiced in many irrigated lands in sub-Saharan Africa.

Table 2 Population and rice production, harvested area and yield during 1970-2001 (Source of data: FAOSTAT)

	1970	2001	Growth rate
Population	3.69 billions	6.13 billions	1.66 %/year
Rice production	316.3 M tonnes	592.8 M tonnes	2.29 %/year
Rice harvested area	133 M ha	155.5 M ha	0.39 %/year
Rice yield	2,377 kg/ha	3,912 kg/ha	1.90 %/year

In the near future, the possibility for expanding area under rice-based systems will remain very limited as the competition for land in major rice producing countries in Asia has been very intensive due to the expansion of urban and industrial sectors. In a number of countries in Sub-Saharan Africa and Latin America, there are still considerable land areas that are suited for rice production. The high costs of developing this land, however, are the major constraint. On the other hand, the expansion of the total rice harvested area through rice-cropping intensification may also be limited in the future due to, at least, one of the following factors:

- The scarcity of water resources due to competition from urban and industrial sectors, especially in Asia. Inadequate water supply is also the factor that limits the area cultivated to rice in Spain, Portugal, Egypt and Australia. In fact, the Egyptian government has a plan to limit the area under rice production due to limited water supply.

- A reduction in investments on the development of irrigation infrastructures. For example, there has been a decline of about 60% in investments in irrigation in Asia since the 1960s.
- The promotion of crop diversification of the mono-intensive rice production systems by rice farmers for higher incomes.

The Declining Yield Growth

The average growth rate of rice yield during the last three decades was 1.9% per year, which is about 5 times of the growth rate of the harvested area (Table 2). Therefore, much of the production increase in the past was due to the rice yield growth. However, a close examination of the growth rate of rice yield shows that yield growth rate has rapidly declined since the early 1980s. The average growth rate of rice yield was 3.68% per year in 1981-85 period, 2.28% per year in 1986-90 period, 0.91% per year in 1991-95 period and only 0.74% per year in 1996-2000 period (Fig. 2). Several factors have contributed to the rapid decline in yield growth since the early 1980s; among them the following factors are important:

Stagnation of the yielding potential of high yielding varieties

Several hundred high yielding varieties have been released for cultivation by international and national agricultural research institutions since the introduction of IR8 in 1966. However, the yield per season of IR8 has never been better, although yield per day may be increased with the development of early maturing varieties.

Declining productivity in intensive rice production systems

Reports emerged in the early 1980s showing a yield decline in the intensively cultivated rice plots within research stations in the Philippines. Subsequent studies in several other countries also reported yield declines (Tran and Nguyen, 2001). More recent analyses support the earlier reports of declining yields from research trials but could not ascertain the extent of the decline in other areas of Asia, due to year to year variation in yields (Dave and Doberman, 2001) . There are other observations indicating that continuous cultivation of irrigated rice, where the soil is maintained in anaerobic conditions for prolonged periods, does result in disorders that limit yield (Pulver and Nguyen, 1999).

Pressures from abiotic stresses such as salinity, low temperature, and drought:

- Increased salinity resulting from long-term rice production in irrigated areas worldwide was reported by Pingali and Rosegrant (1996) and in Australia by McDonald (1994). Low rice yields due to salinity have also been reported in southern Spain (Aguilar et al, 1997). In the delta of the Kuban River in Krasnodar, (Russia Federation), there are about 110,000 ha of saline soils and many of them have been grown to rice (Zelensky, personal communication).
- Rice production in temperate and subtropical climate zones is limited by low temperature. Rice yields in Aomori Prefecture in Japan in 1980 dropped to about 3 tonnes/ha from 6 tonnes/ha due to cool summer damages (Kushibuchi, 1997). In 1993, the average night temperature went down to 11^{0}C in summer, causing a high rate of grain sterility in northern Italy, Japan and Korea (Tran, 1994). Low temperature during reproductive stage was also reported to be a major constraint to rice production in California, USA (McKenzie et al., 1994), Australia (McDonald, 1994), Southern Brazil (Terres *et al*., 1994).

- Yield of rice planted under rainfed conditions and in some cases under irrigated conditions is limited by drought stress due to variation in rainfall and its distribution.

Pressures from biotic stresses

Full yield potential of modern rice varieties are not realized because of the toll taken by the attack of diseases and insects, while weed limits rice through competition for sunlight, water and nutrients. It is estimated that diseases and insects cause yield losses of up to 25% annually. Stem borers are most destructive and common rice insects, while rice blast caused by the fungus *Pyricularia oryzae*, and bacterial blight caused by *Xanthomonas oryzae pv.oryzae* are two of the most serious and widespread diseases in rice production. Heavy weed competition may lead to total crop failure. Barnyard grass (*Echinochloa crusgalli*) is the dominant weed in lowland rice production. With the increased adoption of direct seeding method, weedy rice has become another major weed in rice production (Catala, 1995, Ferrero and Vidotto, 1997; Pulver and Nguyen, 1999, Ferrero, 2003).

Figure 2. Average growth rate of rice yield, 1980-2000 (Source of data: FAOSTAT)

The Low Return from Rice Production

Rice production activities provide main employment and income to the majority of people in rural areas in developing countries. For example, on the average, rice production activities employ 243 person-days/ha in Bangladesh, 195 person-days/ha in India, 156 person-days/ha in Indonesia and 60–80 person-days/ha in the more mechanized production systems of the Philippines and Thailand (Pingali et al, 1997). Initially, the increase in rice yield and production increased the return from rice production and incomes of farmers. However, since 1995 international rice prices have followed a marked declining trend (Calpe, 2003), while prices of production inputs such as fertilizers, other agro-chemicals, labour, fuel and rice machinery and equipment either increased or at best remained unchanged. These factors have led to an increase in production costs and subsequently a sharp reduction in the return from rice production. This has been one of the major causes of poverty and hardship for many small farmers in developing countries. The low return - poverty cycle, in the long run, may affect the capacity of farmers to invest in production inputs, which would eventually lead to lower yield in a number of developing countries.

In industrialized countries the increase in production costs has made rice-based farming activities less competitive. The production costs in Europe, USA, Brazil and Japan are substantially high (Sanint and Zeigler, 1994; Chataigner and Salmon, 1996; Romero, 1996). Traditionally, rice production in industrialized countries is highly subsidized. However, under the pressures from the World Trade Organization, the subsidies in rice production may be substantially reduced. Therefore, rice production in industrialized countries could rapidly decline, unless significant progress is made in technology which could greatly reduce the production costs. For example, both rice production and harvested area in Japan have steadily declined in a substantial way since 1985, although rice is an important food crop of the population (Fig. 3).

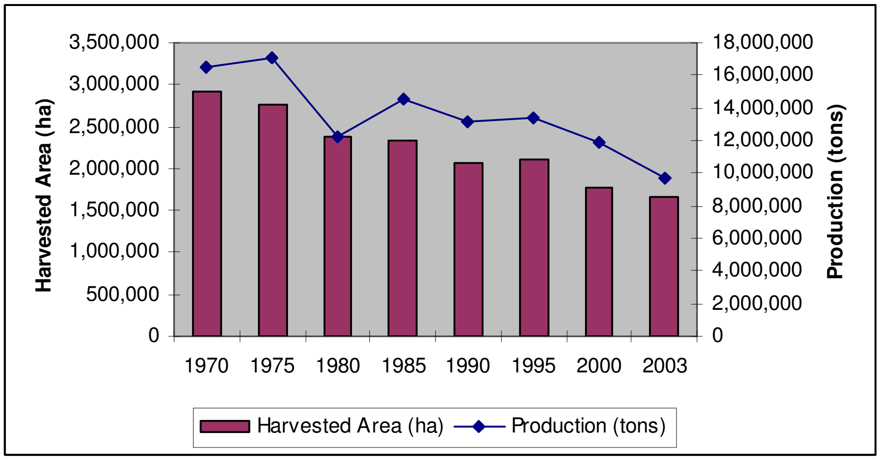

Figure 3 Rice harvested area and production in Japan, 1970-2003 (Source of data: FAOSTAT)

Other Socio-economic Factors

Taste, texture, color and stickiness of the rice variety all influence consumer preferences. Consumers in South Asia and the Middle East prefer dry flaky rice, while in Japan, Taiwan, Korea, Egypt and north China moist, sticky rices are preferred, in parts of southern India, red rice is preferred. Fragrant rices, such as Basmati are highly prized during festivals and special occasions (Kennedy et al., 2003). In the recent past the tendency to prefer long grain, soft and aromatic rice has become more prevailing among rice consumers around the globe. The demand for long grain or "*indica*" type has gradually increased in Europe (Ferrero and Nguyen, 2004). Yield of high quality rice varieties, especially the aronmatic ones, however, is generally still low.

Environmental resource protection is of increasing public concern, and has been reflected in a growing number of international agreements such as the Convention on Biological Diversity and the Framework Convention on Climate Change. Government environmental and chemical regulations and urban growth also may restrict farmers' use of optimum management practices.

Technical Options For Meeting The Challenges

Science provides the basis for improving the productivity and efficiency of rice-based systems. During past decades increasing demand for rice has been met mainly through yield-enhancing measures of the "Green Revolution" in the 1970s, which introduced improved rice varieties and improved production technologies. Hybrid rice and the new rice for Africa (NERICA) are two additional important advances in the field of rice varietal for achieving higher or more stable productivity in different ecological zones. The successful mapping of the rice genome in 2002 has further increased the potential contribution of rice science to sustainable increase in rice production.

Most existing high-yielding and hybrid varieties have a potential yield that exceeds actual yield that are obtained by farmers. The gap between the potential yield and the actual yield is due currently to the deficiencies in crop mangement. There are fortunately a number of improved crop management techniques that are ready to be widely introduced for enhancing the productivity of rice-based systems in a sustainable manner. In addition, rice integrated crop management (RICM) systems have recently been developed for efficient transfer of the crop management technologies.

Hybrid Rice for Irrigated Rice Production

The application of *heterosis* in rice production in China in 1974 was another major step in raising the yield potential. The first generation of hybrid rice varieties (mostly 3-line hybrid) have a yielding advantage of 15-20% over high-yielding varieties and its commercial production started in 1976. The two-line hybrid rice varieries, which have 5%-10% higher yield than that of 3-line hybrid rice varieties, were successfully commercialized in 1995. Several super hybrids with a yield advantage of around 20% over current three-line hybrids were recently developed. In recent years, hybrid rice covers 50% or 15 million ha of the total rice area in China. The nationwide average yield of hybrid rice is 7t/ha, which is about 1.4 t/ha higher than that of inbred varieties (Yuan, 2004). The adoption of hybrid rice in China has enabled a steady increase in rice production, allowing at the same time, to save several million hectares of rice land for crop diversification.

The adoption of hybrid rice technology outside of China has taken place since 1990 and in 2003 about 800,000 ha planted to hybrid rice in countries such as Vietnam, India, Philippines, Bangladesh, Indonesia and Myanmar. The areas under hybrid rice production in many Asian countries outside of China, however, are only a small fraction of their irrigated rice areas, suggesting the potential of hybrid rice for increasing rice yield and production in the next decade. High prices of F1 seed, however, limit the wide adoption of hybrid rice outside China. Technologies to increase the yield of F1 seed production will be needed in order to lower the prices of F1 seed. Improved crop management could increase yield of F1 seed production, but the development of CMS lines with stable sterility, higher outcrossing rate and good grain quality will be the vital step for promoting wide-adoption of hybrid rice in the near future (Nguyen, 2000).

NERICA Rice for Upland Areas in West Africa

Upland rice yields are still generally low due to a number of reasons. During the last decade, WARDA has devoted efforts to develop new rice varieties for upland rice production in West Africa by crossing *O. sativa* L. with *O. glaberrima* Steud; thay are called NERICA or new rice for Africa. Initial field tests showed that under low-input-management systems in the region, NERICA rice varieties produced higher yield than the currently used varieties (WARDA, 2000). In general, NERICA rice varieties have shorter growth durations than the existing varieties. The shorter growth duration could help rice crop to escape the adverse effect of drought stresses on yield. It also would facilitate the adoption of diversified rice-based cropping systems such as rice-legumious crop, rice-cover crop, and other similar systems in order to maintain soil fertility, thus yield of upland rice (Nguyen, 2003).

Other Advances in Rice Breeding

The efforts to develop a new plant type (NPT) by scientists at the International Rice Research Institute have resulted in the released of three NPT lines with 15-20% higher yield than the existing high-yielding varieties in China and one in Indonesia (Khush, 2004). The broadening of crop gene pools through hybridization between an *Oryza rufipogon* accession from

Malaysia and cultivated rice resulted in rice lines that out yielded the recurrent parent by as much as 18% (Xiao *et. al.,* 1996). Moreover, rice is the first food crop for which complete genome sequence is available. This offers an unprecedented opportunity to identify and functionally characterize the genes and biochemical pathways that are responsible for increasing rice yield, resistance to biotic and abiotic stress and consumer quality. Following are promising development in the field of rice biotechnology:

- \- Matsuoka el al (2001) are trying to alter the photosynthesis of rice from C3 to C4 pathway by introducing cloned genes from maize into rice. If successful, the yield potential of rice may increase substantially. C4 plants such as maize and sorghum are more productive as compared to C3 rice and wheat, because C4 plants are 30-35% more efficient in photosynthesis.
- \- *Bt* genes have been introduced into rice for resistant to yellow stem borer - a widespread rice insect in Asia (Datta et al 1997), while the resistance against striped stem borer and pink stem borer has been enhanced by developping of transgenic rice carrying cowpea trypsin inhibitor (*Cpti*) gene (Xu *et. al.,* 1996). Similarly, genes for resistance to blast and bacterial blight have been transferred form *O. minuta* to improved rice germplasm (Brar and Khush, 1997).
- \- Garg *et. al.*, (2002) introduced *ots A* and *ots B* genes for trehalose biosynthesis from *Escherichia coli* into rice and developed transgenic lines with improved tolerance to abiotic stresses such as drought and salinity.
- \- Potrykus (2003) reported the successful development of a genetically modified rice that contains Vitamin A -- commonly called "golden rice" – using agrobacterium mediated tranformation techniques to transfer genes from the daffodil (*Narcissus pseudonarcissus)* and a bacterium (*Erwina uredovora)* to rice. Also, the soybean ferritin gene was transferred into rice to increase the iron content (Goto *et al,* 1999).

Reviewing the 307 rice biotechnology patents filed from 404 organizations, Brooks and Barfot (2003) reported that transgenic technologies under development in rice can be broken out into four categories: (1) herbicide tolerance (2) biotic stress resistance, (3) abiotic stress resistance and (4) nutritional traits. According to them, however, uptill today no commercial release of Transgenic or Genetically Modified Rice has occurred.

Narrowing the Yield Gap in Irrigated Systems with Rice Integrated Crop Management Systems

Irrigated rice is cultivated on about half of the total area planted to rice, but it is responsible for approximately 75% of total rice production. The yielding potential of high-yielding and hybrid rice varieties released for irrigated rice production in tropical climate areas (10 tonnes/ha or more) is much higher than yields that were obtained by best farmers (7 or 8 tonnes/ha), while, on the average, yields of irrigated rice in many cases are still only 4 to 5 tonnes/ha. This shows a large yield gap in irrigated rice production and the closing of this gap could substantially increase rice production. In September 2000, FAO organized the Expert Consultation on the Yield Gap and Productivity Decline in Rice Production. During the Expert Consultation, a number of recently-developed rice integrated crop management (RICM) systems were found promising for yield gap closing. They are:

- The Australian **RiceCheck** system: From 1973 to 1985, rice yield in Australia stagnated at around 6 tonnes/ha. The **Ricecheck** system was developed and transferred in 1986. Australian national yield increased rapidly and steadily to 9.65 tonnes/ha in 2000. Half of the observed yield increase since 1986 can be attributed to the adoption of new rice varieties and another half to the adoption of **Ricecheck** system (Clampett et al, 2003).

- The Egyptian **"Markbouk" system**: It was developed and disseminated for rice production in 1985 (Balal, 1994) and has been modified considerably with time. Rice yield in Egypt has steadily increased from about 5.5 tons/ha in 1985 to above 9 tons/ha in 2002.
- The **"P-7"** or package of technologies for 7 tons/ha of irrigated rice in Burkina Faso (Nguyen et al., 1994). From its initial test site in the Kou Valley in Burkina Faso, the P-7 was widely adopted by the Special Programme for Food Security in Burkina Faso and in Senegal. The adoption of the P-7 for irrigated rice has significantly contributed to sustaining the productivity of rice production systems in these two countries. In the Delta of the Senegal River, not only was the yield increasing, but also production costs were decreasing as a result of the spread of the P-7 system through the Farmer Field School approach (FAO-Senegal, 2000).
- The WARDA's Rice-ICM system for irrigated rice in the Sahel zone of West Africa: It was developed and transferred since 1995. Results of trials conducted in 1998 and 1999 season showed that the application of Rice-ICM system increased rice yield by 1.7 to 1.8 tons/ha. The economic analysis of the application of Rice-ICM system showed that the net benefit of rice production in Senegal also increased by Euro 184/ha and that in Mauritania by Euro 241/ha, although the production costs with Rice-ICM system increased slightly. WARDA concluded that through RICM system farmers were able to increase rice productivity, while maintaining or even enhancing the quality of the natural resource base (Wopereis et al., 2001).

Among the above RICM systems, **the RiceCheck** is the only system that provides, for each crop management area, the following guidances: (1) the reason why the recommendations should be followed, (2) the recommended actions or management practices, and (3) the expected outputs of the correct application of the recommended actions or management practices. For example, for crop nutrition the stem provides the following:

- *Reason:* Split Nitrogen Strategy - two steps which are important to high yields.
- *Recommended Actions:* Apply sufficient Pre-Flood nitrogen to achieve optimum growth at panicle initiation, apply phosphorus if a deficiency is indicated by paddock and/or soil test, and top dress nitrogen at panicle initiation based on shoot counts and NIR analysis using the Rice NIR Tissue Test.
- *KeyCheck or Expected Outputs:* At panicle initiation the Amaroo, Bogan, Illabong and Jarrah (rice varieties) should have 700 - 1100 shoots/m^2 and a leaf nitrogen content (NIR) of 1.2 - 2.2% N; whilst the Pelde, Doongara, Goolarah, YRF9 and YRL34 (rice varieties) should have 500-900 shoots/m^2 and a NIR 1.2 - 2.0%.

Another innovative aspect of the **Ricecheck** system is the use of an Action Framework, which has the following step:

- To UNDERSTAND the purpose of crop management
- To MANAGE the crop using the recommended actions.
- To OBSERVE the performance of the application of the recommended actions. This requires farmers to get "inside" the crop and inspect and observe.
- Observations must be supported by a MEASURE of what is actually occurring; observations must be objective.
- The measurements must be RECORDED.
- At the end of the season, crop performance and management results can be COMPARED with **KeyChecks or Expected Outputs** (e.g. yield, quality and environmental outcomes) and INTERPRETED: The successes (what went according to plan) and the problems (what went wrong) can be identified and judgements made about how to make improvements in the future.
- The farmer can learn from these experiences and TAKE ACTION to correct management weaknesses in the future.

The FAO International Rice Commission, in collaboration with research and extension programmes in member countries have conducted the modification and test of the performance of the Australian's RiceCheck system in rice production under the conditions in developing countries. Following are the initial results:

- Indonesia: farmers achieving all of the (five) target "Check values" had a yield increase of 23 % and a gross-margins increase of 165 % compared to those farmers achieving none of the targets.
- Thailand: Thai RiceCheck system with 10 target checks increased rice yield and reduced production cost. The test results from the application of the Thai RiceCheck system in 2002 and 2003 have led the governors of 10 rice-producing provinces in the country (Ayuthaya, Suphanburi, Ratchaburi, Pathumtani, Chainat, Chiengmai, Prachinburi, Nakhonratchasrima, Surin, Lopburi) to adopt the system.
- Vietnam: The application of the Vietnamese RiceCheck system helped farmers to save 50% of the seeds used in direct rice seeding crops in the Delta of the Mekong River. The results also showed that the use of the Leaf Colour Chart in the application of nitrogen fertiliser at the panicle initiation stage increased rice yield, while reduced the application rate by 20%.
- Brazil and Venezuela: the application of the **RiceCheck** system increased rice yield by about 30%.

Meeting The Challenges Of Rice Production Through Implementation Of The International Year Of Rice

FAO has always felt that rice is one of its major concerns. The FAO International Rice Commission (IRC) was established in 1949 to promote co-operative action in matter related to rice production, conservation, distribution and consumption. The Secretariat of the IRC was established in the Crop and Grassland Service of AGP to assist in the promotion of co-operative action on rice within FAO and between FAO and external institutions and Member Countries. In order to promote of the harminization of global action on rice development and production, the IRC secretariat has worked in partnership with national systems and external institutions through four major networks, which are: the Inter-Regional Collaborative Research Network on Rice in the Mediterranean Climate Areas (MED-RICE), the Wetland

Development and Management Network/Inland Valley Swamps (WEDEM/IVS) in Sub-Saharan Africa, the International Task-Force for Hybrid Rice (INTAFOHR), and the Working Groups to form the Working Group on Advance Rice Breeding in Latin America (GRUMEGA). The FAO International Rice Commission, IRRI and their member countries started the initiative for establishing an IYR in 1999, which led to the declaration of the International Year of Rice by the United Nations General Assembly in 2002.

As requested by the UN General Assembly, FAO has facilitated the implementation of the International Year of Rice – 2004 through partnership under the guidance of the Informal International Working Group for IYR whose members include representatives from 17 major rice producers and consumers, 5 UN agencies, 3 international research institutions, NGOs and private sectors. The fundamental objective of IYR implementation is to promote and help guide the efficient and sustainable development of rice and rice-based production systems now and in the future. In order to meet this overarching goal, the IYR strategy will focus on the following intermediary objectives:

- Increase public awareness, at all levels, on the contributions of rice-based systems for food security, better nutrition, poverty alleviation and livelihood improvement.
- Increase public awareness on the diversity and the complexity of rice-based production systems, as well as the challenges and opportunities for sustainable development of rice-based production systems.
- Promote, and provide technical support to ensure sustainable development of rice and rice-based systems at the global, regional, national and community levels.
- Promote the conservation and enhancement of rice-based products for economic, social, cultural and human health aspects of the population.

Concluding Remarks

Rice is the staple food for over half of the world's population. Almost a billion households in Asia, Africa and the Americas depend on rice systems for their main source of employment and livelihood. Rice systems support a wide variety of plants and animals, which also help supplement rural diets and incomes. Rice is therefore on the frontline in the fight against world hunger and poverty. While the Green Revolution of the 1970s greatly alleviated the global burden of hunger in some parts of the world, these benefits have been leveling off. The world's population is still growing at a steady rate and today there are still 840 million people suffering from chronic hunger, over fifty percent of whom live in areas dependent on rice production for food, income and employment.

Fortunately rice science has continued to advance and there are existing improved rice varieties and crop management practices for exploitation to enhance the sustainability of rice-based systems. Harnessing the improved rice technologies for maintaining of the productivity of rice production systems is too large for any single country, institution or organization to handle individually. The International Year of Rice presents a unique opportunity for farmers, consumers, environmentalists, government ministries, international agencies and stakeholders from civil society to work together for a shared goal. The need for a sustainable increase in rice production affects everyone. The International Year of Rice provide us with a chance to improve food security, alleviate poverty and preserve the environment for the billions of people for whom *Rice is Life.*

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INFLUENCE OF SOWING AND FERTILIZING RATE ON RICE PLANT DEVELOPMENT AND DISEASE EVOLUTION IN VALENCIA (SPAIN)

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Abstract

Rice in Valencia (Spain) includes an area of about 15.000 ha around the Lake of Albufera, a high environmental area, declared Natural Park. In the fields where rice is grown (only possible crop) there are restrictions concerning fertilizers and pesticide applications. The most important pest in this area is the Lepidoptera *Chilo suppresslis* and the most important diseases are those caused by the fungi *Helminthosporium oryzae* and *Pyricularia grisea*, which in the last campaigns have had certain importance, therefore increasing the pesticide treatments. It is well known that factors like fertilizer increase sensitivity of the crop to fungi attack. Since 2001 The Universidad Politécnica de Valencia makes field trials with the purpose of studying different fertilizer combinations and sowing rates in order to diminish the risks of important damages by fungi. In this work, results of these trials (2.001 to 2.003) are shown.

Keywords

Rice; Pyricularia grisea.

Introduction

Rice is one of the most cultivated crops in the world. In Europe, it is mainly cultivated in the Mediterranean Basin, representing Spain, with 113.076 ha in year 2001, a 28,2% of the total rice cultivated surface. In Spain, this crop is usually cultivated in fields situated within the borders of natural areas in which agriculture and wildlife and flora have to live together.

Some examples of the mentioned areas in Spain are Doñana National Park, Delta del Ebro Natural Park and Albufera Natural Park (ANP). The last one is located to the south of Valencia City very close to the Mediterranean sea, as seen in Figure 1.

Rice cultivation in Valencia starts in early march and since that data to middle September rice growers perform many agricultural practices, including: soil cultivation, fertilisation, sowing, water management, weed control, pest and disease control and harvesting.

All of the mentioned practices have to be integrated within the environment where rice grows, especially those involved with pesticide spraying, in order to minimise any risk to non target organisms of the ANP.

Rice blast caused by *Pyricularia grisea* is a serious threat to rice production in Valencia, especially when climatic conditions are favourable. As in other parts of the world substantial efforts to use resistant rice varieties have been made but fungicide application is an important component of the disease management of rice blast by farmers in the ANP in the last years, even when there are no risk of disease or evident damages.

However, apparition of *P.grisea* in rice plants is not only a climatic question, some agricultural techniques may increase plant damage caused by the fungus and as a consequence more pesticide treatments have to be done.

The objectives of this work is study the relationship between different cultural practises (sowing dose and nitrogen fertilisation dose) and rice-blast under field conditions in different locations in the PNA rice fields. It would be desirable, that rice growers perform good agriculture practices like sowing tolerant varieties, sowing the appropriate dose, and fertilizing with the appropriate nitrogen dose, just in case those years when climatic conditions would be favourable to *P. grisea* developing, pesticide treatments could be minimised.

Materials and methods

Since 2001 several field experiments were conducted in three different rice fields within the ANP, with three water origins (water-treatment plant; Albufera lake; Júcar river) and located, one from the other, far enough to ensure different microclimates, as seen in figure one.

In each field the experimental design was a split-plot with three replicates with nitrogen dose as main plots and sowing dose as subplots. All subplots (3x3 m^2) were sown with the same rice variety (S*enia*). but at different nitrogen fertilization doses (0, 110, 155, and 200 $kg.ha^{-1}$) and different sowing doses as secondary factor (120, 180, 240 $kg.ha^{-1}$ in 2001 and 2002 trials; 60, 120, 180 in 2003 trial). Changes in 2003 sowing doses were introduced due to the results of previous years.

Nitrogen fertilizer application was splitted in two times, 110 $kg.ha^{-1}$ were applied before rice sowing time and the rest at the end of June when growers drain the fields. Rice sow was done by hand during the first days of May over the flooded fields. Crop management was the usual one in this rice area, including water management and weed control.

Climatic data were recorded with a HOBO logger and a HOBO shuttle was used to transfer data.

Fungus damage was evaluated three times every 15 days between last of July and beginning of September. Ten hills per plot were randomly examined for disease severity, with two different scales: panicle damage (Clive, 1971 adapted scale) and leaf damage (Marín, 2001 scale, personal communication). Both scales are based on % of damaged surface.

To compare relative levels of damage of rice treatments to blast in the paddy field, the area under the disease-progress curve (ADPC) according to the formula describes by Shaner and Finney (1977) was calculated for each subplot and these were analysed by analysis of variance.

Each treatment was hand-harvested and the grain was also hand-separated from the panicle in 2001/2002 and in 2003 the grains were separated with an "Wintersteiger LD350" machine, determining paddy rice yield, damage grains and weight of 1000 grains. Milled rice was also determined in laboratory conditions with a "Nuova emmebienne" mill.

The analysis of the results was performed studying correlations between variables and ANOVA analysis with the statistic package SPSS v 11.5.

Results

Year 2001. The results of two of the three fields can be observed in table 1, as the third one, located in Silla, was useless due to a heavy local storm occurred in August.

The climatologic conditions during the crop cycle were quite appropriate for rice. Fungus developing risky periods (extended period with moderate temperatures and high air moisture content) did not appear along the summer.

Statistical significant differences were obtained between both assays concerning panicle density, final yield and hectolitre weight .

Table 1 Yield and others parameters of rice cultivated in two assays in year 2001

Assay	Panicle density (Units.m^{-2})	Paddy rice yield (kg.ha^{-1}, 14% MC)	Hectolitre weight (kg. 100 L^{-1})	Milled rice yield (%)
Alfafar	365.6a	7000^{a}	40.57a	65.3 a
Sueca	325.4b	8975b	45.45b	65.3 a

A negative correlation between paddy rice yield and sowing dose was obtained in both assays: the more sowing dose, the fewer yields obtained. This negative correlation was statistically significantin Sueca at the 240 kg ha^{-1} sowing dose (Figure 1).

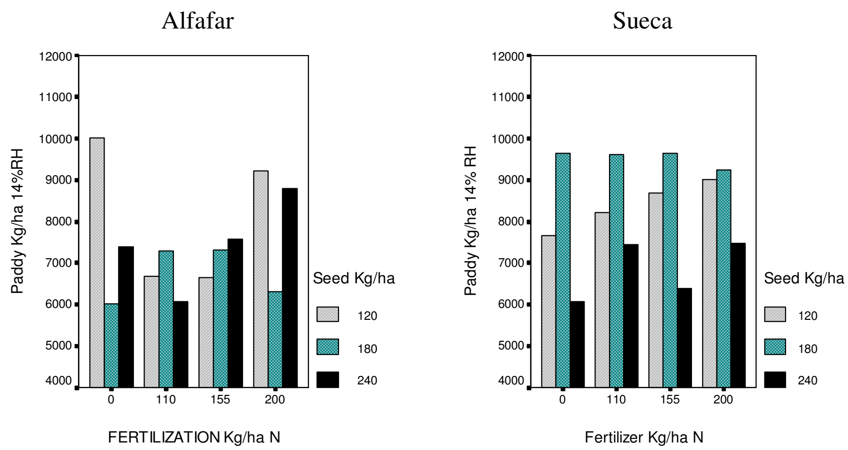

Figure 1 Paddy rice yield in relation with N fertilization and showing dose in two assays in year 2001.

The incidence of leaf blast lesions was very high in both locations, but severity was inconsiderable (a few more in Sueca assay) and not statistically significant in all the studied treatments. For this reason the ADPC was not calculated. Correlations between fertilizing/sowing dose and plant (leaf and stem) damage were not found either, although it could be observed a statistically significant effect of fertilizing dose over the panicle blast damages in both locations, increasing those damages when nitrogen fertilization was also increased (Figure 2).

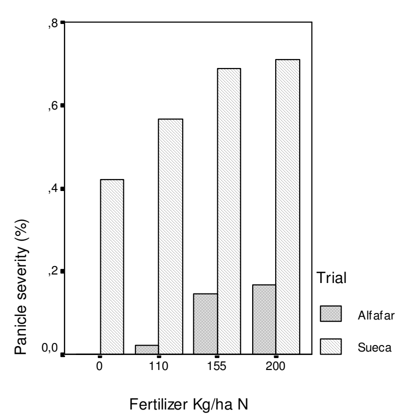

Figure 2 Panicle blast severity in relation with N fertilization in two assays in 2001.

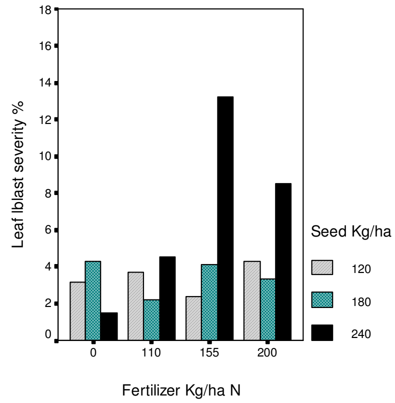

Figure 3 Leaf blast severity in relation with N fertilization and sowing dose in Alfafar assay in 2002.

Year 2002. Only one assay was conducted, located in Alfafar. At the beginning of September there were heavy rain episodes that bend the plants making harvesting and yield evaluation very difficult. No correlations were found between the studied variables (fertilizer and sowing dose) and disease damages. An increment of severity in leaf lesions was observed when increasing sowing dose but differences were not significant.

Year 2003. The climatic conditions were very unusual this year, with heavy rain episodes at the end of April that delayed sowing date, high temperatures during summer that shortened rice cycle and two periods characterized by moderate temperatures and high air moisture content during July and the end of August in which dew and guttation drops remain over plants for long periods of time.

There was a big algae infestation during the first two crop months in Alfafar.

It can be observed (Figure 4) that the obtained yields and blast damages were very high in the Silla and Sueca assays. Analysis of variance showed significant differences between assays in yields and ADPC.

In Alfafar, yield and fungus damage were significantly lower than in the others. The marginal effect of fertilization and sowing doses on yields and blast severity (ADPC) was not significant. In this assay the correlation analysis of studied variables showed no correlation between yield and leaf blast damage (ADPC).

In Silla location, a statistically significant effect of sowing dose over yield was obtained, but not for fertilizer dose. Both factors had no significant effect on ADPC, but the correlation analysis showed a negative correlation between panicle blast severity and fertilizer dose and positive correlation between leaf blast severity (ADPC) and yield.

In Sueca location any statistically significant effect of sowing/fertilizing dose over yield was not observed, but the correlation analysis showed a statistically significant correlation between yield and fertilizer. There was negative correlation between leaf blast severity (ADPC) and fertilizing dose.

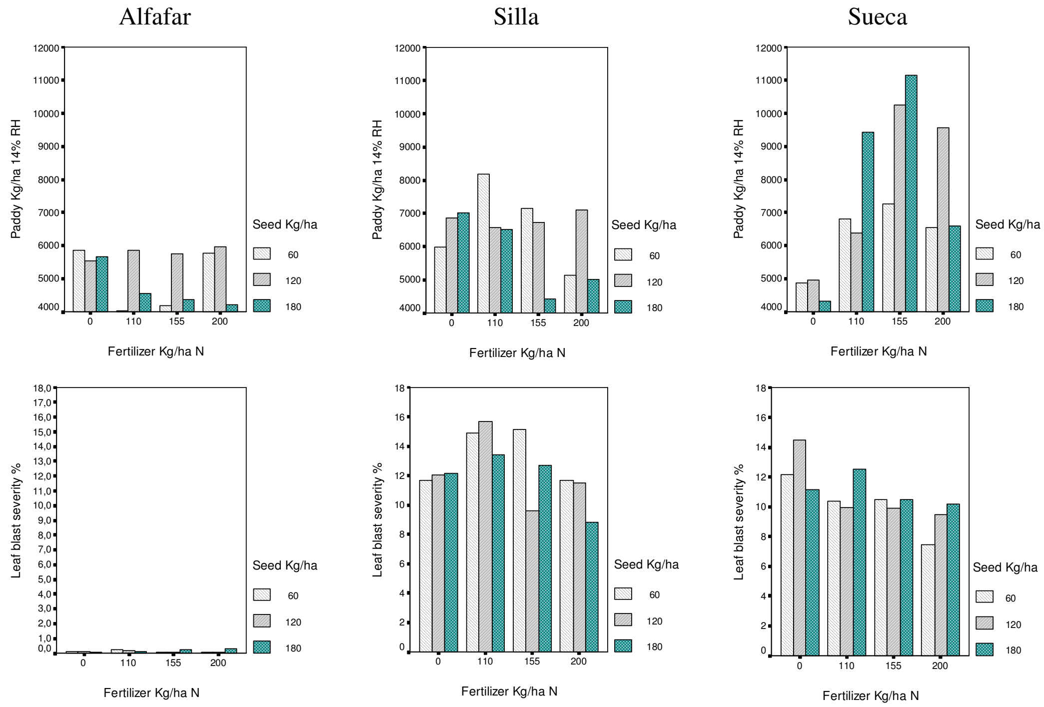

Figure 4 Paddy yield (upper) and leaf blast severity (lower) in three locations in Valencia (Spain) in the year 2003.

Discussion

The results show a great variability among years and locations, what implies many difficulties to extract consistent conclusions. The development of the disease is very complex. Both pathogen and plant and its interaction are influenced not only by meteorological conditions, rice variety and cultivation practices used (Suzuki, 1975). In this case variety and practices were the same and the differences in yields and blast rice damages between locations and years could be explained by the logical differences in soil fertility, water quality and meteorological factors between locations and years.

The effect of increasing sowing dose over yield and disease apparition after the first two years, did not show any effect in the studied range of 120-240 kg.ha^{-1}, but affected the stem bent at the end of the cycle (Figure 6). A superior leaf blast severity was also observed with de greatest dose, especially if combined with high nitrogen dose (Figure 3), and it justified the decrease of the studied sowing doses (60-180 kg.ha^{-1}) in 2003. In this year, only in Silla location, a statistical significant effect of sowing dose over yield was observed, being 120 kg.ha^{-1} the sowing dose that gave better yields. This sowing dose is possibly the optimum one in this area, because fewer doses give more irregular yields and bigger ones do not increase them. At present many farmers use 180 kg.ha^{-1} sowing doses or more, that represent higher costs and, more risk of bended tills, and those doses could be reduced.

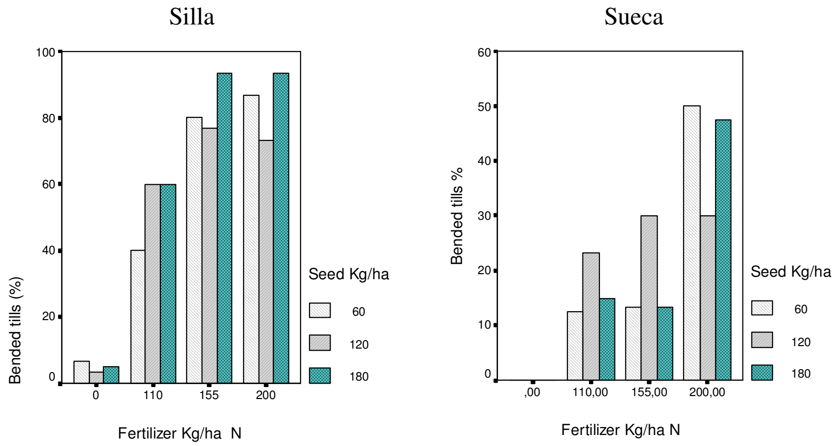

Figure 6 Relationship between fertilizer, sowing dose and bended tills in the year 2003 in rice assays in Valencia. Alfafar not draw because 100% of assay was bended.

The fertilizer factor has a narrower relation with yields (in general they are maximum with medium fertilizer doses) and with disease developing.
In 2001, contradictory results in yield were obtained in Alfafar, specially in control without fertilizer (Figure 1). A possible explication of this phenomenon is that the source of water irrigation in this field is the Valencia water-treatment plant. In different water analysis along crop season nitrates content vary between 8.9-39 $mg.L^{-1}$ supplying a extra high quantity of N to the crop. Furthermore, organic matter in soil is higher in Alfafar (5,35%) than in the other assays (Silla, 4.87; Sueca, 4,34) that explains an excess of vegetative development to detriment of grain yield and higher risk of bended tills (Figure 6).
Contradictory results have been found concerning leaf lesions. In those years with less favourable climatologic conditions for *Pyricularia* disease apparition and with very little severity (2001 and 2002) a nitrogen fertilizer increase favoured leaf and panicle blast lesions. And in those years with great severity the mentioned relation was not so clear in the range studied and even leaf severity decreased when nitrogen fertilizer was increased. In 2003 there was a clear influence of the nitrogen fertilizer over panicle damage in the location of Silla. Perhaps, those plants with a better nutrient availability at the end of the crop cycle could fill better their grains, in spite of the important fungi attack.
Next results from 2004 and 2005 assays will gave more consistences to these first results.

Conclusions

Rice Blast epidemics appear some years in Rice Production Area of Valencia and its virulence differs between locations. Sowing dose is the studied factor that has less influence over the obtained yields and over the *Pyricularia grisea* development, rather than fertilizer dose, in the most cultivated variety (*Senia*), and no high sowing doses are necessary for good rice production. In those years with fewer fungi infestation, the increase of fertilizer doses, promotes an increase of leaf damages, whilst those years with favourable fungi developing conditions and higher damages, the fertilizer influence has less relative importance. It is in this particular situation when the use of rice resistant varieties and chemical control obtain more importance in the prevention of the disease damage.

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OCCURRENCE OF PSOCOPTERA SPECIES IN RICE STORES AT CALASPARRA (MURCIA, SPAIN)

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Abstract
A study of the psocid species occurring during the storage period in rice stores at Calasparra (Murcia, Spain) was carried out during 2001 and 2002. Catches in pitfall traps placed in paddy bulks and insects developed after sample incubation showed the abundance of *Lepinotus reticulatus* (Enderlein), *Liposcelis mendax* (Pearman) and *Liposcelis entomophila* (Enderlein). *Liposcelis bostrychophila* (Baldonnel) and *Liposcelis paeta* (Pearman), to a lesser extent, were also recovered from organic paddy or rice. The presence of psocoptera was associated with warm and humid conditions within the stores. Booklice survived in the husk and broken rice so such subproducts could be a source of reinfestation.

Keywords
Lepinotus reticulates; *Liposcelis mendax*; *Liposcelis entomophila*; *Liposcelis bostrychophila*; *Liposcelis paeta*; psocids; paddy; rice.

Introduction
Psocoptera are a relatively small order of insects with aproximately 4400 species worldwide. Some of these small sized insects are adapted to live in food stores, food industries, bulk grain, houses, etc. (Sedlacek et al., 1996). Indoors their occurrence is more frequent in humid environments (Baz and Monserrat, 1999).
They are regarded as secondary pests, often overlooked due to its small size and the existence of other more damaging primary pests specially in cereal grains. Nevertheless, Rees and Walker (1990) reported that psocids can cause contamination and weight and quality loss in rice.
Liposcelidae is one of the commonest families of Psocoptera in stored products (Kalinovic and Ivezic, 1996). *Liposcelis* species have been cited as pests in the UK (Turner, 1994), Australia (Rees, 2002), Indonesia (Leong and Ho, 1990), Mexico (García Aldrete and Gutierrez Díaz, 1995) and the United States (Mockford, 1991) among others.
In this paper, we report psocid catches in traps placed in stores of Calasparra (Murcia, Spain) during the storing period (one year) and also booklice development after incubation of rice products and subproducts.

Materials and methods
Location of stores
The study was carried out at Calasparra (Murcia) in Spain, within the rice producing area of guarantee of origin named by the locality. The two stores and milling facilities of the area were considered for the study. Here, paddy is usually stored in bulk. Environmental

conditions of the stores were monitored by means of Data Loggers (testostor 171-3), placed 10 cm deep in the bulks, which were able to register data of temperature (ºC) and relative humidity (%) every hour.

Sampling
Rice products such as paddy, organic paddy and rice and subproducts like husk and spoilt or broken rice were sampled in February and April 2002 for the rice harvested on November 2001. 250 g samples were incubated at 30ºC in the dark during 4 months and afterwards inspected visually for the presence of psocids which were counted and identified. In addition, phosphine treated or untreated rice was sampled from the store in December to check psocid survival after incubation.

Trapping
Pairs of pitfall traps (CSL PC trap) were placed one in the surface and another 10 cm deep in paddy bulks. They were inspected every month from December 2001 to September 2002 and catches were counted and identified.

Species identification
Psocoptera species were identified using the keys of Broadhead (1950), Lienhard (1990) and Lienhard (1998).

Results and discussion
Lepinotus reticulatus, *Liposcelis entomophila* and *Liposcelis mendax* fell in great numbers in the traps and *L .paeta* is a rare capture (Table 1). In Store 1, psocids appear mainly in organic paddy during the summer. In Store 2 they are present all over the year but specially during winter in bulks of paddy (Bomba) and organic paddy (Balilla x Sollana). Survival of psocids (*L .mendax)* in December can be explained by the favourable humid (88% r.h.), though suboptimal cold (6.8ºC), conditions. Rees and Walker (1990) noted that both factors interact and have effects on psocid survival and breeding.
L. reticulatus catches were more abundant in traps located over the surface of grain bulks. Numbers in surface or 10 cm deep traps were about the same for the other species.
The literature reports that psocid infestations are seasonal and peak at summer and autumn months (Sinha, 1988; Baz and Monserrat, 1999). According to Mashaya (1999) this is related with prolonged conditions over temperatures of 18ºC and 70% relative humidity.
Phosphine fumigation is not effective in killing booklice. In Table 2 we can see that in either treated or untreated rice, psocids develop after sample incubation. Other authors (Nayak et al., 1998; Riudavets, 2001; Rees, 2002) report that outbreaks of psocids have recently been detected when grain protectants or phophine fumigations are applied.
In the stores of the study area, psocids live both in rice products and subproducts (Table 3). Paddy is the prefered substrate for the insects specially if it comes from organic production. In addition, subproducts such as husk and spoilt and broken rice are reservoirs of the insects during the whole year and therefore could be a considerable source of paddy infestation if they are left nearby. *L. bostrychophila* appeared after incubation of the husk mainly in the April sampling (Table 3) indicating the risk of rice infestation at the end of the storing period.
The problem of booklice in Calasparra could avoided by reducing the relative humidity values within the stores, by removing subproducts and by testing alternative control methods.

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Table 1 Number of psocid catches (males + females) in pitfall traps placed in stored paddy

Store	Species	Dec 2001[3]	Jan 2002	Feb 2002	Mar 2002	Apr 2002	Jun 2002	Jul 2002	Sep 2002
1	*L. reticulatus*						0+1[b]	0+2[b,c]	0+23[a]
	L. entomophila					1+3[b]	36+196[a,b,c]	11+95[a,b,c]	15+185[a,b,c]
	L. mendax				0+1[c]	0+3[c]	222+400[a,b,c]	183+107[a,b,c]	906+3181[a,b,c]
2	*L. reticulatus*		0+650[a]	0+3186[a,c]	0+181[a,c]	0+43[a]		0+1[a]	0+5[a]
	L. entomophila		0+7[a]	0+96[a]	0+14[a,c]	0+19[a]	1+17[a]	0+8[a]	0+1[a]
	L. mendax	0+25[c]	0+96[a]	9+250[a,c]	0+14[a]	1+3[a]	0+16[a]	0+5[a]	0+25[a]
	L. paeta			0+1[a]	0+3[c]				

[a] *paddy variety Bomba*
[b] *paddy variety Balilla x Sollana,*
[c] *organic paddy variety Balilla x Sollana*

Table 2 Effect of fumigation with phosphine on psocid survival

Product	Fumigation	Psocids after incubation (4 months at 30°C)	
		species	number[a]
rice	no	*L. entomophila*	10 ± 9
rice	yes	*L. entomophila*	5.5 ± 0.5
		L. reticulatus	9.5 ± 9.5

[a] mean number (two replications) of insects in 1 Kg of rice (harvested on October 2001 and sampled from the grain store on December 2001)

Table 3 Effect of storage period in psocid development after sample (250 g.) incubation during 4 months at 30ºC

Store	Sample (variety Balilla x Sollana)		Psocids	
	Product	Months in storage	Species	Number of insects
1	paddy	Nov 2001 - Feb 2002	*L. entomophila*	18
		Nov 2001 - Apr 2002	*L. entomophila*	4
			L. mendax	1
	organic paddy	Nov 2001 - Feb 2002	*L. entomophila*	39
			L. reticulatus	1
		Nov 2001 - Apr 2002	*L. mendax*	5
	spoilt/broken rice	Nov 2001 - Feb 2002	*L. entomophila*	25
		Nov 2001 - Apr 2002	*L. entomophila*	120
2	paddy	Nov 2001 - Feb 2002	*L. entomophila*	2
		Nov 2001 - Apr 2002	*L. entomophila*	12
			L. reticulatus	27
	organic paddy	Nov 2001 - Feb 2002	*L. entomophila*	5
			L. reticulatus	6
		Nov 2001 - Apr 2002	*L. reticulatus*	19
			L. mendax	22
			L. paeta	12
	spoilt/broken rice	Nov 2001 - Feb 2002	*L. entomophila*	5
		Nov 2001 - Apr 2002	*L. entomophila*	8
	husk	Nov 2001 - Feb 2002	*L. entomophila*	7
			L. reticulatus	11
			L. bostrychophila	21
			L. mendax	2
		Nov 2001 - Apr 2002	*L. reticulatus*	1
			L. bostrychophila	139

AIRSPORES DETECTION OF *PYRICULARIA GRISEA* (COOKE) SACC. AND *BIPOLARIS* SPP.: A THREE YEARS MONITORING IN DIFFERENT RICE FIELDS IN NORTHERN ITALY

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Abstract

Concentrations of *Pyricularia grisea* and *Bipolaris spp.* airborne spores in different rice fields located in Northern Italy were estimated by using an automatic volumetric spore trap (VPPS 2000, Lanzoni Bologna). Measurements of their concentration were made between June and October over three years (2001-2003). Temperature, humidity, rainfall and leaf wetness were monitored during the vegetative seasons. Both fungi showed spores peaks just 6-7 days before the onset of the typical symptoms of blast and brown spot on leaves. The possibility of the spread of the neck blast was always detected before the end of the growing season. Although peaks of *Bipolaris spp.* and *P. grisea.* often occurred after a short rainy period, a correlation between airborne spores and the chosen parameters was not easily demonstrated. It is necessary to carry on the record of meteoclimatic data in order to build a predictive model; we propose the aeromycological monitoring as a useful diagnostic method to forecast rice diseases at present.

Keywords

airspores detection; blast; brown spot; rice; *Pyricularia grisea*; *Bipolaris* spp.

Introduction

Aerobiological monitoring of pathogens spores may be of great importance in agriculture, as it can provide farmers with an early warning of the risk of infection. In Italy, the most important European rice growing country (219,987 cultivated ha in 2003; ENR, 2004), use of chemical sprays to prevent infections in rice fields is a normal practice, because this crop can be affected by more than 80 biotic and abiotic diseases (Ou, 1985). Low yield and poor quality of grain are often due to nutritional lacks, bad weather conditions, weeds, bacteria, algae and viruses. Most of the diseases seriously affecting rice production are caused by fungi, particularly by *Pyricularia grisea* (Cooke) Sacc., causal agents of blast, and *Bipolaris* spp., including *B. oryzae* (causal agent of brown spot) and other different species of the genus, such as *B. australiensis, B. hawaiiensis, B. spicifera* (reported by Webster and Gunnel, 1992, as agent of obscure diseases of the foliage). Blast is most severe in irrigated rice fields of temperate regions and drought-prone environments (Bonman, 1992). Symptoms may occur on the upper part of the plant, reducing photosynthetic activity and damaging the plant by toxins production; in Italy, the major problem is when the fungus attacks the neck of the panicle, causing losses between 5 and 30% in rice production, depending upon environmental conditions. Brown spot may infect all parts of the plant and its lesions are easily confused with those caused by *P. grisea*: severely infected leaves may die before maturity and plants will produce lightweight or chalky kernels. As the life cycle of these fungi is obviously related to general climatic conditions and both the incidence and the risk of infection by airborne fungal spores are correlated to local microclimatic conditions, knowledge of airborne spores concentration can be a useful indicator for infection risks. In this study we tried to connect airspores presence to temperature, relative humidity and rainfall, to reach a more complete monitoring that can be useful to reduce and direct antifungal treatments. We suggest to carry on this kind of analysis in order to build a predictive model as a tool to forecast rice diseases in the field. This investigation covered a three year vegetative period, two of which under standard climatic conditions and one with considerable peculiarities.

Materials and methods

The quantitative sampling was conducted during the vegetative seasons of the years 2001, 2002 and 2003 in Northern Italy, in the centre of the rice production area. Rice fields were located at: Castello d'Agogna (PV), Sali Vercellese (VC) and Vidigulfo (PV). We used an automatic volumetric spore trap (Hirst, 1952), VPPS 2000 Lanzoni (Bologna, Italy), as described in a previous work by Picco and Rodolfi (2002). The obtained slides were scanned under an optical microscope (x 400) and the results were expressed as the number of spores/m^3 per day. The sampling method, slide preparation and data interpretation were performed according to the standard method of the aerobiological monitoring (Mandrioli et al., 1991). The Meteoclimatic data were monitored through a "µMetos" station (Pessl Instruments, Weiz Austria).

Results

The microscopic observation of the collected slides showed incidence and concentration of *P. grisea* and *Bipolaris* spp. spores. In 2001, only one field, located at Castello d'Agogna (PV), could be monitored. *Bipolaris* spp. was first detected, in the middle of July, and its peak of abundance was reached on 25th August, with 28.49 spores/m^3. *P. grisea* spores were detected at the end of July, and reached the peak of abundance on 2nd September, with 56.11 spores/m^3. In 2002, two spore traps were placed at Vidigulfo (PV) and Sali Vercellese (VC); spores of *P. grisea* reached their maximum value on 12th August, with 12.38 spores/m^3 and on 10th September with 55.73 spores/m^3 in the first and the second field respectively. *Bipolaris* spp. showed peaks of abundance on 13th September, with 16.10 spores/m^3 at Vidigulfo and on 18th July, with 71.21 spores/m^3 at Sali Vercellese. In the vegetative season of 2003, we monitored again a field located at Sali Vercellese and another at Castello d'Agogna: in the former, three big peaks of *P. grisea* spores were observed on 3rd, 5th and 6th September (56.97, 33.43 and 48.92 spores/m^3) while spores of *Bipolaris* spp. were observed on 4th September with 17.33 spores/m^3; in the latter field, peaks of *P. grisea* spores were detected on 16th and 24th July (33.25 and 44.72 spores/m^3) and on 13th September (32.62 spores/m^3) while *Bipolaris* spp. spores reached the peak of abundance on 8th August with 24.08 spores/m^3.

Regarding meteoclimatic data, the summer of 2001 was characterized by a long period of warm temperature and by a lack of rainfalls. The summer of 2002 had temperatures slightly under the average; relative humidity reached 90% at the beginning of July and remained high till the end of August, rainfalls were poor during the whole vegetative season. A particular focus is required on the summer of 2003, because it was exceptionally warm, with temperatures over the average from May till September; it was also a very dry season, and sometimes water supply was difficult for farmers.

Examples of the recorded data are shown in Figures 1 and 2.

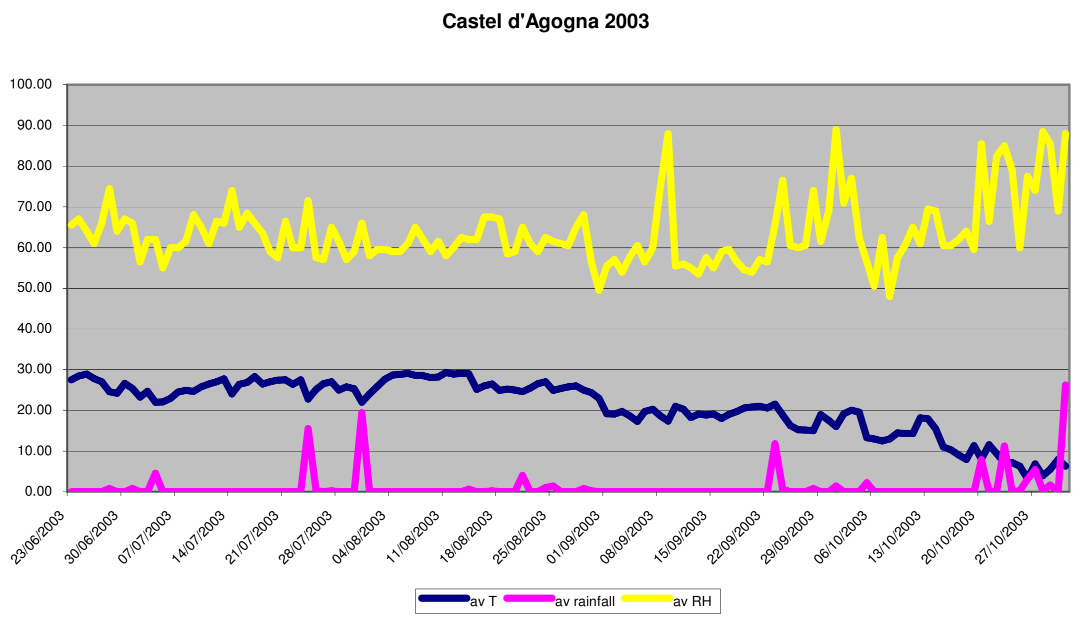

Figure 1 Average data on temperature, rainfalls and relative humidity recorded at Castello d'Agogna during 2003.

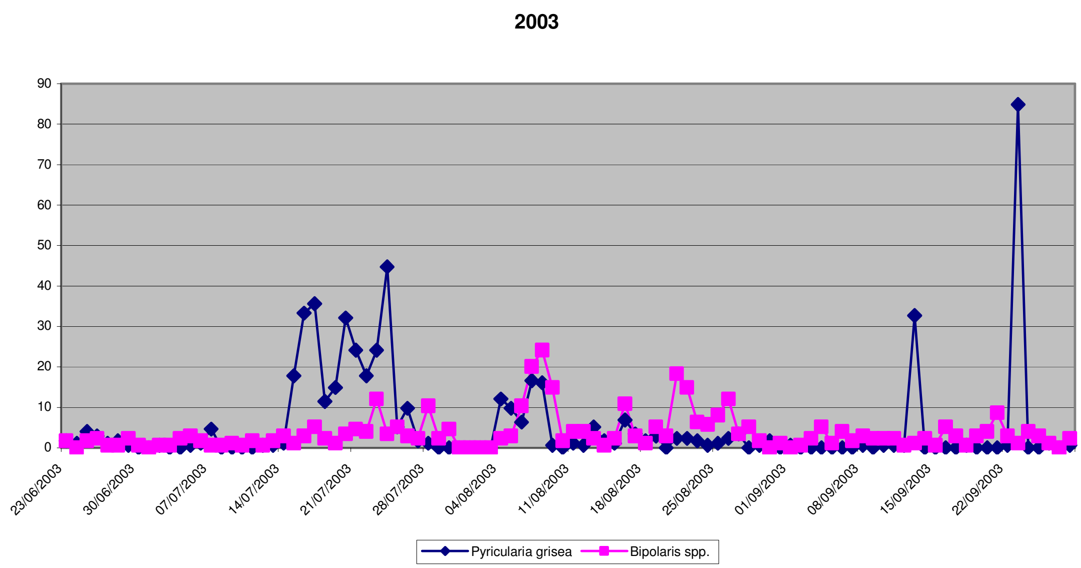

Figure 2 Airspores concentration of *Pyricularia grisea* and *Bipolaris* spp. at Castello d'Agogna during the vegetative season 2003.

Discussion and conclusion

By comparing aerosporological and meterological data, it is possible to infer a useful correlation that allows to forecast diseases. Management of diseases relies on the choice of the best crop defence strategy, that should be based on some main points: the correct identification of the pathogen, the dynamic of the disease and the losses that occur in crop production (Cortesi and Giuditta, 2003). Literature provided lots of information to explain these concepts especially on the two fungi considered in this study; unfortunately, results

already obtained by other Authors are often not applicable in our country. Studies on the rice blast fungus and its prevention by the use of chemicals were done by Suzuki in Japan (1969, 1974); in Italy, different weather conditions, different rice cultivars, and different populations of the pathogen, make Japanese tests unemployable.
This quantitative evaluation highlights the behaviour of the rice blast and brown spot fungi. Regarding *P. grisea*, we noticed that the appearance of a peak of abundance was followed by the presence of the typical blast symptoms in the field, when environmental conditions were favourable. For instance, the peak of blast fungus airspores recorded at the end of July 2001 should be regarded as an index of foliar blast, the same occurring in the other monitored years. When spores concentrations reached maximum values in September, that was the case of neck blast. An interesting correlation between rainfalls and P. *grisea* spore concentration is evident looking at figures 1 and 2. After every episode of rain occurred in summer 2003, an increase of spore number in the air of the rice field were observed. This should be taken in account when building up a model, but, obviously, rain is not the only factor that can influence spore presence. Temperature and relative humidity are two important factors to be considered in blast development: in our region, the optimum lying between 25 and 28°C, and a mean of 70% of RH is sufficient for conidia to be relased. In contrast, in Japan, no sporulation occurs below 89% RH (Hemmi and Himura, 1939).
Even if losses due to brown spot disease are not as high as those caused by blast, *Bipolaris oryzae* is important because it produces a non-host-specific toxic metabolite called ophiobolin (Nakamura et al., 1960) that can interfere in the normal pathway of plant defence responses. Ophiobolin production starts immediately after pathogen penetration and, in host cells which suffered a sublethal dose of the toxin, an increase of phenolic compounds takes place. This event leads to the formation of a brown pigment that spreads into necrotic lesions typical of this disease (Oku, 1965). When these spots appear, they are similar to blast lesions, so the correct identification of the pathogen is necessary to use the best available treatment. When peaks of *Bipolaris* spp. spores were noticed, oval brown lesions appeared in the fields a few days after microscopic examination of the slide. These lesions were observed in the monitored rice fields during the whole vegetative season, however they produced inconspicuous losses.
In order to create a model applicable to Italian rice, monitoring works like this one should be carried on. The collection of a high number of data on airspores concentration and meteoclimatic conditions is mandatory to obtain a reliable model that can help in reducing the use of chemicals without affecting productivity, thus achieving a more sustainable development.

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INFLUENCE OF CUTICULAR WAXES IN THE ABSORPTION OF CYHALOFOP-BUTYL IN *ORYZA SATIVA* L. AND *ECHINOCHLOA ORYZOIDES* POPULATIONS

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Abstract

The aerial surfaces of higher plants are covered with a cuticle, an extracellular, non-living, lipid covering forming the interface between the plant and its environment. The main function is to minimize water loss from plants when stomata are closed. The cuticle also protects the plant against physical, chemical and biological attacks. Moreover, it represents the initial and main barrier to the penetration of foliar-applied compounds when they are sprayed without the addition of surfactants. The outer surface of the cuticle is covered by epicuticular waxes which can take on many forms, from amorphous to crystalline deposits, and which consist of complex mixtures of long-chain aliphatic and cyclic components. Herbicides have been used for a long time to control weeds, those being post-emergents which are the most important. The formulation of herbicides has been developed to pass through the plant cuticle as efficiently as possible. In the present study, the absorption of cyhalofop-butyl was evaluated in one population of *Echinochloa oryzoides* and *Oryza sativa* L. In addition, the outer surface morphology and the ultrastructure were examined by electron microscopy. The results indicated the relation between waxes (their density, distribution and morphology) and the absorption of cyhalofop-butyl. The *Oryza sativa* L. cuticle was uniformly covered by waxes, with predominantly unshaped large waxes randomly distributed, obtaining absorption values of under 30%, 24 hours after application. The *Echinochloa oryzoides* cuticle was clearly different, forming a non-uniform covered reticule, with less wax density and areas lacking in waxes with emergent plate structures due to wax melting. Consequently, maximum values of absorption were obtained rising to 73% of absorption 24 hours after treatment. Wax composition studies by means of TLC and HPLC techniques are highly recommended to differentiate the role of wax as an effective mechanism to reduce herbicide concentration inside grasses.

Keywords

waxes; cuticle; absorption; translocation; cyhalofop-butyl.

Introduction

As a consequence of chemical and biological evolution, living organisms have developed selected biopolymer structures, which serve as protective barriers against the environment. In aerial organs of higher plants, this protective barrier function is carried out by the plant cuticle. From a morphological point of view, the cuticle covers the outer cell wall of epidermal cells. The plant cuticle is mainly formed by a structural component called cutin, an amorphous biopolyester of hidroxy-fatty acids. Associated with this biopolymer are waxes or soluble cuticular lipids (Holloway, 1982). Waxes can be embedded within the cutin, intracuticular waxes, or deposited on the outer surface of the plant cuticle (epicuticular waxes). Wax biosynthesis and further deposition on the outer surface of aerial parts of plants has been studied (Baker et al., 1982; Laguna et al., 1999). Also, studies on cutin biosynthesis have been completed by Kolattukudy (1996). In the case of weeds, the waxy cuticle protects against the entrance of herbicides, thus reducing toxic effects inside grasses. The most important weeds that reduce the world's rice yield are *Echinochloa* spp. The control of this weed is carried out mainly with herbicides, but due to several factors such as a continuous application and high doses of some products, the appearance of some populations escaping chemical control has led to the development of resistance to some groups of herbicides (ACCase, ALS, PS II...). One of the most important herbicides used for the control of *Echinochloa* spp. is cyhalofop-butyl, an ACCase-inhibiting herbicide used for post-emergence treatments. Formulated herbicides contain wetting agents to improve the absorption through waxy cuticles. However, some weeds are able to increase the production of waxes as a selective mechanism to survive herbicide applications (data not published). The objectives of this work were: a) to study the response of these species to cyhalofop-butyl determining the effective dose (ED_{50}) b) to study the morphology and ultrastructure of the cuticle by means of scanning electron microscopy c) to study the absorption and translocation process of [^{14}C]cyhalofop-butyl in these species.

Materials and methods

Plant Material

Seeds of *Echinochloa oryzoides* were collected from paddy fields in the province of Seville (Southern Spain) without any records of treatments with cyhalofop-butyl. In addition, rice (*Oryza sativa* L.) was also studied to determine the selectivity profile of cyhalofop-butyl.

Growth Assays

Seeds were placed on moistened filter paper in petri dishes. Germinated seeds were planted in pots (five plants per pot) containing peat and sandy loam potting mixture (1:2 wt/wt) in a growth chamber (28/18 C day/night, 16-h photoperiod under 350 µmol photosynthetic photon-flux density m^{-2} s^{-1} and 80% relative humidity).

Dose-response assays

Dose-response experiments were conducted in the greenhouse to quantify resistance to cyhalofop-butyl. The concentration of herbicide causing a 50% decrease in growth with respect to the untreated control (ED_{50}) was determined for each species as described by Menéndez et al., 1994. The R/S ratio was computed as ED_{50}(R)/ED_{50}(S). Doses of cyhalofop-butyl ranged from 0 (non-treated), 20, 40, 60, 80, 100, 140, 160 and 200 g a.i. ha^{-1} for *Echinochloa oryzoides* and 100, 200, 300, 400, 600, 800, 1000, 1500 and 1800 g a.i. ha^{-1} for *Oryza sativa* L. The aboveground fresh weight (%) was evaluated 21 days after the application. Herbicide was applied using a laboratory track sprayer equipped with a Tee Jet 80.02.E.VS flat-fan nozzle that delivered a spray volume of 200 L ha^{-1} at 200 KPa. The herbicide was applied to *Echinochloa oryzoides* and *Oryza sativa* L. plants at 3-4 leaf stage. Treatments were replicated three times. Data were pooled and fitted to a non-linear regression model. A regression analysis was performed using the Graphpad Prism 3.03 statistical software (Martín et al., 1990).

Scanning electron microscopy (SEM)

When plant reached the 3-4 leaf stage, the second leaf was cut in 0,5 x 0,5 cm sections of foliar tissue. Plant material was fixed in glutaraldehyde (2%, v/v, in phosphate buffer 0.2 M, pH 7) overnight at 4 ºC. The samples were thoroughly rinsed in fresh phosphate buffer and then dehydrated through an ethanol solution series: 40, 50, 70, 80, 95 and 100% and increasing times, from 15 min to 1 h 30 min. They were placed on a metallic holder using a doubled-faced adhesive and coated with a 0.05-µm thin film of gold. A JEOL JSM-840 scanning electron microscope operated at 10-20 kV was used for examination of the samples.

Absorption and Translocation

[^{14}C]cyhalofop-butyl was mixed with commercially formulated cyhalofop-butyl to prepare emulsions with a specific activity of 1000 Bq µl^{-1} (both absorption and translocation studies). Concentration of formulated cyhalofop-butyl was 1.5 g a.i. L^{-1} that corresponds to 300 g a.i. ha^{-1} of cyhalofop-butyl in 200 L of final volume. This formulation of labelled herbicide was applied to the adaxial surface of the third leaf of each plant in four 0.5-µl droplets using a microapplicator[1]. A total of 2 KBq (both absorption and translocation studies) were applied on each plant. Plants were harvested in batches of three plants at 3, 6, 12 and 24 hours after herbicide application and separated into treated leaf, upper leaf, root and remainder of shoot. Unabsorbed [^{14}C]-cyhalofop-butyl was removed from the leaf surface by washing the treated area with 3 mL of methanol 80%. Washes from each batch were pooled and analyzed by liquid scintillation spectrometry (LSS)[2]. Plant tissue was dried at 55 ºC for 72 h and combusted in a sample oxidizer[3]. The $^{14}CO_2$ evolved was trapped and counted in a 10-mL mixture of Carbo-Sorb E and Permafluor E^{+} [4] (3:7 v/v). Radioactivity was quantified by LSS. Percent herbicide absorbed was expressed as [KBq in combusted tissue/ (KBq in combusted tissue + KBq in leaf washes)] x 100. The experiment was repeated three times. In the translocation studies, the treated plants were removed from pots at the same intervals after herbicide treatment. Roots were washed and whole plants were blotted dry, pressed against X-ray film, and stored at 4 ºC. The film was developed after three weeks of exposure.

Results

Dose-response assays

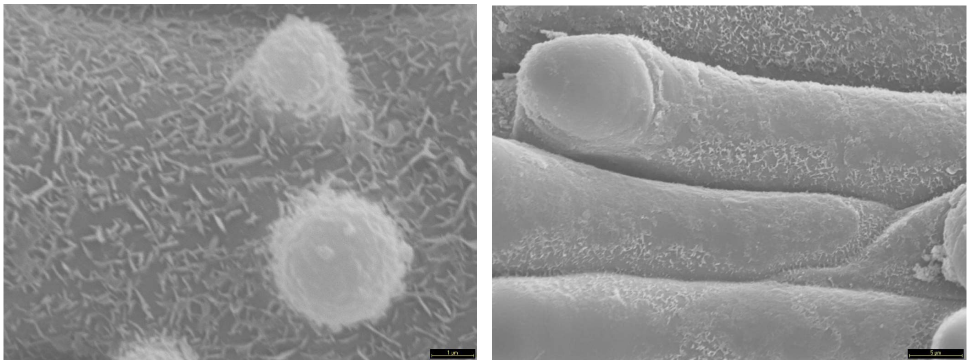
The dose-response assays confirmed the selectivity profile of cyhalofop-butyl in *Oryza sativa* L., displaying ED_{50} values greater than 1800 g a.i. ha^{-1} (Table 1), approximately 6-fold greater than the field dose. A slight reduction in growth during the first days after application was observed, normal growing then being reinitiated. In the case of *Echinochloa oryzoides,* it displayed an ED_{50} value of 64 g a.i. ha^{-1}, which is below the field recommended dose (300 g a.i. ha^{-1}). The effect on susceptible plants was rapid, the first symptoms of phytotoxicity appearing five days after treatment. These symptoms were a visible reduction in plant growth together with chlorotic spots on the cotyledons. At higher herbicide rates, plants displayed a total growth stop, followed by a rapid plant degeneration leading to death in a time period of less than 15 days after treatment.

Table 1 ED_{50} values of cyhalofop-butyl in *Oryza sativa* L. and *E. oryzoides* species**.**

SPECIES	**BEHAVIOUR**	**ED_{50} (g a.i. ha^{-1})**	**Resistance factor ED_{50}(R)/ED_{50}(S)**
Oryza sativa	**T**	> 1800	>28
E. oryzoides	**S**	64	-------

SEM

The outer surface of cuticle of *E. oryzoides* and *O. sativa* L. was studied by means of SEM techniques (Figure 1).



A B

Figure 1 Scanning electron micrographs of *O. sativa* L., bar 1 µm (A) and *E. oryzoides*, bar 5 µm, (B) outer surface in a 3-4 leaf stage. All samples were fixed in glutaraldehide.

Oryza sativa L. cuticle (Figure 1.A) revealed the massive presence of epicuticular waxes provided with a uniform cover that protected them against herbicide penetration. Unshaped large waxes randomly distributed were predominant throughout the cuticle forming a star-shape reticule. The *Echinochloa oryzoides* cuticle (Figure 1.B) was clearly different, forming a non-uniform covered reticule, with less wax density and areas lacking in waxes with emergent plate structures due to wax melting, thus losing the regular structure and letting herbicides pass through the cuticle more easily than rice. Nevertheless, the structure and orientation of waxes in both species were similar, with unshaped shorter waxes forming a star-shaped reticule being predominant.

Absorption and translocation

There were significant differences in the absorption of [^{14}C]cyhalofop-butyl in *E. oryzoides* and *O. sativa* L. (Figure 2). After 24 h, 30% of the recovered radioactivity had penetrated into leaf tissue of the *O. sativa*, with a slight increase in the rate of absorption from 3 h (11%) to 24 h, whereas into the *E. oryzoides*, 77% had penetrated 24 h after treatment. Moreover, high absorption values were reached only 3 h after treatment (47%).

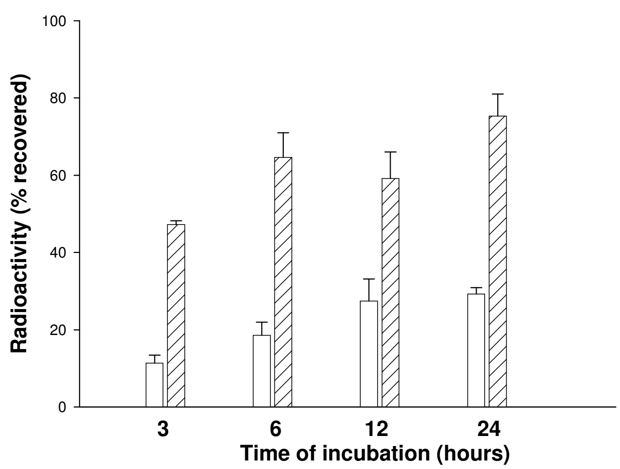


Figure 2 Absorption of [^{14}C]cyhalofop-butyl in *Echinochloa oryzoides* (hatched bars) and *Oryza sativa* L. (open bars). Values are mean of three experiments, and vertical bars represent standard errors.

In the translocation study, the recovered radioactivity of both species revealed a similar distribution of [^{14}C]cyhalofop-butyl or any type of [^{14}C]metabolite formed because of metabolism, into the treated leaves (almost 97% of the recovered radioactivity), with no appreciable acropetal o basipetal herbicide translocation (or both) in both species from 3 h to 24 h after application (Figure 3). These results clearly indicated the immobility of cyhalofop-butyl inside the species examined. No differences were found, either between species, or between times (Figure 3).

A **B**

Figure 3 Corresponding autorradiographs of *Echinochloa oryzoides* (A) and *Oryza sativa* L. (B), 24 h after [^{14}C]cyhalofop-butyl application.

Discussion

Due to the different tolerance observed to cyhalofop-butyl in *Oryza sativa* L. (ED_{50}> 1800 g a.i. ha^{-1}) and *E. oryzoides* (ED_{50}=64 g a.i. ha^{-1}), a differential absorption between both species has been confirmed to be at least one of the mechanisms involved. These results indicated the relation between waxes (density, distribution and morphology) and the absorption rates of cyhalofop-butyl. The *Oryza sativa* L. cuticle was uniformly covered by waxes thus obtaining the lowest absorption values in comparison with the susceptible *Echinochloa oryzoides*, whose cuticle was clearly different regarding its wax distribution and density. The presence of areas lacking of waxes and irregular emergent plate structures mainly determined the absorption obtaining maximum. However translocation was no different in either species. The study of other mechanisms is highly recommended such as susceptibility of the target site (ACCase) and the existence inside grasses of enhanced metabolism carried out mainly by P450 and GST enzymes.

Sources of material

[1] Dispenser, Hamilton PB 6000 TA, Hamilton Co., Reno, NV.
[2] Scintillation counter, Beckman LS 6500 TA, Beckman Instruments Inc., Fullerton, CA 92634-3100.
[3] Oxidizer, Tri Carb Model 307, Packard Instrument Co., Downers Grove, IL 60515.
[4] Scintillation cocktail, Packard Instruments Co., Downers Grove, IL 60515.

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HYDROPHYSIOLOGICAL ANALYSES AND ASPECTS OF ORGANIC FERTILIZATION IN SOILS OF ITALIAN RICE-GROWING AREAS

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Abstract
This paper presents the results of hydrophysiological analyses performed on 24 soils of Italian rice-growing areas, situated in 11 provinces and formed on different geological substratum. Further the results of fertilization obtained by buried and burned straws are compared.

Keywords
Hydrophysiological analysis; chemical agricultural test; geology of substratum; rice-growing areas; Italy.

Introduction

The hydrophysiological analysis of soil (Sasso, 1991, with references) is an objective test of agronomic type, not substitutive of normal physical and chemical analyses and not even of the microbiological ones.
It is based on an hybrid maize culture in little plastic containers without holes in the bottom, realized in conditions of climatic simulation: ambient only artificially lit by fluorescent lamps (12 light's hour at 4000 lux) and kept on a temperature of 25 °C by day and of 20 °C by night.
The gradual and systematic improvement of the analytic method has led to determine 18 agronomical parameters about water capacity, workability, fertility and reactivity on fertilization, weeds.
Every day, at the same hour, weight tests and observations about maize growth are executed.
The data concerning hydrologic parameters are transformed, for an easier utilization in the field, in volumetric values (percentage of water on soil volume).
At present a research to reduce the analysis' time (average 60 days) is in progress.
By this method 24 soils in rice-growing areas have been tested (table 1). From an administrative point of view they belong to 8 Regions, 11 Provinces and 22 Municipalities, and formed on different geological substratum
Further a preliminary chemical agricultural test (Lotti and Galoppini, 1967) has been executed to study the change of the content of organic substance (humus) in two samples of soil collected by Mr Antonio Tinarelli in a rice-growing area near Vercelli (Marchesini, 1984; Tinarelli, 1998; Marchesini and Donna D'Oldenico, 2001-2002). Both the samples (A and B in table 3) show a coarse-grained silt-sandy structure and have been fertilized one (A) by buried straws and the other (B) by burned straws.

Results of the analyses

To make easier the reading and the critical evaluation of the results, 3 tables concerning the different parameters have been elaborated.

In table 1, in addition to the list of the 24 soils divided by Municipality, Region, Province and geology of the substratum, the hydrological parameters (represented as % of water on soil volume) are reported: they are maximum water capacity (column A), water retaining capacity (B), critical humidity limit (C), permanent wilting point (D), hygroscopic point (E) and usable water by plants (F). Permanent wilting point must be considered the more qualifying hydrological parameter and it varies from 3% of Trino (N° 13) to 25% of Ioppolo (N° 24).

Table 1 List, location, geology of substratum (explanation in Appendix) and hydrologic parameters represented as % of water on soil volume. A: maximum water capacity; B: water retaining capacity; C: critical humidity limit; D: permanent wilting point; E: hygroscopic point; F: usable water by plants.

N°	Municipality	Region	Province	Geology of substratum	Hydrologic parameters					
					A	B	C	D	E	F
1	Isola Sant'Antonio	Piemonte	Alessandria	a^1fl^W	50	43	21	11	3	32
2	Pontecurone	”	”	a^1fl^3	56	44	18	8	3	36
3	Pozzolo Formigaro	”	”	fl^3	47	30	19	6	2	24
4	Predosa	”	”	fl^3	44	40	19	8	3	32
5	Rovasenda	”	Biella	fg^R	49	38	17	8	1	30
6	Salussola	”	”	fg^R	59	36	19	10	3	26
7	Borgo Ticino	”	Novara	a^1	65	56	33	7	3	49
8	Caltignaga	”	”	fg^W	51	42	26	5	2	37
9	Caltignaga	”	”	fg^W	56	49	29	6	2	43
10	Fontanetto Po	”	Vercelli	a^1-fg^W	53	38	25	6	1	32
11	Olcenengo	”	”	fg^{W-R}	53	45	28	7	1	38
12	Sali Vercellese	”	”	fg^W	45	40	33	7	2	33
13	Trino	”	”	fg^{W-R}	42	34	21	3	1	31
14	Voghera	Lombardia	Pavia	a^1fl^3	61	47	17	10	4	37
15	Zeme Lomellina	”	”	fl^W	50	39	22	8	2	31
16	Rovigo	Veneto	Rovigo	a	41	36	27	3	2	33
17	Rovigo	”	”	a	45	38	20	18	3	20
18	Baricella	Emilia-Romagna	Bologna		60	51	15	13	5	38
19	Granarolo dell'Emilia	”	”	a^4	45	37	15	9	3	28
20	Ozzano dell'Emilia	”	”	a^4	50	36	18	12	5	24
21	Follonica	Toscana	Grosseto	p^3	42	36	19	11	3	25
22	Pesaro	Marche	Pesaro	f^4	55	45	21	11	3	34
23	Castrignano del Capo	Puglia	Lecce	QP	49	38	19	11	4	27
24	Ioppolo	Calabria	ViboValentia	q^{cl-s}	44	37	28	25	4	12

Table 2 shows hydrological characteristics (reported as % of water on soil volume), density and parameters of soil workability. For an easy reading the first column (A) includes the point of wilting; in the next columns usable water by plants, or available water (B), is divided in optimal water (C) and water of shortaging (D). In the last column (E) shortaging water is given in percentage of total usable water. It is noteworthy that the values of this percentage basically decrease with the rise of the wilting point. The extreme values are 73% (Rovigo 16) and 5% (Baricella), being this last value perhaps related to the typology of the substratum formed by reclaimed land (see Appendix).

The knowledge of these values is very important for the application of the technique of water shortaging.
The apparent density (kg/dm^3) of the soil and the parameters of workability are also given in table 2. In column G is reported the number of waiting days for work with humid soil (column F). This value (number of days) have been obtained with an hypothetical example of work at the depth of 30 cm and with an average daily atmospheric evaporation of 3 mm.
In columns H, I, L, M are reported the penetrometric measurements (kg/cm^2) with humid soil (H), with workable (in "tempera") soil (I), with dry soil (L, wilting point), with parched soil (M, hygroscopic point). Within of an important variability of the 24 soils, depending from their grain size ("texture"), a close relation between an elevated point of wilting and high penetrometric values can be observed.
The last two columns show the fertility index and the control of the weeds. The fertility index is expressed in grams of dry substance (105 °C) for maize plant (average of 10 plants) at the 30th day from the emergency; it varies from the minimum of fertility to the maximum between 0,10 g and 1,0 g.

Table 2 Hydrological characteristics expressed as % of water on soil volume (A: wilting point; B: usable water; C: usable optimal water; D: usable water for shortaging; E: % of shortaging water on usable water), apparent density, parameters of workability (F: % of water in humid soil; G: number of waiting days for work; resistance to penetration (kg/cm^2) in humid soil H, in workable (in "tempera") soil I, dry L, parched M), fertility index (weight in g of dry substance for plant of hybrid maize at 30th day from emergency), number of weeds for m^2 of soil and number of Gramineae (reported in brackets) on the total.

N°	Hydrologic characteristics					Apparent density	Parameters of workability						Fertility index	Number of weeds (n°/m^2) and n° of Gramineae	
	A	B	C	D	E	(kg/dm^3)	F	G	H	I	L	M			
1	11	32	22	10	31	1,196	35	8	1,8	5,0	6,0	8,0	0,38	60	(0)
2	8	36	25	11	31	1,112	26	18	1,0	2,3	5,0	6,0	0,32	60	(0)
3	6	24	11	13	54	1,250	24	6	2,0	2,8	4,3	6,5	0,15	120	(0)
4	8	32	21	11	34	1,142	28	12	1,0	2,8	4,5	5,0	0,13	0	(0)
5	8	30	21	9	30	1,230	30	12	1,3	3,0	5,0	5,5	0,16	420	(30)
6	10	26	17	9	35	1,114	26	10	1,3	2,0	4,5	5,5	0,20	0	(0)
7	7	49	23	26	53	0,701	53	3	1,0	1,3	1,0	1,0	0,07	0	(0)
8	5	37	11	26	70	1,173	36	6	0,5	1,8	4,0	4,0	0,23	150	(0)
9	6	43	20	23	53	1,134	38	11	1,3	2,0	4,5	4,5	0,31	210	(30)
10	6	32	13	19	58	0,838	34	4	0,8	0,8	1,5	1,8	0,19	270	(60)
11	7	38	17	21	55	0,923	33	12	1,0	1,5	2,5	3,5	0,21	1800	(0)
12	7	33	17	16	48	1,030	29	11	1,3	2,5	4,5	5,0	0,17	1800	(0)
13	3	31	13	18	58	1,167	28	6	1,5	1,8	2,3	3,3	0,19	600	(0)
14	10	37	30	7	19	1,127	30	17	1,3	5,0	6,5	11,0	0,24	0	(0)
15	8	31	17	14	45	1,078	31	8	1,0	1,3	2,5	3,5	0,17	0	(0)
16	3	33	9	24	73	1,396	32	4	0,8	1,0	1,3	0,8	0,18	120	(0)
17	18	20	18	2	10	0,996	24	14	1,3	3,3	5,0	8,0	0,19	0	(0)
18	13	38	36	2	5	0,963	29	22	2,0	5,0	6,0	11,0	0,28	0	(0)
19	9	28	22	6	21	1,199	25	12	1,3	3,5	5,5	8,0	0,30	0	(0)
20	12	24	18	6	25	1,133	27	9	1,8	3,5	5,5	8,0	0,28	750	(0)
21	11	25	17	8	32	1,217	24	12	1,5	4,5	6,5	11,0	0,21	120	(0)
22	11	34	24	10	29	0,990	26	19	1,5	2,0	4,0	8,0	0,26	30	(0)
23	11	27	19	8	30	1,256	33	5	1,3	3,0	5,0	5,5	0,54	1350	(240)
24	25	12	9	3	25	1,230	34	3	3,5	5,0	6,5	12,0	0,21	90	(60)

Except for Castrignano del Capo (N° 23), that presents a value of 0,54 and whose geological substratum is very different from the other samples (see Appendix), the fertility index of these soils varies between 0,13 of Predosa (N° 4) and 0,38 of Isola Sant'Antonio (N° 1) and indicates rather modest fertility. A particular and interesting case is the soil of Borgo Ticino (N° 7) with an index of 0,07 that only the chemical analysis could explain. We treat particularly about a soil with a low apparent density (0,701), elevated retaining capacity (56%) and a total absence of weeds. Let us suppose in this case the residual effect of a total herbicide treatment employed in elevate dosage.
Concerning the infesting plants the total number for square meter is calculated, pointing out the number of Graminae. Tested soils present an important variability: detected values comes from zero to 1800 and the number of Graminae range between zero and 240. Particularly we note that 19 soils on 24 (79%) present for Graminae zero value, this condition is probably due to the use of selective herbicides.
In table 3 are listed the results of the preliminary chemical agricultural test on two samples of soil collected near Vercelli: the content in humic substances doesn't show meaningful differences, the content in organic substance is medium-low and fulvic acids are abundant. The organic substance not extracted from soil by alkalis, at last, presents a modest value.

Table 3 Decomposition rate of the organic substance in two soils near Vercelli, fertilized by buried (A) and burned (B) straws.

	A	B
Organic substance %	1,36	1,30
Organic substance not extracted from soil by alkalis %	8,3	8,0
Raw humic acids %	61,53	63,77
Fulvic acids %	38,47	36,23
pH	5,6	6,1

Conclusions
After this work illustrated by this attempt of synthetic statement, documented by 456 numerical data, it seems we can affirm that within the complex system CLIMATE-SOIL-PLANT the SOIL element presents an important variability and the hydrophysiological analysis can point out in a significant way the different agronomic parameters, that are correlated to productivity of the tested soils.
Preliminary data from the chemical agricultural test of soils A and B show most likely that buried straws endure a decomposition process with formation of unstable humus not accumulated in soil. Soil with burned straws presents a variation of pH tending to neutrality: a growth in variation of pH in the future could reduce the fertility after a solubility of the humus in soil.

Appendix
In the table 1 the column "Geology of substratum" reports the symbols used on the sheets of the Geological Map of Italy at scale 1:100˙000 (Geological Map of Calabria at scale 1:25˙000 for the N° 24) for the geological formations of the tested soils.
In follows the references to the number and the name of the sheets and a synthetic description are supplied.
a (64 - Rovigo): alluvium of the different courses followed by Adige river;
a^1 (31 - Varese): terraced alluvium;

a^4 (88 - Bologna, 89 - Imola): plain's alluvium;
a^1-fg^W (57 - Vercelli): sometimes terraced, coarse- to fine-grained pebbly-sandy alluvium, locally containing clayey lenses, covered by a thin stratum of brownish weathering;
a^1fl^W (58 - Mortara): terraced sandy-silty-clayey alluvium of different ages;
a^1fl^3 (70 - Alessandria): mainly silty alluvium of the principal surface in the plain at the S of Po river;
fg^R (43 - Biella): pebbly glaciofluvial alluvium, weathered in clayey, yellow-ochreous soil for a maximum thickness of 3 m; with loess cover;
fg^{W-R} (43 - Biella): pebbly glaciofluvial alluvium, with a thin stratum of brownish or sometimes yellowish weathering, forming the fundamental level of the plain; with loess cover;
fg^W (44 - Novara): pebbly glaciofluvial alluvium and mainly sandy-silty fluvial alluvium (below the northern limit of the resurgences), with a thin stratum of brownish weathering;
fl^W (58 - Mortara): mainly sandy and sometimes silty fluvial alluvium, with a weak ochreous or brown weathering on surface;
fl^3 (70 - Alessandria): pebbly-sandy-clayey alluvium with a modest weathering in surface;
f^4 (109 - Pesaro): pebbly and sometimes partially sandy alluvium and coastal deposit of the IV order of the terraces at 5-10 m on valley floor;
p^3 (127 - Piombino): present-day and recent palustrine deposits, pebbly and sandy silts with pebbly levels and lenses;
q^{cl-s} (245 I N.E. Spìlinga): reddish continental deposits formed by conglomerates, sandy conglomerates and sands;
QP (223 - Capo S. Maria di Leuca): CALCARENITI DEL SALENTO (Salento calcarenites) - calcarenites; coarse-grained, "panchina" type limestones; more or less cemented and sometimes clayey limestones ("tufi").
The sample N° 18 (Baricella) comes from a reclaimed land and for this reason we haven't reported any indication.
About the age of these formations it extends from Plio-Pleistocene for the Calcareniti del Salento (QP) to Holocene. In particular a^1-fg^W, fg^R, fg^{W-R}, fg^W, fl^W, fl^3, f^4 and q^{cl-s} have Pleistocenic ages, a^1fl^W and a^1fl^3 place themselves to the limit of Pleistocene-Holocene and the remainings have got Holocenic ages.

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CROP COEFFICIENT (Kc) DETERMINATION IN SPRINKLER IRRIGATED RICE

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Abstract

Experimental trials were carried out in Sardinia in 2002 and 2003, in order to obtain, among other, a first information on kc values at different phenological stages in sprinkler irrigated rice; in 2003 the trials differed for the sowing date.

An equipment recording soil moisture content every 10 cm up to 100 cm depth was used to obtain the water balance for the first soil layers. Result analysis highlighted that the layer up to 20 cm covers about 80 % of the crop water requirements for the whole cycle.

Kc values were determined as the ratio between crop evapotranspiration and ETo, after Penman-Monteith's equation.

The results show a variability particularly related to the meteorological trend during the trials. Crop coefficient (estimated value) ranged between 0.2 and 0.75 for the initial stage (Kc_{ini}), between 0.85 and 1.0 for mid season stage (Kc_{mid}) and was 0.2 for late season (Kc_{end}).

Keywords

Rice**;** sprinkler irrigation; crop coefficients.

Introduction

Rice growing is generally carried out with flooding irrigation. Water consumption is very high and in temperate areas, depending on environmental conditions such as climate, soil characteristics, cultivar employed, etc., it ranges between an average of 13 and 20,000 m^3 ha^{-1}, with maximum values of about 50,000 m^3 ha^{-1} (Giardini, 2002; Spanu *et al.*, 1996a; 1997a; 1997c; 1998a; 1999a; 2000a; 2001; 2002a; 2003a).

Many experiments were carried out to reduce such water consumptions. An alternative irrigation method to flooding is sprinkler irrigation (Ferguson *et al*., 1977; McCauley, 1990), which besides high water savings, presents environmental, agronomic and economic advantages. Experimental trials with sprinkler irrigation carried out in Sardinia highlighted the possibility of reducing water consumptions by 50 % (Spanu *et al.*, 1989; 1992; 1996b; 1996c; 1996d; 1997b; 1997d; 1998b; 1999b; 2000b; 2002b; 2003b).

In order to increase water savings, knowing single crop coefficient (kc) values at different phenological stages for sprinkler irrigated rice in temperate areas is very helpful. Studies and researches reported in literature only concern kc values for flooded irrigated rice (Lage *et al*., 2003; Shah *et al.*, 1986; Spanu, 1984; Tyagi *et al.,* 2000).

Materials and methods

The trial was carried out in a two-year period (2002-2003) in Sardinia at "S. Lucia" experimental field (34° 59' N and 8° 40' E) of University of Sassari.

Table 1 Main characteristics of the experimental field.

Location	39°59' N and 8°40' E
Altitude (m a. s. l.)	15
Texture	Loam-clay
Field capacity (*in situ*, % vol.)	31.60
Wilting point (-1.5 MPa, % vol.)	20.35
Bulk density (t m^{-3})	1.375
Water table	absent
pH	7.02
$CaCO_3$ total (%)	1.77
Organic matter (%)	1.2
Total N (%)[1]	0.077
P_2O_5 ass. (ppm)[2]	189.5
K_2O exch. (ppm)[3]	249.5

[1] Kjeldhal method; [2] Jackson method; [3] Ammonium acetate method.

In year 2002 sowing was carried out on the 28th of May and crop emergence occurred on the 6th of June. In year 2003 sowing was carried out in two periods: the first on the 2nd of May, with emergence on the 18th of May, and the second on the 27th of May, with emergence on the 4th of June.

In both years 500 viable seeds m^{-2} were sown in 14 cm spaced rows. The cultivar employed was IRAT 190 (upland) in 2002 and EURO in 2003.

Before sowing, both in 2002 and 2003, 90 kg ha^{-1} of N (urea), 90 kg ha^{-1} of P_2O_5 (superfosphate) and 50 kg ha^{-1} of K_2O (potassium sulphate) were distributed. Additional 30 kg ha^{-1} of N (calcium nitrate) were distributed both at the end of tillering and at early heading stages. Weed control was carried out at crop pre-emergence by distributing about 1.300 g ha^{-1} of Pendimethalin.

Soil water content variations during the irrigation season were taken twice a day in 2002 (7:00 a.m and p.m) and once in 2003 (7:00 a.m). The equipment used (Diviner 2000) consisted of a probe which, after being normalized, is introduced in the soil through an access tube, and records water content at different soil depths. The different soil dielectric capacity, that changes in relation to moisture content, was recorded in a data-logger. The equipment allowed to follow soil moisture content variations at regular intervals of 10 cm till 100 cm of depth. The readings were taken on two replications for every sowing time and year.

Sprinkler irrigation was planned to keep soil humidity in the 0-40 cm layer inside field capacity values, previously determined '*in situ*'.

Results and Discussion

These first experimental results on crop coefficient determination for sprinkler irrigated rice were taken from data obtained in still ongoing multi-year trials, aiming at determining the main irrigation parameters and yield response coefficient (ky) of rice to irrigation.

Charts of soil water content fluctuations during 2002 and 2003 irrigation seasons are reported in figures 1, 2 and 3. Readings were carried out in 10 cm deep soil layers, up to a depth of 100 cm. Charts report water content trends of the 40-50 cm and 50-60 cm layers, and average water content trends of the 0-40 cm and 60-100 cm layers. Soil moisture, during the irrigation season, remained almost always above field capacity values.

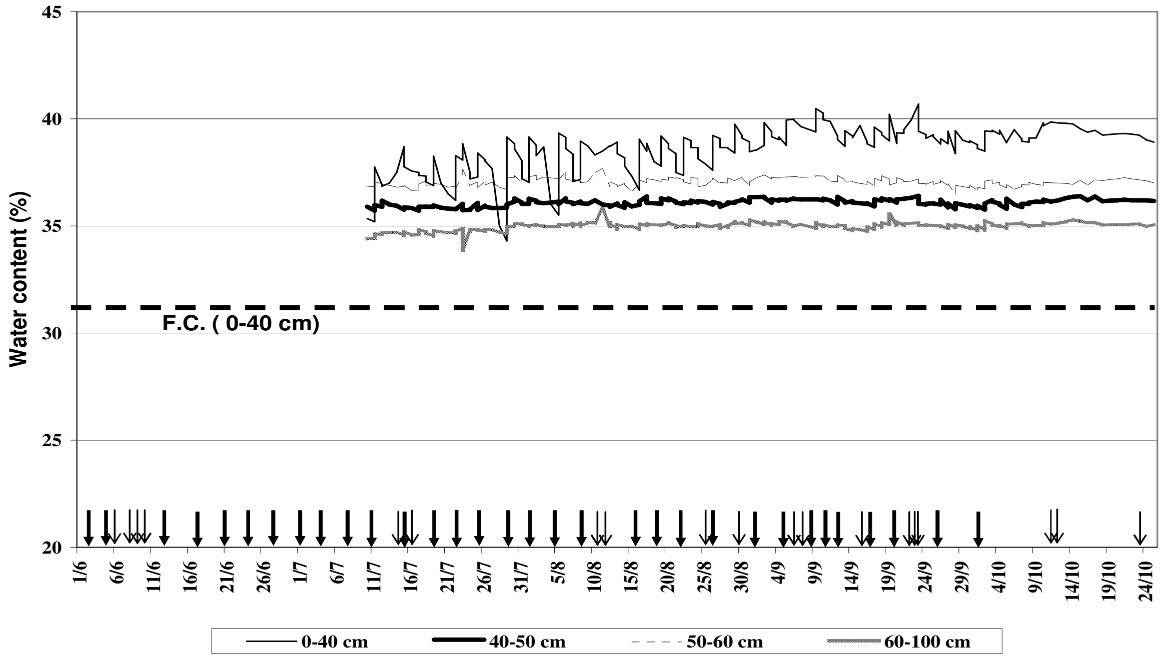

Figure 1 Trend of soil water content at different soil layers in 2002 (irrigation: ↓; rainfall: ↓).

The 0-40 cm soil layer showed the higher moisture variations in all treatments due to both evapotranspiration and rainfall (particularly in 2002) and irrigation water supplies. Deeper layers do not seem to be affected by root uptake and water infiltration, probably due to soil texture.

The constancy of soil moisture values below 40 cm shows that water uptake by rice roots and the related water movements occur only in the upper soil layers.

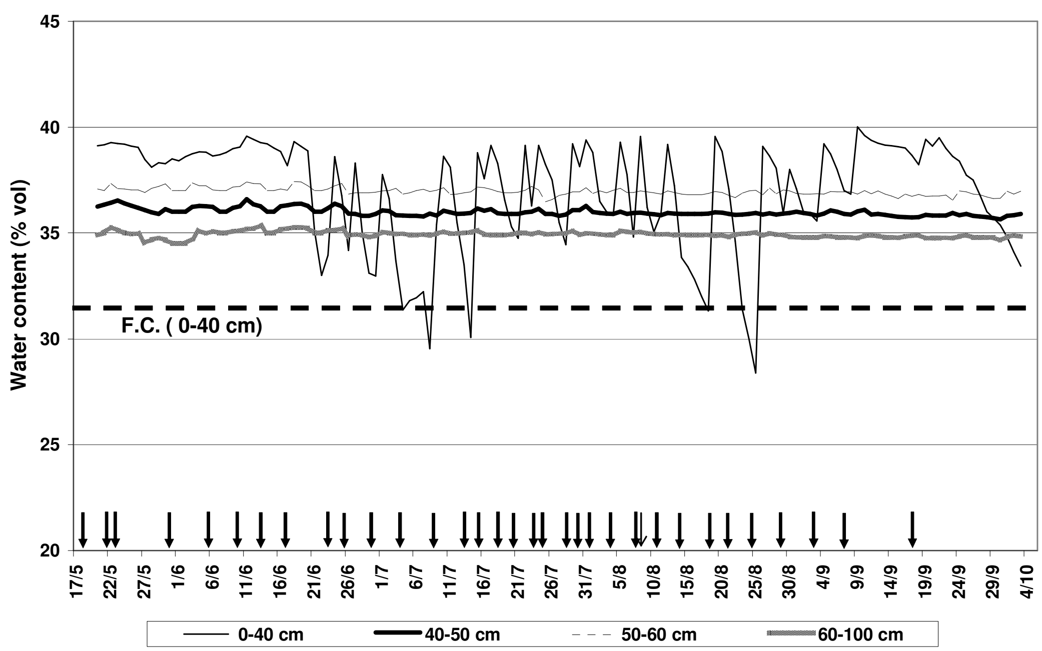

Figure 2 Trend of soil water content at different soil layers in first sowing period of 2003 (irrigation: ↓; rainfall: ↓).

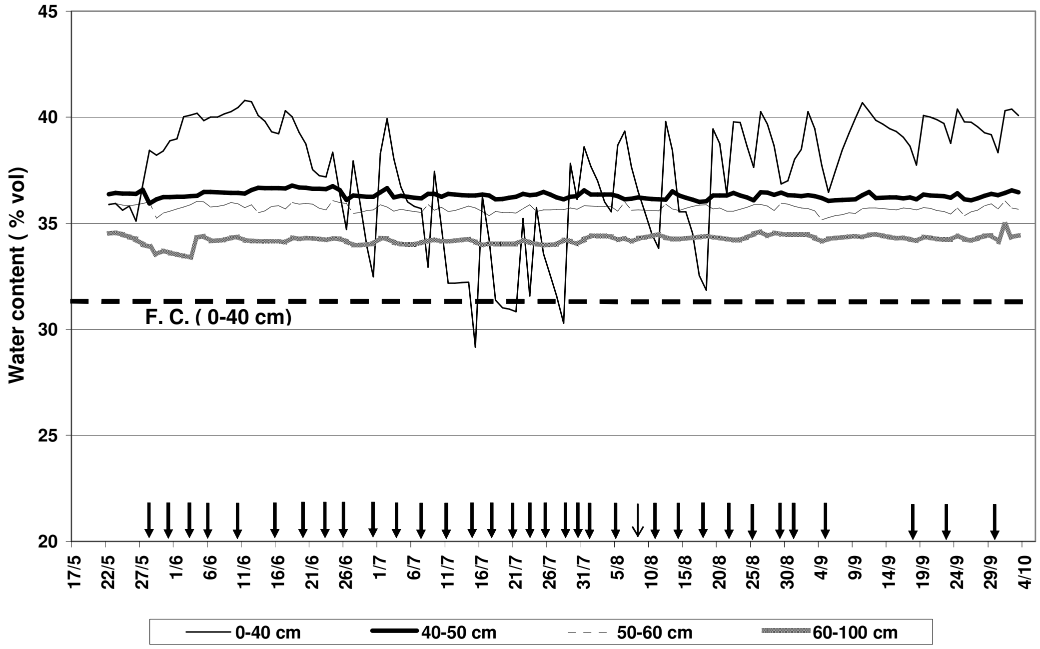

Figure 2 Trend of soil water content at different soil layers in second sowing period of 2003 (irrigation: ↓; rainfall: ↓).

Water losses in the 0-40 cm layer were evaluated one by one for the 0-10 cm, 10-20 cm, 20-30 cm and 30-40 cm soil layers. These results are reported in figure 4.

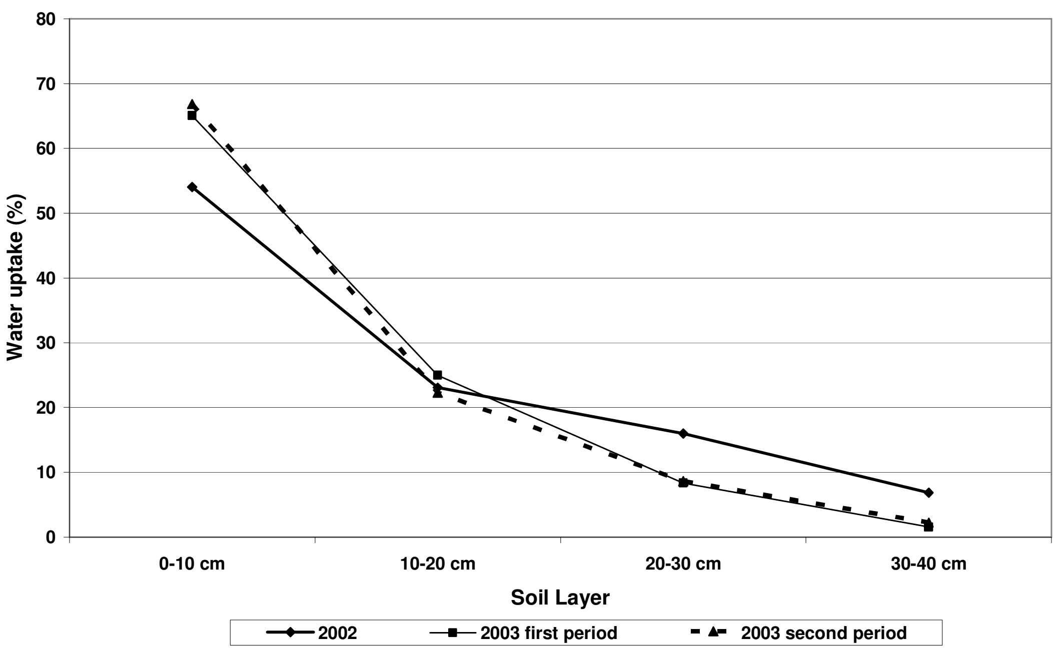

Figure 4 Water uptake by different soil layers.

The 0-10 cm layer highly contributes to crop water supply, probably for the high root system density, as highlighted in other studies. In 2003 no differences were recorded for different sowing periods; in 2002 water extraction patterns do not essentially differ from 2003.

The constancy of soil moisture values in deeper layers (below 40 cm) and the optimal rice water-conditions allowed kc evaluation at different crop stages.
Single kc values were obtained as the ratio between crop evapotranspiration in different periods, calculated through soil moisture content variations of the 0-40 cm layer, and standard evapotranspiration (ETo) over the same periods, calculated by hourly Penman-Monteith's equation.
ETo and kc values are reported in figure 5, 6 and 7. Differences between ETo values in 2002 and 2003 highlight the different climatic trend between the two years: in 2002 temperatures and rainfall were not as high as in corresponding stages of crop growth of 2003, in fact maximum values of standard evapotranspiration did not exceed 5.4 mm d^{-1} , whereas average and minimum values were 3.4 and 0.7 mm d^{-1} respectively. On the other hand, the high temperatures recorded during rice growth in 2003 and the lack of rainfall contributed to raising the average ETo value to 6.4 mm d^{-1} , with a peak of 9 and a minimum of 2.1 mm d^{-1}.
In 2002 total daily standard evapotranspiration in the emergence-physiological maturity period was 447.0 mm; in 2003 the same value was 819.4 mm for the first and 730.1 for the second sowing.
In 2002 (figure 5) kc calculation started on the 10th of July when the crop had completely covered the soil, therefore the average values of previous periods relating to the 1st and 2nd stage, initial and crop development stage respectively, were evaluated with the F.A.O. method reported in paper n° 56 (Allen *et al.,*1998). They were 0.7 for the initial stage and 0.85 for the crop development stage. The average kc value were 1.0 for the mid-season stage (3rd F.A.O. crop stage) and 0.2 for the late season stage (4th F.A.O. crop stage).

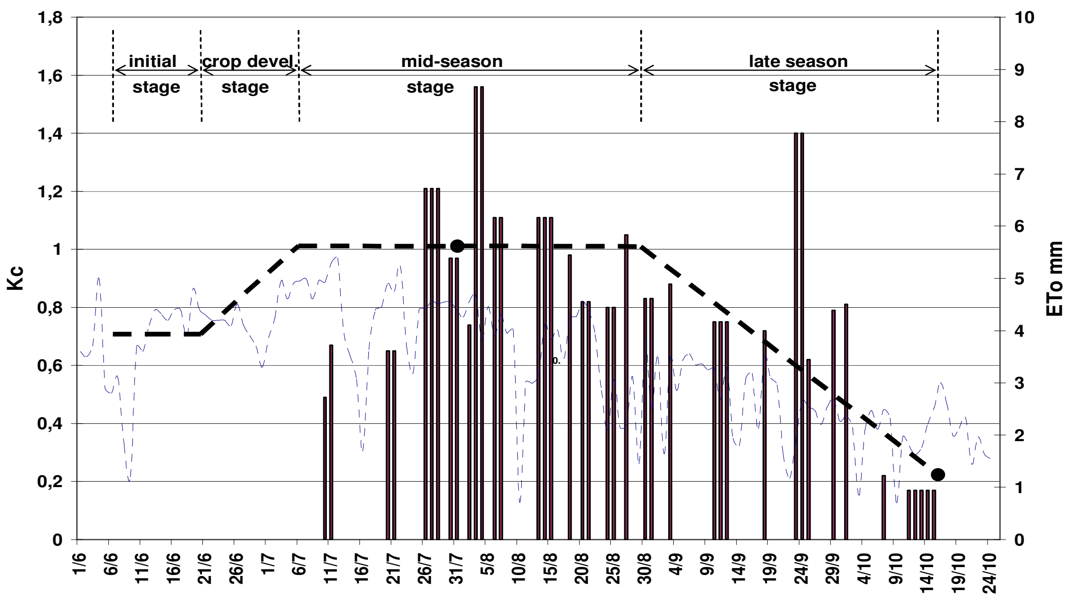

Figure 5 Detected crop coefficients for growth stages and ETo (dotted line) by Penman-Monteith hourly time equation for 2002.

The four growth stages' length in which the biological rice cycle was subdivided was14 days for the first stage (from emergence to 10 % soil cover – coinciding with tillering start), 16 days for the second stage (from the end of the previous period to 100 % soil cover – at half tillering), 54 days for the third stage (from the end of the previous period to the beginning of maturity), which therefore included end-tillering, start-shooting, heading, flowering and fruit-setting, 47 days for the fourth stage (from the end of the previous period to final maturity), which involved different sub-stages of maturity process. The total length of the rice biological cycle (from emergence to physiological maturity) was therefore 131 days.

Figure 6 and 7 report kc values for the first and second sowing period of 2003. The kc average values calculated for the first stage in both periods approach 0.2 and are therefore significantly lower than the value estimated by F.A.O. for 2002 (kc = 0.7). F.A.O. strictly relates the kc value for the first stage to crop water supply and ETo. In 2002 indeed, due to rainfall during the emergence-10% crop cover stage and lower ETo average values, we assume that the ratio between crop and standard (kc) evapotranspiration was higher than in 2003. The latter was characterized by a lack of rainfall and a higher ETo average value, which explain the higher kc value obtained in 2002.

The kc average value for the 2nd stage was 0.6 and 0.55 for the first and the second sowing time respectively.

The trend of kc values during the intermediate period (3rd stage) shows at first an increase for the different sowing periods, and then a decrease to significantly lower values, not entirely explained by the variability of the experimental datum measurement. This decrease of kc values is probably due to partial stomata closing in reaction to high the temperatures recorded in that period (ETo max 9 mm d^{-1}). The high thermal trend, particularly in the second sowing period, caused an early partial wilting of leaf ends, determined by an unbalance between crop evapotranspiration flux and water uptake by the root system. This occurred because of the high atmosphere's evaporation power and despite the high soil water content (figure 1, 2 and 3). The mean kc value calculated for the first sowing time, as a consequence, was 0.85 vs. 1.0, confirming the datum obtained in 2002.

As for the kc value in the late season stage it was 0.2 for both sowing periods.

In 2003 the emergence-final maturity period length was 128 days for the first sowing period (1st stage 14 days, 2nd stage 16 days, 3rd stage 54 days and 4th stage 47 days) and only 118 days for the second sowing period, mainly due to shortening of the 4th stage (flowering-physiological maturity) determined by the interactive effect between the thermal trend and the maturity processes (1st stage 14 days, 2nd, 3rd and 4th stage 16, 52 and 36 days respectively).

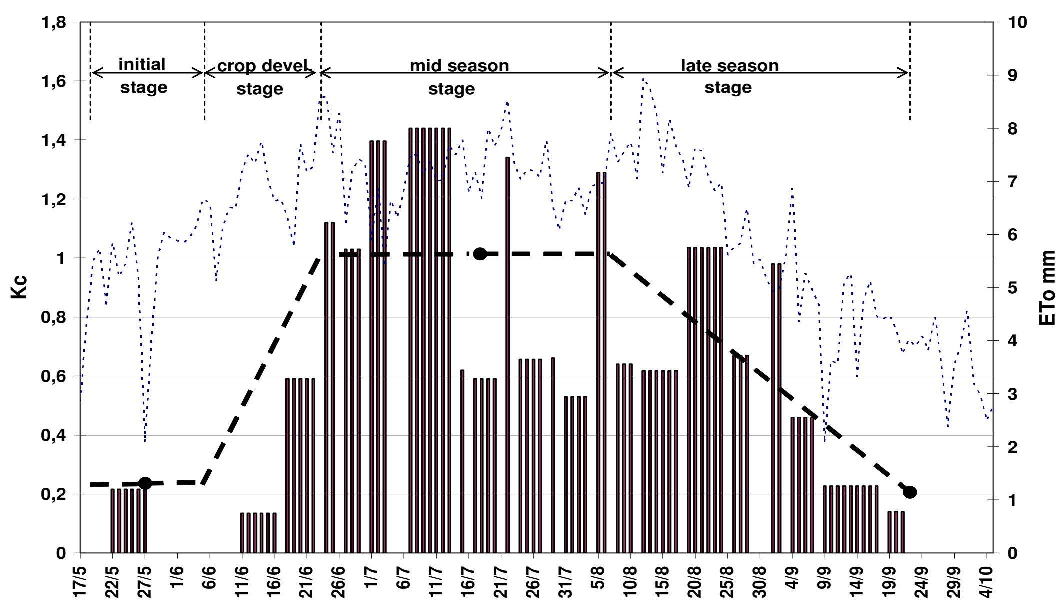

Figure 6 Detected crop coefficients for growth stages and ETo (dotted line) by Penman-Monteith hourly time equation for first sowing period of 2003.

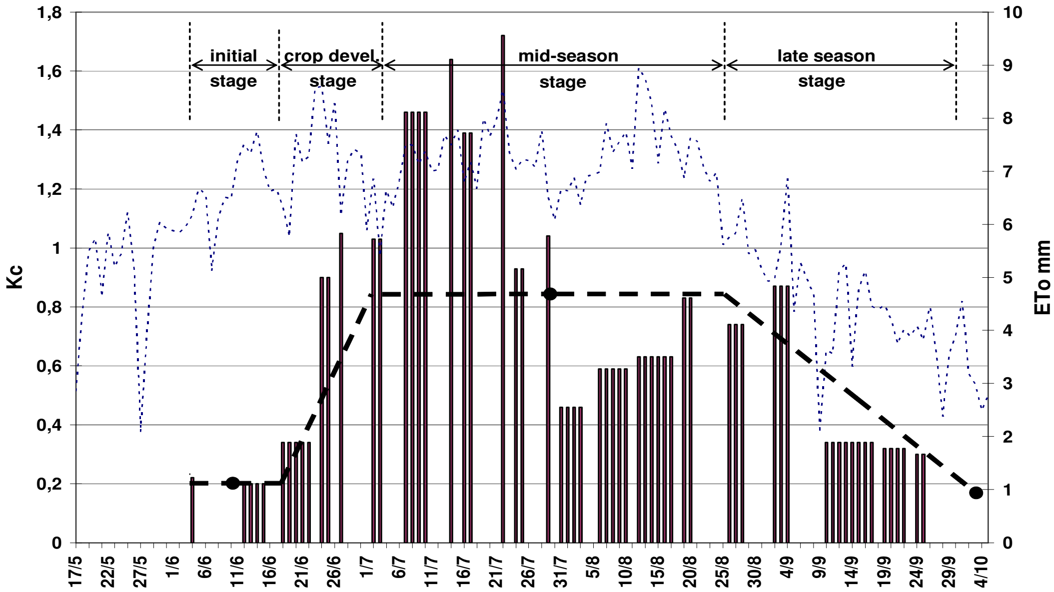

Figure 7 Detected crop coefficients for growth stages and ETo (dotted line) by Penman-Monteith hourly time equation for second sowing period of 2003.

Conclusions

The equipment used (Diviner 2000) allowed to analyse soil moisture content variations every 10 cm of soil up to a depth of 1 meter. Data analysis highlighted the importance of the shallower (0-10 cm) soil layer for water uptake in sprinkler irrigated rice.

Soil water content variations analyses allowed indeed to obtain first and reliable kc values, which will be however further verified and mediated with the ones obtained by rigorous statistical analyses from the multi-year ongoing experimental trials.

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EFFECT OF THE AMOUNT AND FORM OF NITROGEN FERTILIZER ON VEGETATIVE GROWTH AND NITROGEN UPTAKE AT MAIN PHENOLOGYCAL STAGES OF SPRINKLER IRRIGATED RICE

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Abstract

In cereal crops nitrogen availability is strictly linked with biomass production and can therefore become a limiting factor for grain yield. The amount and time of nitrogen distribution plays an important role in limiting losses and in increasing nitrogen fraction uptake by plants.

In flooded irrigated rice, if the fertiliser is incorporated in the reduced soil layer, the denitrification process is reduced as nitrogen is kept in the N-NH_4^+ form by the soil exchange complex.

With sprinkler irrigation the soil environmental conditions pass from a reduced to oxydated state: under these conditions soil NO_3 is formed very rapidly and, not being retained by the soil CEC, is easily leached.

The effects of different nitrogen amounts and formulations on vegetative growth and element uptake at main phenological stages was evaluated. Treatments with 100, 150 and 200 kg ha^{-1} nitrogen supply in ureic nitric and mixed (ureic and nitric in equal parts) form was compared.

The highest values of LAI, particularly at 150 and 200 kg ha^{-1} nitrogen levels, were recorded in the mixed treatment. A higher nitrogen content in leaves, stems and panicles at main phenological stages was also recorded in mixed treatment.

The total amount nitrogen utilised by the crop, at ripening, was highest in the mixed treatment.

Keywords

rice; nitrogen; phenological stages; sprinkler irrigation.

Introduction

The cereals yield increasing is strongly related to nitrogen availability. In irrigated crops, nitrogen fertilizing timing and rate play a key role both on nitrogen losses limiting and on plant uptake increasing.

Several experiment on flooded irrigated rice highlighted deep nitrogen burying after sowing achieved yield increases than split application also when it is applied at mid tillering and/or panicle initiation stage (Craswell *et al*., 1980; De Datta *et al*., 1981; 1983; Flinn *et al*., 1981; Mikkelsen, 1987; Spanu *et al*., 1997a).

Many experimentations in sprinkler irrigation evidenced higher rough rice production than in flooded irrigated condition. By adopting sprinkler irrigation, beside lower environmental impact following the simplified crop cultivation technique, around 45% of water saving is achieved (Spanu *et al*., 1989; 1992; 1996b; 1996c; 1996d; 1997d; 1998b; 1999b; 2000b; 2002b; 2003b).

The elevated soil oxidation, following sprinkler irrigation adoption, leads fast N-NO_3 formation. To avoid nitric nitrogen leaching it is necessary to split nitrogen applications.

Employing ureic nitrogen, a trial on sprinkler irrigated rice carried out in Sardinia evidenced higher yield on split application treatment (Spanu *et al*., 1997b).
To evaluate the influence of nitrogen form, rate and distribution timing on rough rice production, a trial was carried out on sprinkler irrigated rice. This paper reports the effects on vegetative growth and nitrogen uptake efficiency at main phenological stages.

Materials and Methods

Planning a split plot experiment with four replicates three nitrogen forms as main plot were compared: 1) ureic (from urea); 2) nitric (calcium nitrate); 3) mixed (1/2 ureic + ½ nitric). As a second variation source, three level of nitrogen supply were compared: 100, 150 and 200 kg ha^{-1} (N_{100}, N_{150} and N_{200}, respectively).
In relation to nitrogen form, the distribution timing were: 1) on ureic nitrogen treatment the total amount has been divided in three interventions: ½ at the sowing, ¼ at tillering and ¼ at boot; 2) on nitric nitrogen treatment the total amount has been divided in three interventions: ¼ at the sowing, ¼ at the tillering and ½ at the boot; 3) on ureic + nitric nitrogen treatment, the total amount has been divided in three interventions: ½ at the sowing (employing only urea), ¼ at the tillering (employing only calcium nitrate), and ¼ at the boot (employing only calcium nitrate).
On 126 m^{-2} main plot 500 viable seeds m^{-2} of rice cultivar Thaibonnet were sown.
The sowing bed preparation has been performed with a ripper at 20 cm depth followed by harrowing. All the plots were fertilized distributing 90 kg ha^{-1} of P_2O_5 and 50 Kg ha^{-1} of K_2O.
The sowing has been carried out with a drill at 3 cm depth in 14 cm spaced rows; following sowing a rolling has been conducted.
The pre-emergence weed-control has been carried out distributing 1,300 g ha^{-1} of pendimethalin.
On two sampling areas of 2 linear meter each on, data on crop density at emergence and harvest were collected. On two sampling areas (0.5 linear meter each on) per plot, plant samples were collected at each phenological stages (tillering, panicle initiation, heading and maturity), to dry matter (DM), Leaf Area Index (LAI) and nitrogen uptake evaluation. Nitrogen uptake was estimated by the weighted mean of N content (Kjeldahl) determined on stems, leaves and panicles.

Results and Discussion

In Table 1 are reported the Fisher test results carried out on observed variables. Not significant interaction were found between nitrogen form and rates.

Table 1 Fisher test results on observed variables.

	Nitrogen form (A)	Nitrogen rate (B)
LAI (tillering)	n.s.	n.s.
LAI (panicle initiation)	*	n.s.
LAI (heading)	n.s.	*
LAI (maturity)	*	*
Stems (no. m^{-2}, tillering)	n.s.	n.s.
Stems (no. m^{-2}, panicle initiation)	n.s.	n.s.
Stems (no. m^{-2}, heading)	n.s.	n.s.
Stems (no. m^{-2}, maturity)	*	n.s.
Dry matter (g m^{-2}, tillering)	n.s.	n.s.
Dry matter (g m^{-2} panicle initiation)	n.s.	n.s.
Dry matter (g m^{-2}, heading)	n.s.	n.s.
Dry matter (g m^{-2}, maturity)	n.s.	n.s.
Sowing-heading interval (d)	***	***
Sowing-maturity interval (d)	n.s.	***
Nitrogen uptake (kg ha^{-1}, tillering)	n.s.	n.s.
Nitrogen uptake (kg ha^{-1}, panicle initiation)	*	**
Nitrogen uptake (kg ha^{-1}, heading)	n.s.	***
Nitrogen uptake ((kg ha^{-1}, maturity)	n.s.	n.s.

** = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$;*

In figure 1 (a,b,c) are respectively reported the LAI trends for the nitrogen form treatment. Any statistical differences were found between nitrogen form and rate at mid-tillering; therefore in nitrogen nitric form at high rate the higher LAI values were observed. At panicle initiation stage the ureic-nitric treatment showed the higher LAI values, probably caused by the accumulation of released ureic nitrogen and the top dressed nitric nitrogen. At heading no staftistical differences were evidenced between nitrogen form, while on treatment N100 were observed the lower LAI values. At maturity, lower average values were observed on nitric treatment, probably caused by high nitrogen losses. About nitrogen rate, a progressive reduction of LAI values were observed from N200 to N100.

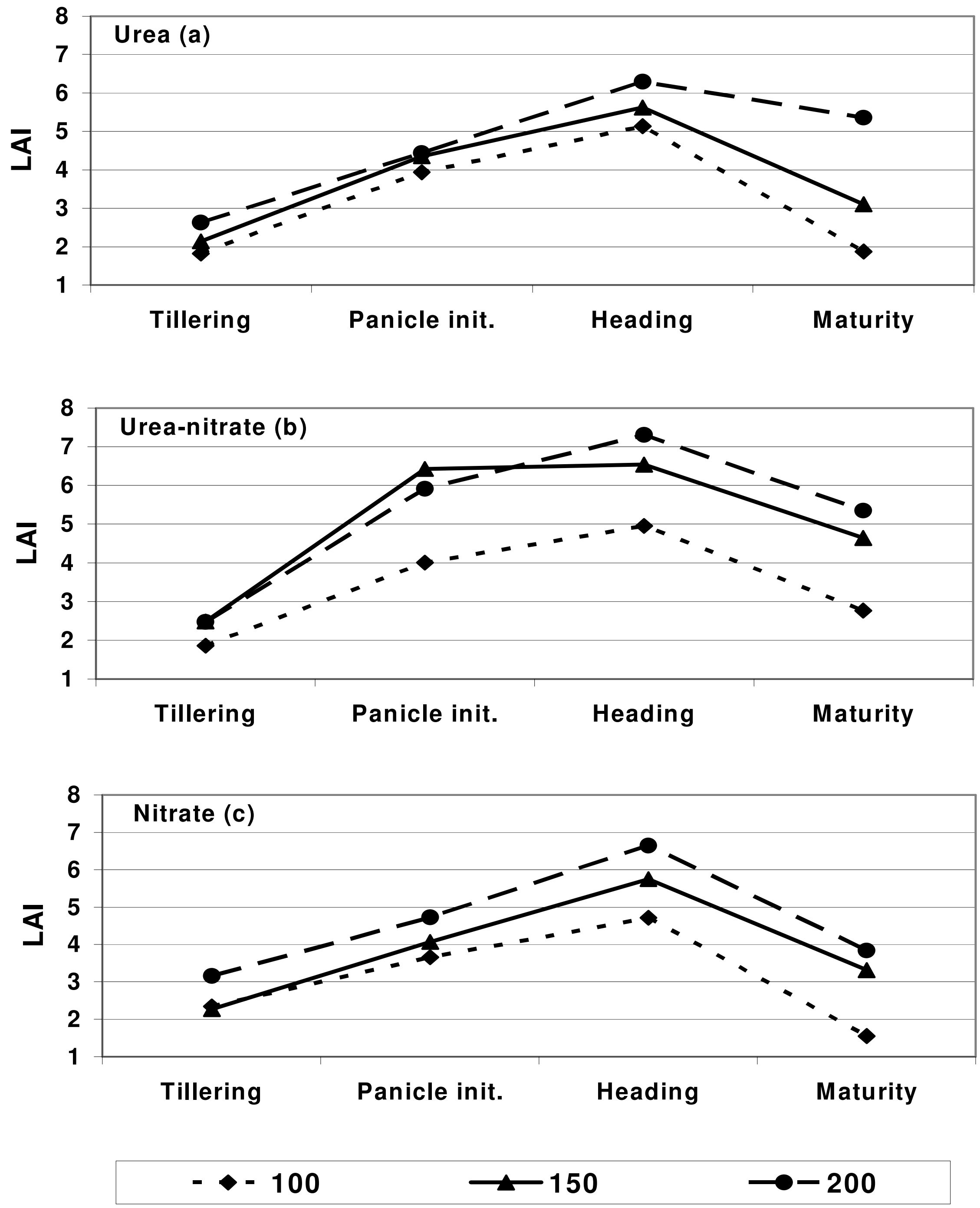

Figure 1 LAI trend for the nitrogen form and rate treatment.

Figure 2 (a,b,c) reports the stems number m^{-2} monitored during the trial. Significant differences were not evidenced between nitrogen from and rate on tillering, panicle initiation and heading stages. As effect of intra-specific competition, a progressive reduction of tiller number (from tillering to heading) was observed. Significant differences were recorded at maturity between nitrogen form: the lower number of stems was observed on nitric nitrogen treatment, particularly at lower rate.

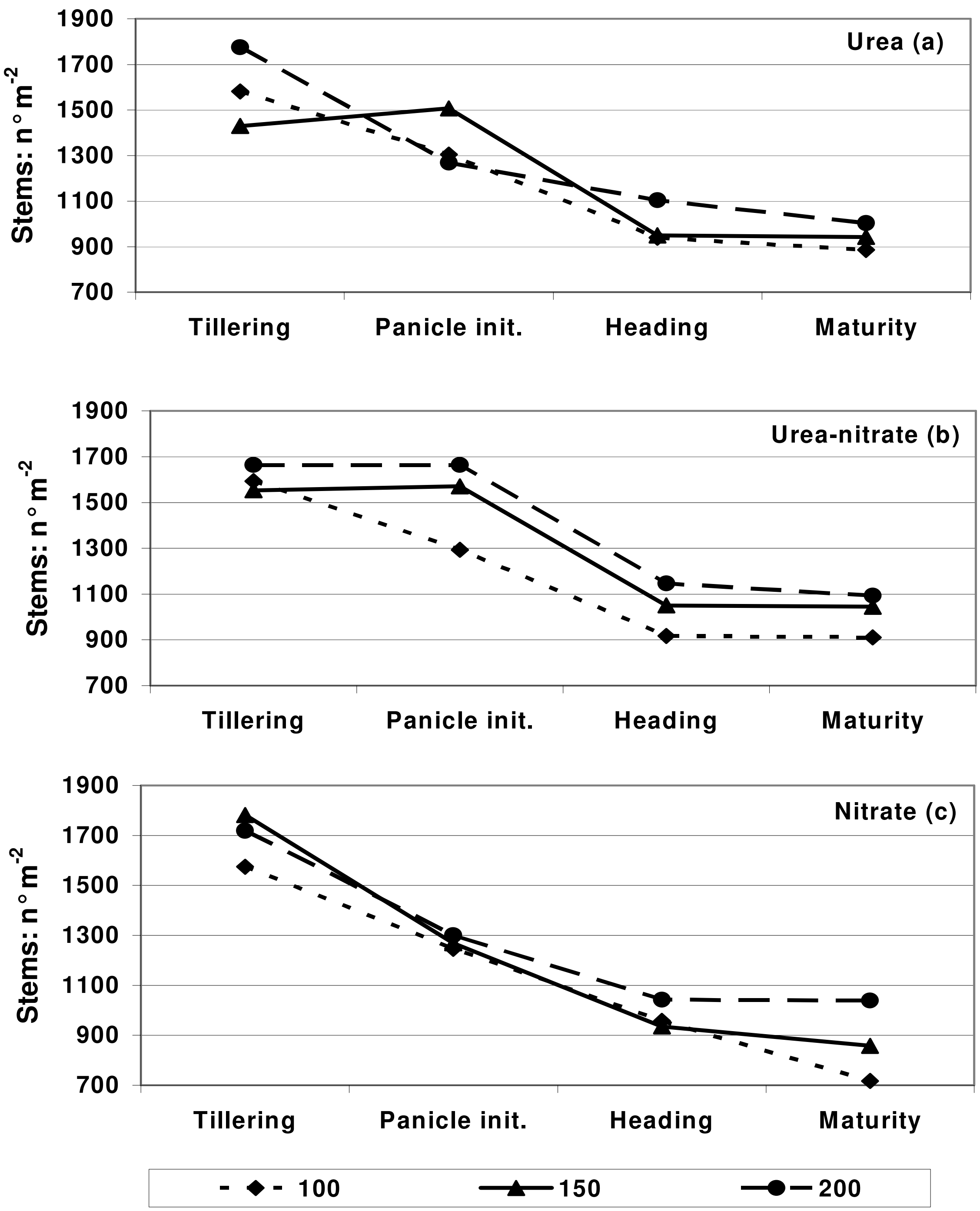

Figure 2 Stems number trend for the nitrogen form and rate treatment.

Total dry matter was not influenced by nitrogen form and rate, showing a progressive increasing only in relation to the phenological evolution (figure 3 – a,b,c).

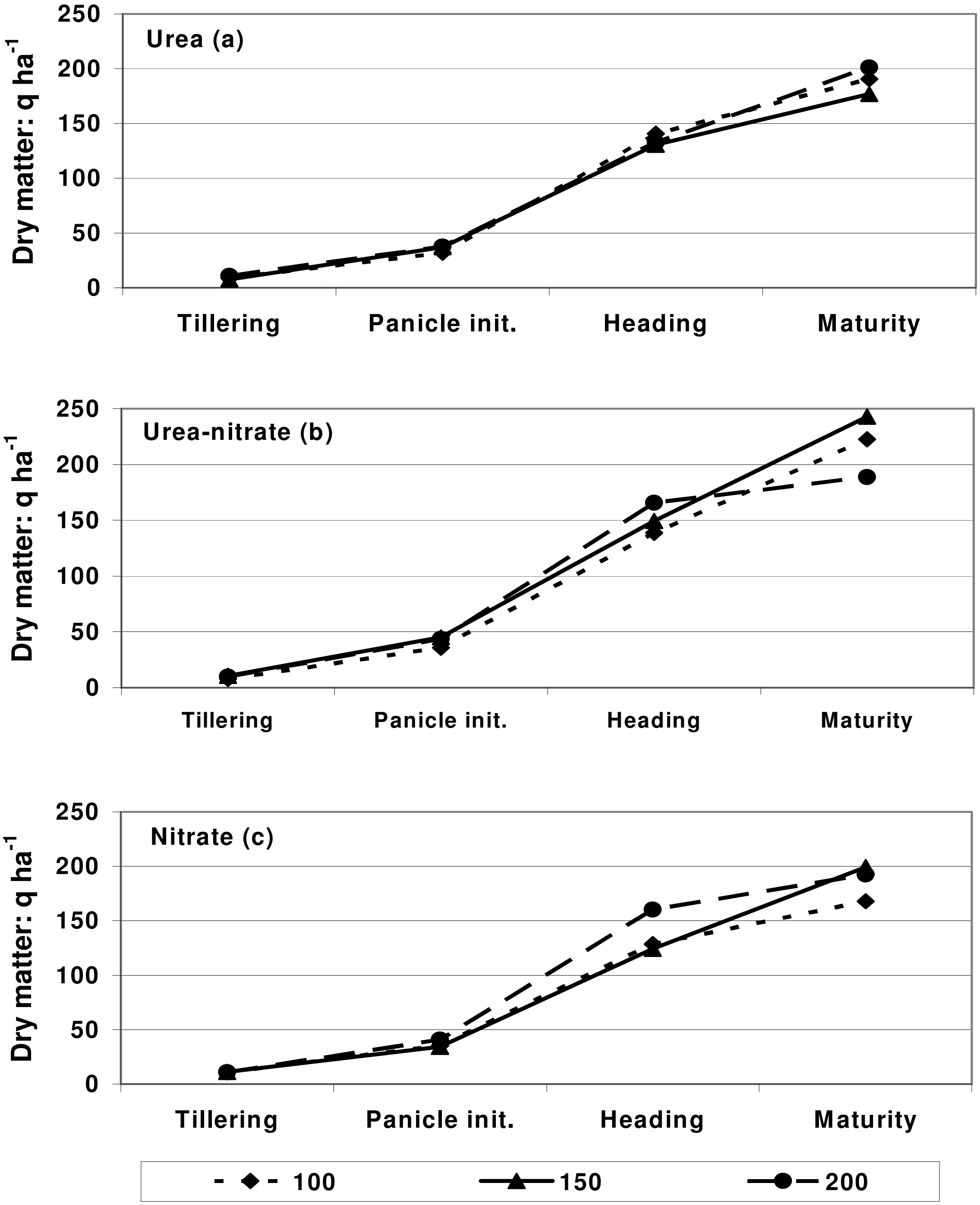

Figure 3 Dry matter trend for nitrogen form and rate treatment.

In figure 4 (a,b,c) are reported the nitrogen uptake trends. Significant differences were not found at tillering stage while nitrogen rate and form influenced nitrogen uptake at panicle initiation: higher values were reached on N200 treatment and the ureic-nitric nitrogen distribution consented the total uptake increasing. At heading statistical differences were observed only between nitrogen rate treatment: higher absorption rates were observed in N200 treatment. No differences were observed at maturity stage.

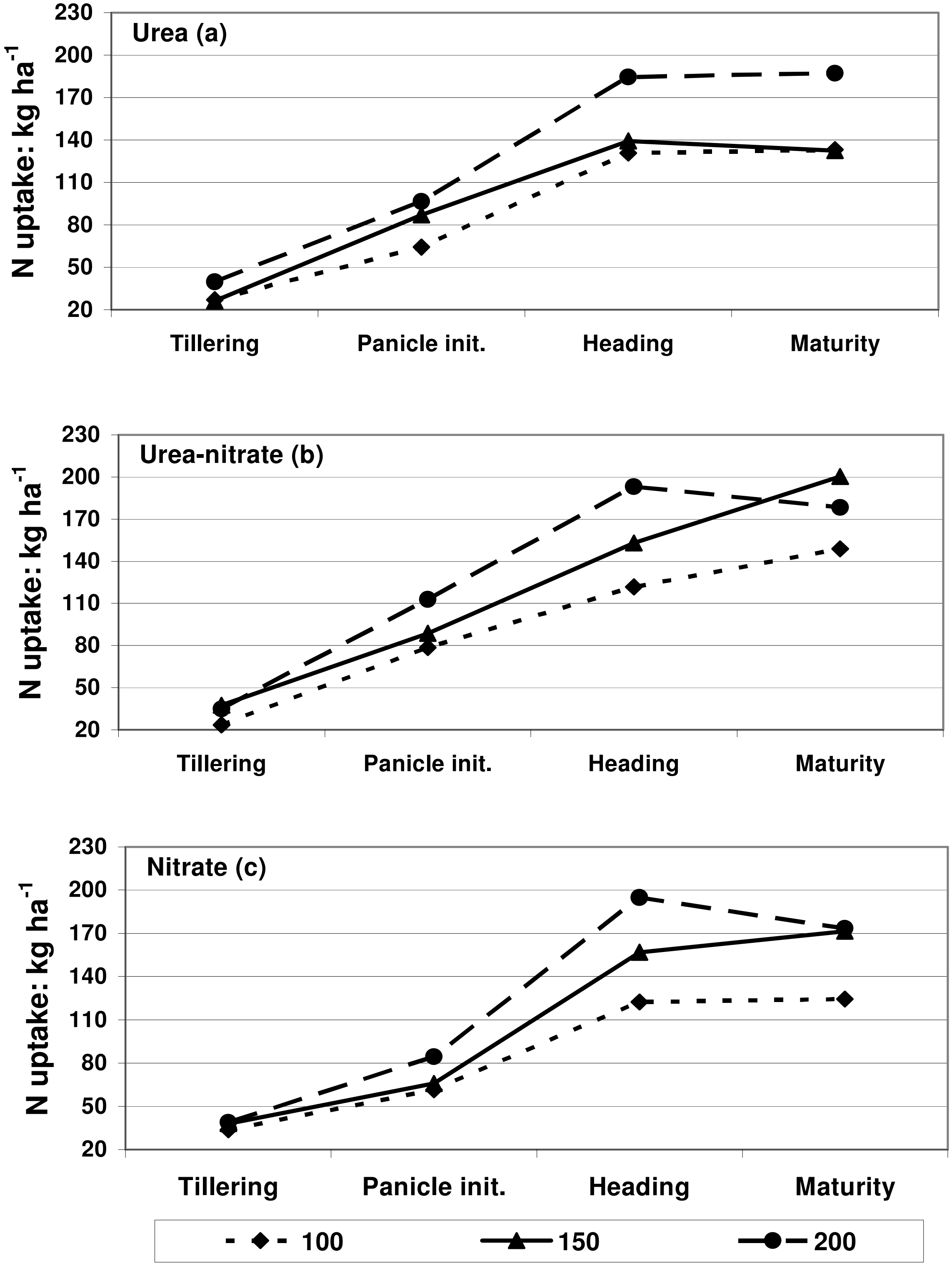

Figure 4 Nitrogen uptake trend for nitrogen form and treatment.

High significant differences were evidenced by the ANOVA and Fisher test carried out on the average value of phenological stage duration (table 2). Considering the heading and maturity stages, high level of nitrogen fertilizing increased the crop cycle duration. The fast availability of nitric nitrogen influenced the crop growing, affecting the shorter duration of the sowing-heading interval; whereas, differences were not evidenced on sowing maturity interval, probably due to the increased absorption of nitrogen from ureic and ureic-nitric form, that elongated the duration of the final stage.

Table 2 Phenological phases duration.

Treatment	Sowing-Heading	Sowing-Maturity
	days	days
U N_{100}	103	144
U N_{150}	104	146
U N_{200}	106	149
U+NO_3 N_{100}	101	144
U+NO_3 N_{150}	104	147
U+NO_3 N_{200}	106	149
NO_3 N_{100}	100	143
NO_3 N_{150}	102	145
NO_3 N_{200}	103	147

Conclusions

For the monitored parameters, ureic + nitric nitrogen treatment highlighted a better crop response than only nitric or ureic ones.

Pre-sowing fertilizing could be awarded to sufficient nitrogen availability, able to cover still limited crop’s nutritive requirements of earliest growing stages.

The higher uptake rate observed at following stages, from mid-tillering to heading, is satisfied both by readily top dressing assimilable nitric nitrogen and by slowly available ureic nitrogen residual. Probably, in only nitric fertilizing the nitrogen is not retained by soil adsorbing capacity and can be partially lost by leaching and/or volatilization, becoming not sufficient for crop’s requirements satisfaction. About ureic-nitrogen treatment, the element availability could not be sufficient for the crop’s requirements satisfaction, probably due to slow nitrogen transformation in readily assimilable form at high requirement stages.

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INNOVATIVE AGRONOMIC TECHNIQUES FOR RICE CULTIVATION

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Abstract

Flooded irrigation is usually adopted for rice growing in temperate areas. The efficiency of this method is generally very low and water consumption is comprised on average between 13 and 20.000 m^3 ha^{-1}, with maximum values reaching over 50.000 m^3 ha^{-1}.

In areas with low water availability, rice growing can be carried out adopting alternative irrigation methods such as sprinkler irrigation and weekly soil saturation.

Many cultivars showed high productivity and high yield in industrial processing in response to this technique.

Main advantages with sprinkler irrigation are: reduction of seasonal water volume of about 40-50 %; lower environmental impact due to easier weed control and total algae absence; possibility of fitting the crop in open rotations; lower total costs as soil levelling and specific machines are not required; possibility to grow the crop even in clay loam to sandy soil; lower costs for certified seed production.

Whereas with the soil saturation technique with turned water distribution: reduction of seasonal water volume ranges about 20-30 %; lower environmental impact due to avoidance of algaecide use and to reduced employment of weed killer.

Keywords

Rice; agronomic technique; soil saturation; sprinkler irrigation.

Introduction

Among the Countries that over-look Mediterranean sea, rice cultivation expansion is highest in Egypt with more than 600.000 ha followed by Italy, with over 200.000 ha, Spain, over 110.000 ha, Turkey, 85.000 ha, and Greece and France, with about 20.000 ha each (FAO, 2002).

Flooded irrigation is usually adopted for rice growing in temperates areas. The efficiency of this method is generally very low and water consumption averages between 13 and 20.000 $m^3 ha^{-1}$, with maximum values reaching over 50.000 $m^3 ha^{-1}$ (Giardini, 2002).

The agronomic technique usually adopted in flooded irrigation does not differ between different environments at several latitudes. It consists of a thorough soil levelling to allow a uniform water supply that is very important, in high latitude Countries, to take advantage of thermal engine effect and to assure a satisfactory and high uniformity of crop emergence. In warmer countries, as those over-looking the Mediterranean sea, it is possible to cultivate without thermal engine to obtain a high and rapid emergence.

In these countries, where water availability is frequently limited, rice cultivation can be carried out with alternative irrigation methods, such sprinkler irrigation and soil saturation.

Different irrigation methods also entails different crop techniques, as described below.

Crop technique with soil saturation irrigation

In permanent paddy fields, seed bed preparation can be carried out with a simplified technique; which involves: after soil levelling a minimum tillage at about 20 cm depth, to allow burial of crop residues and fertilizers; before sowing some longitudinal furrows, with low depth and at 25-30 m distance, to convey irrigation water throughout the length of the field and, for slow overflowing, to uniformly moisten the soil until saturation of the superficial layer; after sowing, to be carried out with a seed-drill at a 3 cm depth with 500 viable seed m^{-2}, harrowing of the superficial soil layer to improve weed-control efficiency and to draw closer higher amounts of soil to the seed employing a ring roller. For weed control one treatment in pre-emergence with 1,300 g ha^{-1} pendimethalin, is usually sufficient (Spanu *et al.*, 1992).

Water supplies, till crop emergence, will be turned to maintain the superficial soil layer moist; afterwards they will be weekly irrigated with water volumes that saturate the superficial soil layer. Adopting the above described crop tecnique, in a experimental trial carried out in 2003, characterized by ETo (Penman-Monteith) and temperatures above multi-year 1958-2002 average, water consumption was 9,290 m^3ha^{-1} and 10,870 m^3ha^{-1} in cultivars which reached full maturity 115 and 135 days after sowing (DAS) respectively (Spanu *et al.*, 2004a). Adopting flooded irrigation, in the same environment and in previous years, water consuption was between 12 and 14,000 m^3ha^{-1} (Spanu, 1984; Spanu *et al.*, 1996a; 1997a; 1998a; 1999a; 2000a; 2001; 2002a; 2003a); therefore water savings can be estimated between 2,500 and 3,000 m^3ha^{-1}.

Crop technique with sprinkler irrigation

Sprinkler irrigation allows a further simplified cultivation. In this case, in fact, soil levelling is not required and seed bed preparation can be carried out with traditional or minimum tillage according to high or low weed and/or crop residues presence. Sowing is in rows with 500 viable seed m^{-2} at a 3 cm or more depth, according to soil texture. A following ring rolling increases soil-seed adhesion and improves weed-control efficiency. The best experimental results for weed control were obtained employing 1,300 gha^{-1} pendimethalin as a pre-emergence treatment.

The irrigation, to be carried out mostly with low or average intensity sprinklers, will be carried out every two days with 50-70 m^3ha^{-1} and such as to maintain moist the superficial soil layers to assure a uniform and quick crop emergence. After emergence, following irrigation with volumes of about 200-250 m^3ha^{-1}, according to the climate trend, can be carried out with a timetable of 4-5 days during emergence-end shooting and waxy-full maturity and with a timetable of 3-4 days during start boot-end of waxy maturity. In case of availability of ETo (Penman-Monteith) daily values, water supplies can be executed when sum of ETo multiplied for Kc reaches 20 mm. The first results of experimental trials carried out in Sardinia on Kc determination in sprinkler irrigated rice (Spanu *et al.*, 2004b), showed values of 0.2, Kc_{ini}; 1.0, Kc_{mid}; 0.3, Kc_{end}.

Many experimental trials carried out in Sardinia with sprinkler irrigation showed water consumptions between 7,500 and 9,000 m^3ha^{-1}, according to climate trends and crop cycle duration of the cultivar employed (Spanu *et al.*,1989; 1992; 1996b; 1996c; 1996d; 1997b; 1998b; 1999b; 2000b; 2002b; 2003b). Comparing these results with flooded irrigation trials in the same environment, water savings can reach even 50%.

Agronomic results in cultivar-comparison trials with different irrigation methods

For more than twenty years in Sardinia, experimental field 'S. Lucia' (OR) at 34° 59' N and 8° 40' E, 15 m a.s.l., experimental trials were in process to set innovative agronomic techniques for rice cultivation aiming to decrease water requirements. More comforting results were obtained adopting sprinkler instead of flooded irrigation.

Trials without flooded irrigation, adopting soil saturation, were carried out recently (Spanu *et al.*, 2004a).

This paper reports the results of cultivar-comparison trials carried out in 2001, 2002 and 2003 adopting flooded, sprinkler and soil saturation irrigation respectively. Statistical analysis results refer to single years of trial and not to the comparison of years and irrigation methods.

The meteorological trend recorded at the experimental field in trial years is reported in figure 1.

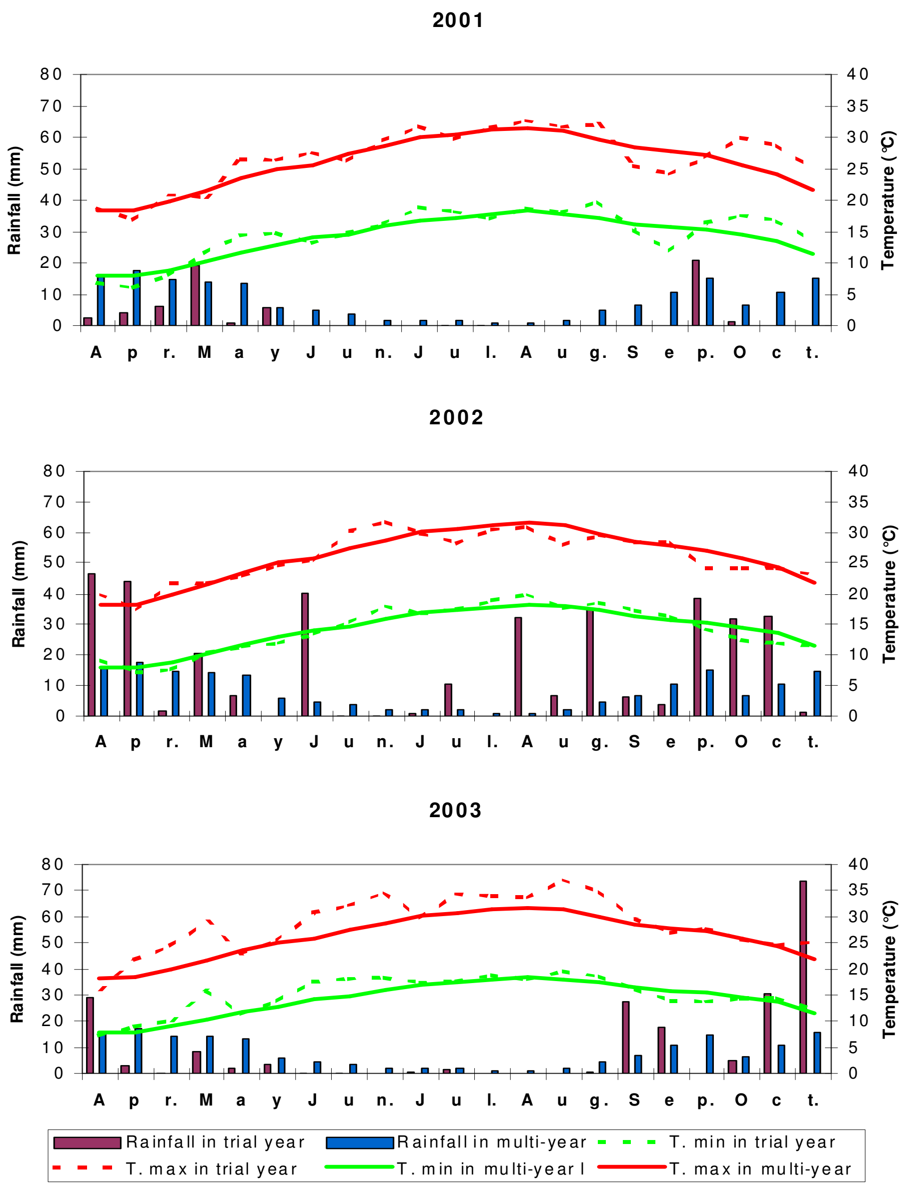

Figure 1 Ten-day average values for minimum and maximum temperatures and 10-day cumulate rainfall recorded at the site in 2001, 2002 and 2003 and multi-year.

The thermo-pluviometric trend registered during the trial, between April and October, for the years 2001 and 2003 did not differ from the average values referred to the long-term period: high temperatures, both minimum and maximum, and lack of rainfall. In 2002 indeed it was different, particularly for the high rainfall (110 mm) in April and at the beginning of May, contributing to establish a good water supply in the soil. From sowing to harvest about 200 mm of total rainfall, unusual for Sardinia, were registered vs. a multi-year average value of about 20 mm. Rainfall trend affected temperatures and average maximum values were almost always lower than in the long-term. Such meteorological trend extended the maturity duration in many cultivars and fostered higher elaborate accumulation in kernels.

Main results of twenty-six cultivars employed, of which thirteen were common to all trials, are reported in tables 1-2-3; for every cultivar the irrigation method employed for comparison is reported as a subscript (f = flooded; s = soil saturation; sp= sprinkler irrigation).

With flooded irrigation, grain yields were comprised between 7.5 and 10.1 t ha^{-1}, for Fragrance and Ambra respectively; they reflect average yields of Sardinian rice growing.

Table 1 Results obtained with flooded irrigation in 2001.

Cultivars	Sowing - maturity (days)	Plant total height (cm)	Stems fertile (n. m^{-2})	Rough rice 13% moisture (t ha^{-1})	Yield whole grain (%)
Short Grain					
Ambra $_{f, s, sp}$	121 d	65.7 cg	740 ac	10.1 a	68.4 ac
Balilla $_{f, s, sp}$	130 b	72.0 bc	657 ce	8.9 bd	71.6 a
Marte $_{f, s, sp}$	125 c	62.1 eg	637 cf	9.2 ad	71.3 ab
Medium Grain					
Alpe $_{f,s}$	114 h	81.0 a	460 g	8.7 cd	61.7 e
Lido $_{f,s}$	114 h	70.7 cd	654 ce	8.6 cd	62.7 de
Long Grain A					
Ariete $_{f,s}$	119 f	82.4 a	474 g	8.9 cd	66.5 ae
Astro $_{f, s, sp}$	121 d	70.7 ab	532 fg	8.7 cd	66.6 ae
Delfino $_{f, s, sp}$	117 g	72.8 bc	491 g	9.3 ad	65.0 ce
Sis R-215 $_{f, s, sp}$	119 f	62.8 eg	725 ad	9.5 ac	67.4 ad
Euro $_{f, s, sp}$	119 f	69.5 ce	553 eg	9.1 bd	68.5 ac
Long Grain B					
Albatros $_{f, s, sp}$	120 de	71.8 bc	656 ce	8.7 cd	68.9 ac
Eolo $_{f, s, sp}$	121 d	63.8 dg	816 a	9.4 ad	66.5 ae
Gladio $_{f, s, sp}$	119 f	65.1 cg	671 bd	9.2 ad	66.3 be
Lamone $_{f,s}$	119 f	61.2 fg	781 ab	9.8 ab	64.3 ce
Thaibonnet $_{f, s, sp}$	130 b	68.3 cf	727 ad	8.6 d	67.3 ad
Scented					
Asia $_{f, s, sp}$	130 b	66.3 cf	739 ac	7.7 e	52.2 f
Fragrance $_{f, s, sp}$	116 g	58.6 g	615 df	7.5 e	55.8 f
Gange $_{f,s}$	134 a	63.2 dg	658 ce	7.6 e	66.5 ae

Means averaged by the same letter did not differ per $P \leq 0.05$.
f = flooded; s = saturation of soil; sp = sprinkler irrigation.

Grain yield obtained with sprinkler irrigation was between 11.3 t ha^{-1} for Gladio and 8.3 t ha^{-1} for Karnak; Loto production, damaged by Passeriformes, is not to be considered. Half of the cultivar compared registered rough rice production over 10.0 t ha^{-1}.

Table 2 Results obtained with sprinkler irrigation in 2002.

Cultivars	Sowing - maturity (days)	Plant total height (cm)	Stems fertile (n. m^{-2})	Rough rice 13% moisture (t ha^{-1})	Yield whole grain (%)
Short grain					
Ambra $_{f, s, sp}$	138 de	56.0 h	676 ac	9.9 be	67.1 eg
Balilla $_{f, s, sp}$	145 b	72.0 cd	592 ce	10.0 be	70.3 bc
Marte $_{f, s, sp}$	141 cd	62.3 fh	496 ef	9.6 ce	74.3 a
Medium grain					
Irat 190 $_{sp}$	141cd	82.2 b	592 ce	9.8 be	63.7 ij
Irat 212 $_{sp}$	141 cd	91.0 a	649 bd	10.2 ae	64.2 hj
Long grain A					
Astro $_{f, s, sp}$	140 cd	82.4 b	569 cf	9.6 ce	66.3 fi
Delfino $_{f, s, sp}$	136 e	73.6 c	510 df	10.2 ae	70.8 b
Euro $_{f, s, sp}$	139 cd	70.3 ce	623 ce	10.9 ab	68.9 bf
Karnak $_{f, s, sp}$	140 cd	72.4 cd	532 df	8.3 f	65.2 gj
Loto $_{s,sp}$	116 f	62.3 fh	451 f	6.3 g	67.7 cg
Sirmione $_{sp}$	139 cd	57.3 gh	567 cf	9.1 ef	66.2 fi
Sis R-215 $_{f, s, sp}$	140 cd	66.6 dh	627 ce	10.5 ad	69.2 bc
Long grain B					
Albatros $_{f, s, sp}$	140 cd	69.3 ce	637 cd	10.7 ac	70.1 bd
Eolo $_{f, s, sp}$	140 cd	64.1 eg	769 ab	11.2 a	67.4 dg
Gladio $_{f, s, sp}$	140 cd	63.9 eg	780 a	11.3 a	66.9 eh
Thaibonnet $_{f, s, sp}$	142 bc	61.9 fh	556 cf	10.3 ad	67.7 cg
Scented					
Asia $_{f, s, sp}$	148 a	65.3ef	620 ce	9.5 de	62.8 j
Fragrance $_{f, s, sp}$	145 b	59.8 fh	605 ce	9.6 ce	66.5 eh

Means averaged by the same letter did not differ per $P \leq 0.05$.
f = flooded; s = saturation of soil; sp = sprinkler irrigation.

In the soil saturation trial the highest production was reached by Albatros (11.6 t ha^{-1}) and the lowest with Alpe and Giano (8.3 t ha^{-1}). Among twenty-four compared cultivars, height exceeded 10 t ha^{-1} and nine-ten 9 t ha^{-1}.

Table 3 Results obtained with soil saturation irrigation in 2003.

Cultivars	**Sowing - maturity (days)**	**Plant total height (cm)**	**Stems fertile (n. m^{-2})**	**Rough rice 13% moisture (t ha^{-1})**	**Yield whole grain (%)**
Short grain					
Ambra $_{f, s, sp}$	115 ef	68 j	671 ac	9,9 bh	67,2 ac
Balilla $_{f, s, sp}$	131 ab	83 c	588 cf	10,7 ac	70,7 a
Marte $_{f, s, sp}$	131 ab	69 hj	503 fj	10,2 ag	70,5a
Medium grain					
Alpe $_{f,s}$	112 fh	79 df	476 hj	8,3 i	60,1 fi
Lido $_{f,s}$	108 gh	73 gh	637 bd	9,2 ei	53,9 j
Long grain A					
Ariete $_{f,s}$	110 fh	82 cd	464 ij	8,9 fi	68,2 ab
Astro $_{f, s, sp}$	114 ef	84 c	510 ej	9,6 bi	63,4 cg
Carnaroli	127 bd	115 a	410 j	9,3 di	63,3 cg
Delfino $_{f, s, sp}$	112 fh	76 fg	468 hj	9,3 ci	64,2 cf
Euro $_{f, s, sp}$	114 ef	73 gh	516 ei	11,0 ab	62,6 eg
Loto $_{s,sp}$	107 h	70 hj	529 ei	9,4 ci	59,7 gi
Karnak $_{f, s, sp}$	134 a	89 b	479 gj	10,3 af	62,8 dg
Sis - R 215 $_{f, s, sp}$	115 ef	69 hj	600 bf	9,0 fi	66,9 ad
Long grain B					
Albatros $_{f, s, sp}$	118 e	77 eg	604 bf	11,6 a	63,2 cg
Eolo $_{f, s, sp}$	123 d	66 ik	654 ac	10,6 ad	56,8 ij
Giano $_{s}$	113 fg	67 ij	572 ch	8,3 i	61,3 eh
Gladio $_{f, s, sp}$	113 eg	65 jk	704 ab	9,3 ci	60,3 fi
Lamone $_{f,s}$	111 fh	62 k	677 ac	8,8 hi	62,4 eh
Prezioso $_{s}$	125 cd	65 jk	583 cg	9,3 ci	64,0 cf
Sirmione $_{s}$	127 cd	81 ce	535 di	9,8 ch	65,3 be
Thaibonnet $_{f, s, sp}$	133 a	75 fg	742 a	10,5 ae	58,5 hi
Scented					
Asia $_{f, s, sp}$	130 ac	77 eg	593 cf	10,4 ae	40,5 k
Fragrance $_{f, s, sp}$	125 d	66 ik	642 ad	9,8 bh	41,2 k
Gange $_{f,s}$	133 a	70 hi	614 be	8,8 gi	62,9 dg

Means averaged by the same letter did not differ per $P \leq 0.05$.
f = flooded; s = saturation of soil; sp = sprinkler irrigation.

Conclusions

Experimental results obtained in many trials in Sardinia confirm that with sprinkler irrigation rice production and yield in industrial processes are similar or higher than with traditional cropping techniques.

The higher efficiency of the irrigation method allows water savings of about 50% and it could increase, in Countries with limited water availability, the rice cultivated area or other irrigated crops area.

With sprinkler irrigation land levelling in paddy fields is not required, getting round the problem of repeated monoculture cultivation and easily fitting the rice in open crop rotations. Moreover it makes possible to cultivate rice also to basically loose textured soils.
The simplified crop technique allows a relevant cost reduction because of the use of mechanical equipments employed also in other autumn-winter cereal crops, both for soil tillage and for sowing and harvest; for production of certified seeds, field expulsion is simpler and less expensive.
Such crop technique is also very important for the lower environmental impact, because of the absence of algae and of the development of aquatic species that require specific weed-control.
Even if related to only one year of trial, productions obtained with soil saturation irrigation were very encouraging. Moreover, main advantages of this technique with respect to flooded irrigation were water savings of about 20-30% and lower environmental impact both for algae absence and simplified weed-control.

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ROOT SYSTEM DEVELOPMENT IN FLOODED AND SPRINKLER IRRIGATED RICE CULTIVARS

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Abstract

One of the most important factors to obtain high yield is a good root system development. The exploration of a greater soil mass allows a higher water and nutrient uptake and increases crop yield. Numerous authors noticed a significant correlation between yield and root system dimension.

The growing environment, and therefore the agronomic technique, affect root system development.

Traditionally rice is grown by flooded irrigation that, due to oxygen lack in deep soil layers, brings to a low root system deepening. In upland rice, where the growing does not take place with continuous flooding, some authors noticed a good root system development.

A comparison trial, with national and selected for upland conditions rice cultivars, was carried out to evaluate root system development adopting both traditional flooded irrigation and sprinkler irrigation. Root density was determined by Newman method for different soil depths.

Results highlight a greater root density in flooded crop in comparison to sprinkler irrigation. The higher yield obtained with sprinkler irrigation, even higher than flooding irrigation, suggest a greater efficiency of the root system in water and nutrient uptake in oxidized environment.

Keywords

Root system; rice; flooded irrigation; sprinkler irrigation.

Introduction

The soil water status greatly affects root system development. Several studies show that under continuous submersion conditions rice plants develop a higher root density ((Tarafder *et al*., 1995; Kondo *et al*, 1998).

In lowland cultivars, therefore in submerged conditions, root development, as well as water and nourishing elements uptake, is strictly related to the edaphic parameters of the crop's environment (Nieuwenhuis *et al.*, 2001). A different rate of water and nutrient uptake was observed at different roots lengths (Teo *et al*., 1995, Beyrouty *et al*., 1992). Trials carried out with nourishing solutions showed that the root system also affects the enzymatic kinetic of nutrient uptake, particularly nitrogen (Baptista *et al*., 2000; Grigg *et al*., 2000). Also in upland conditions the effect of soil on root development and depth is markedly linked with the cultivar employed (Kondo *et al*., 2002; Price *et al*., 2002). Studies were carried out on upland cultivars to establish root system depth: about 80-85 % of the root system seems to be located in shallow soil layers (Murty *et al*., 1999).

In sight of water shortages it is essential to take under consideration irrigation methods with a higher efficiency, that allow water savings maintaining productions. In the last years numerous experimental trials were carried out with sprinkler irrigation on rice in Mediterranean climate environments (Spanu *et al.,* 1989; 1992; 1996a; 1996c; 1996d; 1997c;

1997d; 1998b; 1999b; 2000b; 2002b; 2003b). A cultivar-comparison trial was carried out to evaluate root development in relation to soil water status, thus to adopted irrigation methods. The cultivars employed were selected among national lowland and foreign upland varieties. Root density measurements were carried out both with flooded and sprinkler irrigation at different soil depths.

Materials and methods

The trial was carried out at the experimental field of "Santa Lucia" of University of Sassari, located at 39°59' N and 8°40' E, at 15 m a.s.l..

The flooded irrigated trial was carried out on a permanent paddy field (over 25 years) soil characterized by sandy-loam texture for the first 40 cm layer and loam texture in the lower layer. Sprinkler irrigated trial was carried out in a sandy-clay-loam soil. The soil bulk density was 1.35 and 1.25 t m^{-3} for flooded and sprinkler irrigated trial respectively.

Neutral pH, poor organic matter and nitrogen content, high assimilable phosphorus and exchangeable potassium are the soil chemical characteristics of both trials.

In the flooded trial 18 cultivars with different biological cycle, of both *indica* and *japonica* sub-species, registered in the National Varieties List and widespread were compared. The same and other 11 cultivars, selected by IRRI and Ibaraki Agriculture Centre for upland cultivation, were employed for the sprinkler irrigation trial.

In both trials the experimental design was a randomized block with four replicates.

Sowing was carried out in both trials in early may; broadcasting in a 30 m^2 plot area in the flooded irrigation trial and at a 3 cm depth and 14 cm in spaced rows in a 10 m^2 plot area in the sprinkler irrigation trial.

For flooded irrigation the cultural technique adopted was the traditional one (Spanu *et al.*,1996b; 1997a; 1997b; 1998a; 1999a; 2000a; 2001; 2002a; 2003a), whereas for sprinkler irrigation it was a system developed in several years of experimentation carried out in Sardinia. In the traditional technique irrigation was carried out with continuous submersion for the whole crop cycle, while in sprinkling water intervention was carried out when the Class A pan evaporation, corrected by a planned empirical coefficient, reached 20 mm. Further information on crop technique adopted are reported in Table 1.

Table 1 Crop management.

	Flooded	**Sprinkler**
Crop precession	rice	bare fallow
Tillage	rotary hoeing and harrowing	ripping and harrowing
Fertilizing pre-sowing	N: 125 kg ha^{-1} P_2O_5: 90 kg ha^{-1} K_2O: 75 kg ha^{-1}	N: 125 kg ha^{-1} P_2O_5: 90 kg ha^{-1} K_2O: 75 kg ha^{-1}
Sowing density	500 m^{-2} viable seeds	500 m^{-2} viable seeds
	1,125 g ha^{-1} Pretilachlor + 562 g ha^{-1} Fenclorim + 225 g ha^{-1} Molinate	1,300 g ha^{-1} pendimethalin
Top dressing	30 kg ha^{-1} N	46 kg ha^{-1} N
Seasonal volume	11,183 m^3 ha^{-1}	7,460 m^3 ha^{-1}

For all cultivars and replicates of both trials phenological stages and, in representative test areas, the main agronomic parameters were recorded. After harvest, a 14 cm of diameter and until 25 cm of depth soil core (Figure 1) was taken from every plot. In the flooded trial plot

sampling was carried out in an area with uniform density and representative of average investment, while in the sprinkler trial plot the samples was taken in the centre of two rows.
Each soil core was cut into 5 cm slices and in which, after hand reduction in clods of 1 cm of diameter, root density was determined by the Newman method (Newman, 1966). Unlike the latter, a 0,2 mm mesh sieve (Amato *et al*., 1994) was employed for roots separation, while a stereoscopic microscope with 32 magnifications was employed for the reading of contact point between roots and mesh.


Figure 1 Soil sampler (left) and soil core (right) taken in the field trial.

Results and Discussion

The results of comparison trials carried out in the last five-years at “S. Lucia” field, highlighted that sprinkler irrigation allows grain yields similar to continuous flooded irrigation, and in some cultivars clearly higher. The best yield response with sprinkler irrigation can be ascribed to a more simultaneous and uniform emergence, due to algae absence and an almost no weed competition and, consequently, to a higher number of fertile stems m^2 at harvest.
National cultivars showed a higher root density (cm of root per cm^3 of soil), higher in flooded than in sprinkler irrigation. In both irrigation methods a higher density was recorded in shallow layers. In flooded irrigation the values ranged between about 70 and 100 cm cm^{-3} in the 0-5 cm layer up to minimum values between 5 and 15 cm cm^{-3} in the 20-25 cm layer.
Most sprinkler irrigated cultivars, including those selected for upland cultivation, recorded root density ranging between 30 and 40 cm cm^{-3} in the shallow soil layer, up to values of 5 and 10 cm cm^{-3} at higher depths.
Table 2 reports the variance analysis of root density for 18 flooded and sprinkler irrigated cultivars.

Table 2 Analysis of variance.

Source of variation	D.F.	F value	Significance
Irrigation method (I.m.)	1	229.4	**
Cultivar (cv)	17	1.32	ns
I.m. x cv	17	1.57	ns
Layer (L)	4	907.17	**
I.m. x L	4	144.99	**
cv x L	68	1.43	*
I.m. x cv x L	68	1.34	*

** Significant at the 0.05 probability level.*
*** Significant at the 0.01 probability level.*

Figure 2 reports the root density of some cultivars; the linear equation refers to flooded irrigation (dotted line) and the quadratic equation to sprinkler irrigation (continue line).

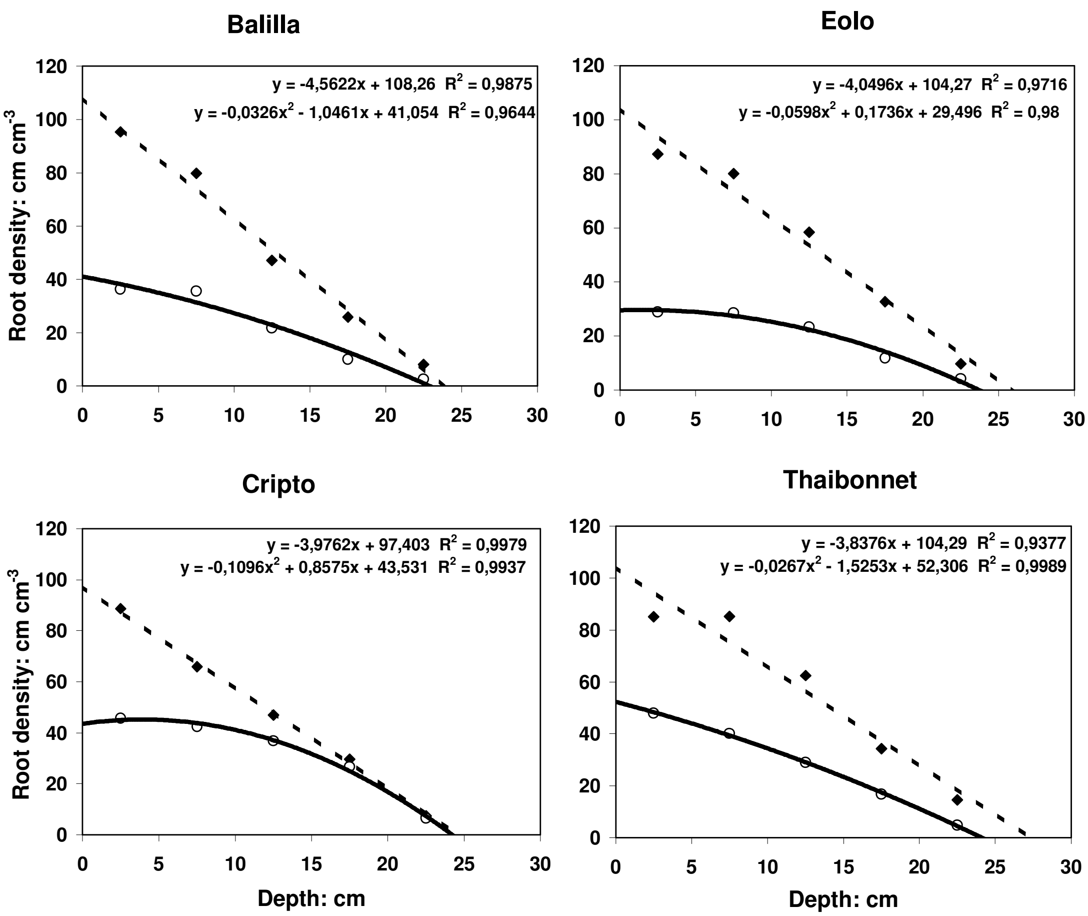

Figure 2 Root density of some cultivar flooded (dotted line) and sprinkler (continue line) irrigated.

Figure 3 reports the root density of some sprinkler irrigated cultivars selected for upland conditions.

Difference in root system density with respect to the irrigation method and soil layer was significant, while it was not between cultivars. Interactions, with the exception of irrigation method x cultivars, were significant.

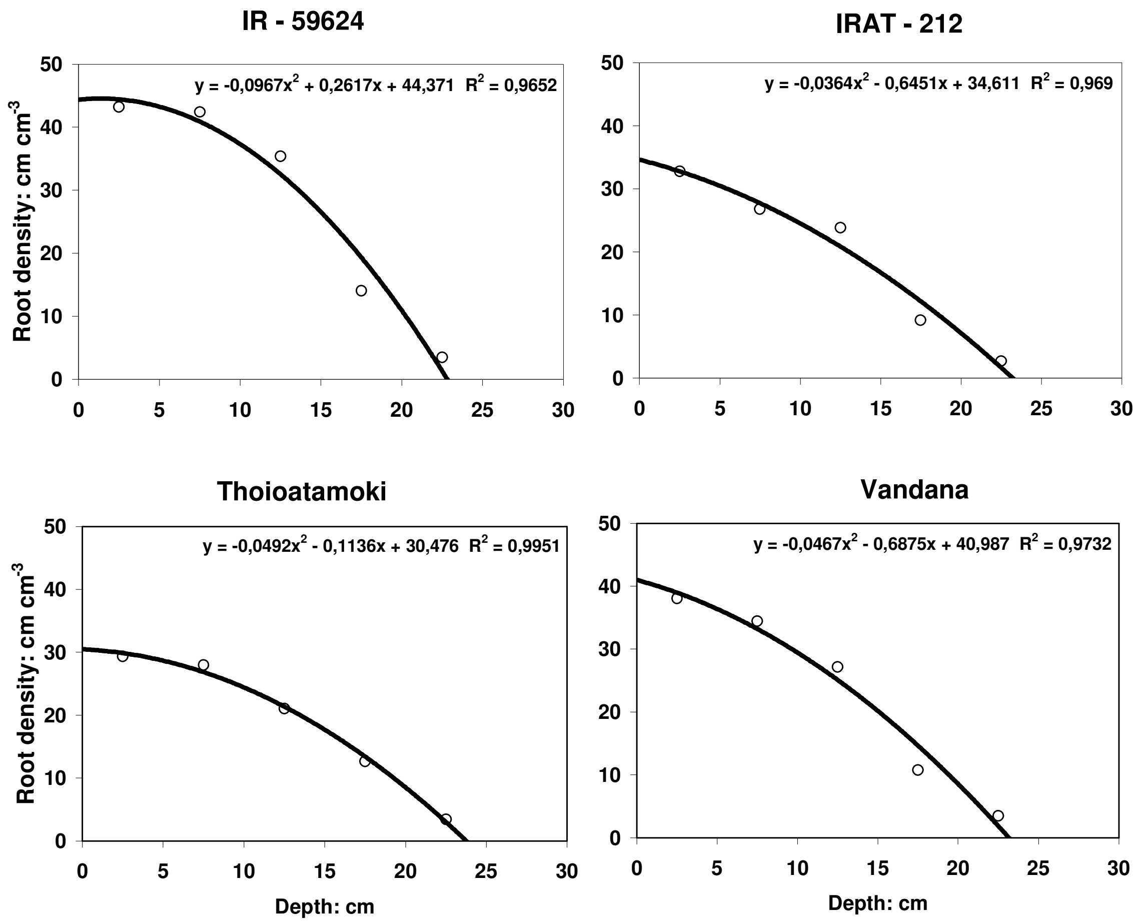

Figure 3 Root density of some sprinkler irrigated cultivars.

Root density of the observed five layers, for 18 flooded irrigated cultivars and 29 sprinkler irrigated cultivars, is reported in Figure 4. Unexpectedly, root densities of upland cultivars were similar to national cultivars.

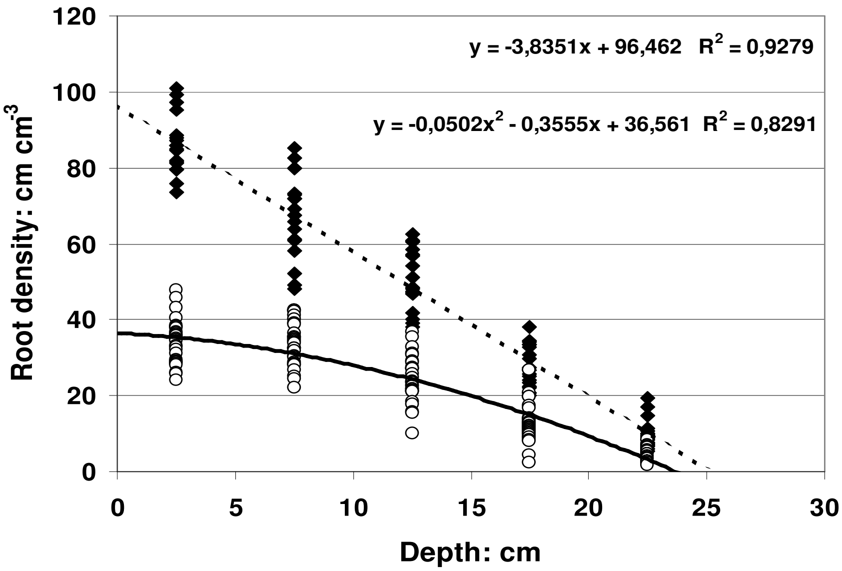

Figure 4 Average values of all cultivar, flooded (dotted line) and sprinkler (continue line) irrigated.

Conclusions

Results underlined that root system development (cm of roots for cm^3 of soil) is always lower in sprinkler than in flooded irrigated plants. Many experimental trials carried out in the Mediterranean environments highlighted that rough rice production achievable with sprinkler irrigation crop technique is equal or higher than with flooded irrigation. Thus, rough rice production is not positively related with root system dimension. Probably this parameter is more significantly affected by the higher water and nourishing elements absorption efficiency in the oxidized environment.

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HERBICIDE RESISTANCE IN ITALIAN RICE CROPS: A LATE-DEVELOPING BUT FAST-EVOLVING STORY

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Abstract
Herbicides have been used intensively in Italian rice crops for the last 40 years. Use of ALS-inhibitors began in 1988 and now more than 80% of rice fields are treated with these herbicides. Since 1994, three species (*Alisma plantago-aquatica, Schoenoplectus mucronatus* and *Cyperus difformis*) have evolved resistance to ALS inhibitors, while three populations of *Echinochloa crus-galli* have evolved resistance to propanil. A population of *Echinochloa erecta* recently proved to be multiple-resistant to quinclorac and propanil. It is estimated that resistance exists in more than 25,000 ha, with *S. mucronatus* and *C. difformis* showing the highest rate of increase. Field histories show that high selection pressure had been imposed for several years prior to resistance development, as well as a lack of any resistance prevention strategy. Most ALS-resistant populations show a wide pattern of cross-resistance with a high level of resistance to SU and a lower level to the TP metosulam. The ALS-resistance mechanism is due to an altered herbicide site of action. Resistance management strategies are discussed.

Introduction
The development of herbicide resistance is an evolutionary process mainly driven by selection pressure imposed by herbicides (i.e. herbicide efficacy, persistence and frequency of application), initial gene frequency and fitness of the resistant and susceptible biotypes. Individual plants do not change from being susceptible to resistant, but the proportion of resistant plants within a population increases over time (Moss, 2002). The majority of resistant biotypes have evolved in situations where rotation of crops and/or herbicide modes of action were absent or limited (Heap and LeBaron, 2001).
Most temperate rice crops are grown using very intensive cropping systems, where rice monoculture and heavy reliance on herbicides for weed control are common agronomic practices. Generally, only a few herbicide modes of action are used in rice: acetolactate synthase (ALS) inhibitors, synthetic auxins, thiocarbamates, acetyl co-enzyme A carboxylase (ACCase) inhibitors and amides being among the most frequent groups. Under these conditions herbicide resistance has become a major and widespread problem in many areas, involving several chemical families (Valverde and Itoh, 2001).
Until the early 90s herbicide resistance was not widespread in Italy, with the triazines being the only chemical family involved (Porceddu et al., 1997). One of the reasons for this is probably related to the delay in marketing new herbicides in Italy (Table 1). The situation began to change after the introduction of new highly-active herbicide groups, which are target site specific (i.e. ALS and ACCase inhibitors).
Bensulfuron-methyl, the first ALS inhibitor marketed for rice crops in Italy, was introduced in 1988 (Table 1). Since then six new herbicides have been introduced with the same mode of action, though belonging to three different chemical families (sulfonylureas - SU,

triazolopyrimidines – TP, and pyrimidinyl thiobenzoates - PTB). It is now estimated that more than 80% of Italian rice fields are treated with ALS inhibitors.

Table 1 Weed species and herbicides used in Italian rice crops that are involved in resistance (SU = sulfonylureas, TP = triazolopyrimidines; PTB = pyrimidinyl thiobenzoates, AM = amides, QC = quinoline-carboxylic acids. ALSPA = *Alisma plantago-aquatica*, SCPMU = *Schoenoplectus mucronatus*, CYPDI = *Cyperus difformis*, ECHCG = *Echinochloa crus-galli*, ECHER = *Echinochloa erecta*).

Herbicide	Chemical family	Year of introduction		First documented resist. pop. in Italy	Species with resistant populations
		In the world	In Italy		
bensulfuron	SU	1985	1988	1994	ALSPA, SCPMU, CYPDI
cinosulfuron	SU	1987	1992	1994	ALSPA, SCPMU, CYPDI
metsulfuron	SU	1981	1993	(*)	ALSPA, SCPMU, CYPDI
metosulam	TP	1994	1996	1997	ALSPA, SCPMU
azimsulfuron	SU	1995	1997	1998	ALSPA, SCPMU, CYPDI
ethoxysulfuron	SU	1995	1998	1998	ALSPA, SCPMU, CYPDI
bispyribac-Na	PTB	1997	2004	2002	SCPMU, CYPDI
propanil	AM	1960	1962	1999	ECHCG
quinclorac	QC	1985	1992	2003	ECHER

(*)In Italy metsulfuron-methyl for use in rice crops is sold only in combination with bensulfuron-methyl and has never been included in resistance tests. However, field observations strongly prove that the herbicide mixture did not adequately control some resistant populations.

Evolution of herbicide resistance in Italian rice weeds

In 1994, six years after the introduction of bensulfuron, the first populations of *Alisma plantago-aquatica* L. resistant to ALS inhibitors were found near Novara and in 1995 the first seed samples of *Schoenoplectus mucronatus* (L.) Palla syn. *Scirpus mucronatus* L. were collected from plants that had not been controlled by bensulfuron; field and laboratory tests revealed that those seeds produced ALS-resistant populations. Since then resistance to ALS inhibitors has spread substantially (Figure 1): in three years (i.e. samples harvested in 1998) the total no. of cases tripled (Sattin et al., 1998 and 1999). For *S. mucronatus*, the no. of local administrative districts (in Italian called *comuni*) where resistant populations have been found is still increasing, although the rate is decreasing, while the diffusion of ALS-resistant *A. plantago-aquatica* stabilised after 1999. This is encouraging and proves that the work and consequent resistance management indications given to farmers by the Italian Herbicide Resistance Working Group (GIRE) have had some success (Sattin et al., 2004).

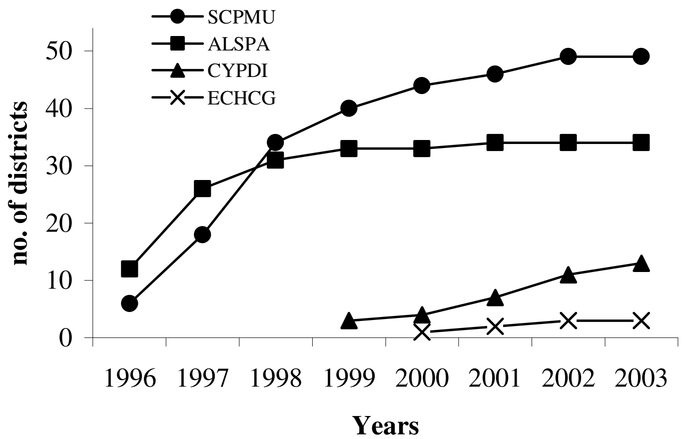

Figure 1 Evolution of herbicide resistance in four Italian rice weed species in terms of no. of local districts where resistant populations were found: SCPMU, ALSPA and CYPDY resistant to ALS-inhibitors; ECHCG resistant to propanil. Most 1996 data were provided by DuPont and refer to seed samples collected from 1994 to 1996 for ALSPA and in 1995-96 for SCPMU (G. Trainini, pers. comm.). Resistance tests were subsequently done at the Weed Science Section of IBAF-CNR.

The first ALS-resistant biotype of *Cyperus difformis* L. was found in 1999 and first documented by Sattin et al. (2002); since then the no. of cases has steadily increased and has now reached 13. Interestingly, four cases are not in the major Italian rice growing area between Turin and Milan: three come from the Po delta area and one from central-western Sardinia. Even the nine sites in the "classic" rice growing area are quite spread out, thus indicating that most populations have been independently selected, as already documented for the other two species (Sattin et al., 1999). Although no extended random survey has yet been done, it is now estimated that resistance to ALS inhibitors is present in well over 25,000 ha (i.e. more than 11% of the Italian rice cropping area), with *S. mucronatus* being the most widespread and *C. difformis* having the fastest rate of increase.

The first of three propanil-resistant populations of *Echinochloa crus-galli* was sampled in 1999 in the Lombardy region (province of Pavia), the second a year later in Tuscany (Siena province) and the third in Piedmont (province of Alessandria). Notwithstanding the slow rate of increase of these biotypes (Table 1), herbicide performance against *Echinochloa* spp. is being carefully monitored because of the worldwide history of herbicide-resistant *Echinochloa* (Valverde et al., 2000; Heap, 2004) and the biological characteristics of this genus that makes it one of the most troublesome rice weeds (Tabacchi, 2003). The herbicide history of the "resistant-fields" we have been able to collect cover a period of six to ten years (farmers records do not generally go further back) and highlight that where propanil-resistant *E. crus-galli* was found, at least eight-nine treatments with propanil had been done (probably more, given that propanil has been on the market since 1962), often with two treatments per year.

Field herbicide histories where ALS-resistant populations were found indicate that during the 90s the most frequent selecting agent was bensulfuron followed by cinosulfuron, while in the last four years azimsulfuron has often substituted the former two SU. In a few fields, resistance evolved even if a crop rotation alternating three consecutive years of rice with three of maize was adopted. Only a few cases of resistance had evolved where a timely pre-emergence treatment with oxadiazon at recommended dose had frequently been applied.

A recent case involving a biotype of *Echinochloa erecta* syn. *Echinochloa hispidula* (Costea and Tardif, 2002) multi-resistant to quinclorac and propanil will be described briefly below.

Characteristics of resistant populations

Materials and methods

Greenhouse experiments

Seeds samples were collected from 1997 to 2003 in fields where field technicians of the member companies of GIRE or the Rice Research Centre observed unjustified poor control of a weed. Seeds were taken from plants that had survived a herbicide treatment: A. plantago-aquatica, S. mucronatus and C. difformis treated with an ALS inhibitor, E. crus-galli treated with propanil and E. erecta treated with quinclorac. All the resistance tests have been done in a greenhouse at the Weed Science Section of IBAF-CNR in Legnaro (north-eastern Italy) following the procedures described by Sattin et al. (1999) for ALS-resistant populations and Scarabel et al. (2002) for Echinochloa spp.

The herbicides used in the experiments and relative field dose (dose 1x) are as follows: azimsulfuron 20 g a.i. ha^{-1}, bensulfuron-methyl 60 g a.i. ha^{-1}, cinosulfuron 80 g a.i. ha^{-1}, ethoxysulfuron 60 g a.i. ha^{-1}, metosulam 70 g a.i. ha^{-1}, propanil 4800 g a.i. ha^{-1}, quinclorac 550 g a.i. ha^{-1}, cyhalofop-butyl 300 g a.i. ha^{-1}, fenoxaprop-P-ethyl 90 g a.i. ha^{-1}, profoxydim 200 g a.i. ha^{-1}. An adjuvant was added where appropriate. Plants were sprayed using a precision bench sprayer delivering 300 L ha^{-1}, at a pressure of 215 kPa and a speed of about 0.75 m sec^{-1}, with a boom equipped with three flat-fan (extended range) hydraulic nozzles (TeeJet 11002). Plants were treated at three-four leaves stage; herbicide efficacy was evaluated about 30 days after application of ALS inhibitors and about three weeks after treatment for Echinochloa experiments.

Field experiments

Three field experiments were done by the Rice Research Centre at Balzola (Alessandria province, 2003 and 2004a) and Villarboit (Vercelli province, 2004b) (north-western Italy, in the major Italian rice growing area) in two fields where accessions of *S. mucronatus* and *C. difformis* had been previously confirmed as resistant to azimsulfuron and cross-resistant to bensulfuron-methyl, cinosulfuron and ethoxysulfuron by greenhouse experiments (at the Weed Science Section – CNR). The aim was to verify the efficacy in the field of the newly marketed (2004) ALS-inhibitor bispyribac-sodium (PTB) in controlling resistant populations of the two above-mentioned weeds.

The herbicides used in the experiments and relative field dose are reported in table 4. An adjuvant was added, where appropriate. Plants were treated at five-six leaves stage. The treatments were applied on saturated soil and the fields re-flooded three days later. The herbicides were applied using a backpack sprayer delivering a spray volume of 400 L ha^{-1} at 220 kPa with a single Albuz 150 nozzle.

Treatments were arranged in a randomised block design, with three replicates. Data were arcsine transformed before being subjected to ANOVA.

Results and discussion

Greenhouse experiments

Most of the screened populations proved to be resistant, thus confirming that the herbicide failures observed in the fields were due to resistance (Table 2). It should be pointed out that the type of sampling almost invariably produced black-and-white results, with most populations showing very high percentage survival of the herbicide treatments. This means that any action is generally taken when resistance has already evolved.

ALS-resistant populations of *A. plantago-aquatica* and *S. mucronatus* generally showed a wide pattern of cross-resistance with a high level of resistance to SU and a lower level to the TP metosulam. As evinced in other experiments, a few populations were highly resistant to both chemical families (Sattin et al., 1999; Scarabel et al., 2004) with a resistance index above 100 (R.I. = ED_{50} of R biotype divided by ED_{50} of S biotype). All biotypes of *C. difformis* were cross-resistant to the three SU included in the screenings. As already found in other countries (Osuna et al., 2002; Kuk et al., 2003), cross-resistance between SU and the PTB herbicide have been documented in Italian populations of *C. difformis* (Vidotto et al., 2003). Some of the populations cross-resistant to metosulam or bispyribac-sodium had never previously been treated with these herbicides.

The resistance mechanism, as documented in many other species that evolved biotypes resistant to ALS inhibitors (Heap, 2004), appears to be related to the decrease in sensitivity of the ALS enzyme (Osuna et al., 2002; Busi et al., 2004) caused by an alteration of the encoding gene (Scarabel et al., 2004).

Table 2 Number of populations of *A. plantago-aquatica*, *S. mucronatus* and *C. difformis* ascribable to three categories based on the percentage of plants surviving herbicide treatment at 3x (three times field dose, herbicide dose used from 1996 to 1998) or 1.1x (herbicide dose used since 1999).

	Herbicide				
Survival (%)	bensulfuron methyl	cinosulfuron	ethoxysulfuron	azimsulfuron	metosulam (*)
A. plantago-aquatica					
0 - 20	**6**	**4**	**4**	**8**	**36**
21 - 60	**0**	**2**	**0**	**5**	**2**
61 - 100	**42**	**25**	**23**	**13**	**1**
No. of tested populations	48	31	27	26	39
S. mucronatus					
0 - 20	**11**	**7**	**7**	**14**	**35**
21 - 60	**3**	**2**	**2**	**4**	**8**
61 - 100	**39**	**11**	**11**	**24**	**17**
No. of tested populations	53	20	20	42	60
C. difformis					
0 - 20	**4**	**-**	**2**	**5**	-
21 - 60	**1**	**-**	**0**	**0**	-
61 - 100	**13**	**-**	**10**	**13**	-
No. of tested populations	18	-	12	18	-

(*) Metosulam was not included in the screenings of C. difformis *because it does not sufficiently control this weed.*

All susceptible standards were 100% controlled by all herbicides when dose 3x was used and control was always above 93% (with most above 98%) with dose 1.1x.

An association has been postulated between each mutation/s and the level and pattern of cross-resistance to different ALS inhibitors (Tranel and Wright, 2002). Amino acid substitutions at the Pro_{197} position have frequently been found and these usually result in high

SU resistance, medium-high TP resistance and no, or low-moderate, IMI (imidazolinones) resistance. Analysis of the molecular basis of resistance in Italian populations of *S. mucronatus* showed that this is a predominantly self-pollinating polyploid species and that a substitution at the Pro_{197} position is involved. However, the analysis of single plants in resistant populations revealed that there are two resistant alleles with different mutations as well as one allele showing multiple mutations (Scarabel et al., 2004). The situation appears complex, with different biotypes having co-evolved in the same resistant population. Caution is therefore required with this type of species before associating a certain pattern of cross-resistance to a mutation and, anyway, molecular tests should be based on a reasonable number of plants.

Screening and dose-response experiments showed that all three resistant populations of *E. crus-galli* have medium-high resistance to propanil. The resistance index of the first population found in 1999 varied between 5 (based on plant fresh weight) and 18 (plant survival). Scarabel et al. (2002) showed that the photosynthetic electron flux was hardly affected by propanil in this population. Preliminary screenings of the other two populations with two propanil doses showed very low mortality even at dose 3.3x, suggesting that their resistance level is likely to be higher than that of the first population. All three accessions proved to be susceptible to azimsulfuron, fenoxaprop-p-ethyl and profoxydim.

In 2003, seeds were collected of a population of *Echinochloa erecta* poorly controlled by double treatments with a quinclorac plus propanil mixture. The field had been treated for at least 6 years with oxadiazon (pre-sowing) and then quinclorac+propanil (post-emergence). Two greenhouse experiments confirmed that the population is multi-resistant to both chemicals, especially quinclorac (Table 3). The experiments, plus field observations, showed that the population is still adequately controlled by azimsulfuron, cyhalofop-butyl and profoxydim.

Table 3 Effect of different herbicides sprayed at two doses on a susceptible and resistant population of *Echinochloa erecta*. The experiment was repeated twice. V.E.B. = visual estimate of biomass in relation to the untreated check (100%); S.E. = Standard Error.

Herbicide	Dose	Check S		Pop. 03-4	
		Survival (%) ± S.E.	V.E.B. (%) ± S.E.	Survival (%) ± S.E.	V.E.B. (%) ± S.E.
quinclorac	1x	**4** ± 4	**2** ± 2	**98** ± 3	**98** ± 0
	3x	**0**	**0**	**96** ± 4	**94** ± 2
propanil	1x	**2** ± 2	**1** ± 1	**46** ± 8	**51** ± 7
	3x	**0**	**0**	**50** ± 12	**59** ± 9
cyhalofop-butyl	1x	**12** ± 4	**5** ± 1	**6** ± 6	**1** ± 1
	3x	**0**	**0**	**0**	**0**

Both populations were completely susceptible (0% survival) to azimsulfuron and profoxydim at both doses and therefore the data have not been included in the table.

Field experiments

The differences in weed control between the two ALS inhibitors and the double treatment were always highly significant (Table 4). In all three experiments the two populations of both species proved to be highly resistant to both azimsulfuron and bispyribac-sodium, a PTB herbicide that appeared on the market this year, and were almost completely controlled by the double application of propanil mixed with MCPA in the first treatment. This is the first report of a *S. mucronatus* biotype cross-resistant to SU and the PTB bispyribac-sodium in Italy.

Table 4 Field experiments: efficacy of different herbicides or mixtures on resistant populations of *S. mucronatus* and *C. difformis*. V.E.C. = Visual Estimate of weed Control in relation to the untreated check.

Herbicides	Rates	V.E.C. (%)				
		S. mucronatus			*C. difformis*	
	g a.i. ha^{-1}	2003	2004a	2004b	2004a	2004b
azimsulfuron*	20	**5**	**8**	**10**	**12**	**7**
bispyribac-Na**	30	**5**	**6**	**13**	**6**	**5**
propanil + MCPA* fb propanil*	4160 + 400 fb 4160	**98**	**98**	**99**	**100**	**98**
	LSD (P=0.01)	8.4	11.2	13.1	12.6	6.4

** with added non-ionic surfactant at 0.1% v/v.*
*** with added Biopower® surfactant at 1 L ha^{-1}.*

Conclusions

Since 1994, five species have evolved resistance to different herbicides in Italy, with the most recent showing multiple resistance. Resistance development must be carefully monitored in species with certain biological characteristics (e.g. ploidy, physiology, plasticity, large numbers of persistent seeds, ability to produce several germination flushes) (Costea and Tardif, 2002; Scarabel et al., 2003 and 2004; Tabacchi, 2003) because once evolved, long-term resistance management strategies will have be adopted, often implying extra costs and higher environmental impact.

The use of ALS inhibitors for more than 4-5 years as the sole means of weed control should be avoided. They should instead be rotated or mixed with other herbicides with different modes of action. From a resistance management perspective it is also important that each herbicide in a mixture controls the same weed spectrum (Shaner and Heap, 2002). However, chemical weed control should be responsibly used, tailored to the weed flora and, where required and feasible, integrated with other measures such as crop rotation and mechanical means. This is particularly true for herbicides like ALS inhibitors, i.e. highly-active herbicide groups which targets specific enzymes. For several reasons their use is constantly increasing and a vast literature (see Tranel and Wright, 2002) and database (Heap, 2004) demonstrate that resistance frequently evolves even after a few treatments (3 to 5).

Herbicides are highly “technological” tools and are often fundamental for profitable agriculture (rice crop being a good example); the decrease in the rate of introduction of new herbicides, and especially new modes of action, should induce all stakeholders to take action to preserve their efficacy over time. More effort is still required to convey the right message to farmers, stressing the importance of implementing efficient and cost-effective resistance prevention and control strategies. Worldwide experience shows that herbicide resistance can be contained through integrated weed management.

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GROWTH OF *MURDANNIA KEISAK* AS AFFECTED BY WATER MANAGEMENT AND TIMING AND RATE OF SEVERALE RICE HERBICIDES

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Abstract

Murdannia keisak (Hassk.) Handel-Mazz. is a member of the Commelinaceae family and it is reported in the spontaneous vegetation of Italy since 1974, while first significant infestations in paddy fields are dated from the end of 1990's. Despite the importance of *M. keisak* in several rice growing areas, only few information on biological and ecological traits related to the agronomical management of this weed are available in the literature.

The aim of this paper was to study the effect on *M. keisak* of different water management strategies and to investigate the sensitivity of the weed to widely used rice herbicides applied at different growth stage.

M. keisak and rice plants were grown in pot in greenhouse under different water management conditions: saturation (A), intermittent irrigation (B), and continuous flooding (C). Aboveground fresh weight of both species were measured 50 days after seeding.

M. keisak plants grown in pots at 3- or 7-leaf leaf stage were sprayed with one of the following herbicides: azimsulfuron, bensulfuron-methyl, cinosulfuron, bispyribac-sodium, bentazone, ethoxysulfuron, glyphosate and triclopyr. The herbicides were applied at the rates of 0 (untreated check), 0.5, 1 or 2 times the recommended field rate. Herbicide efficacy was determined by measuring aboveground fresh weight 15 days after spraying.

Plants of *M. keisak* grown under continuous flooding conditions showed the lowest values of aboveground fresh weight (9.6% of that recorded in saturation conditions).

Bispyribac-sodium, bentazone, triclopyr and glyphosate effectively controlled *M. keisak* already at 0.5x field rate, either when sprayed at 3- or 7-leaf stage plants. Bispyribac-sodium, triclopyr and glyphosate were more effective against older plants.

A successful control strategy of this weed can be achieved with a correct choice of water management, herbicide and stage of application.

Key words

Marsh dayflower; water management; herbicide; dose-reponse study.

Introduction

Murdannia keisak (Hassk.) Handel-Mazz. (Marsh dayflower) is a member of the spiderwort (Commelinaceae) family. Together with several other species of the genus *Murdannia*, such as *M. nudiflora* (L.) Brenan, M. *triquetra* Wall. and *M. vaginata* (L.) Bruckn, *M. keisak* is native of eastern Asia, where it is reported both in cultivated and natural areas (Baki et al., 1997; JinHao et al., 2000; Kitano et al., 1999b; LiuQing et al., 1998; Sago et al., 1996; Wilson, 1981).

M. keisak was first noted as weed in 1935 in cultivated rice paddies in South Carolina (Swearingen et al., 2002) and has become established and naturalized also in other cultivated or natural areas in the USA, often showing an aggressive growth and competing against native vegetation by forming dense vegetation layers (King et al., 2000; Rundell and Diamond, 1999). Other species of *Murdannia*, and *M. nudiflora* in particular, are important weeds in several Latin America rice areas (Bastidas López, 1996; Plaza and Forero, 1998; Rojas et al., 2002; Wilson, 1981).

In Italy this species is reported in the spontaneous vegetation since 1974 (Pignatti, 1982), while first significant infestations in paddy fields are dated from the end of 1990's (Ferrero and Vidotto, 2004).

M. keisak grows in damp soil and may form dense floating mats wherever there is standing water. This plant has become highly invasive in marshes, swamps, ditches, creek and riverbanks, and around ponds and lakes, rapidly crowding out desirable native herbaceous vegetation. Even in its native region this species is a difficult weed.

M. keisak presents alternate linear or lanced-shaped leaves about 6 cm long and 1 cm wide. The base of the leaves have closed tubular, hairy sheaths. The stems are succulent, rooting at the nodes, growing prostrate. The flowering generally occurs in September-October. The flowers, solitary or 2-4 in axillary racemes, are generally developed at the end of the stems or from the leaf axil, show 3 green sepals, ovate to oblong, and 3 pink petals, a little longer than the sepals. The stamens are 6 (only 2 or 3 fertile) and the ovary is superior. Fruits are capsules 6-7mm long, ellipsoid to oval, pointed at the tip, smooth, containing about 48 grey and flat seeds (Pignatti, 1982). Reproduction is by fragmentation and seed production. In field conditions, burial and submersion affect seedling emergence, which can occur when mean air temperature is higher than 8 °C (Sago et al., 1996).

Despite the importance of *M. keisak* in rice growing systems of different areas of the world, only few information on biological and ecological traits related to the agronomical management of this weed are available in the literature. Few investigation were carried out in particular in sensitivity of this weed to herbicides. *Murdannia* species are reported to be successfully controlled with application of bispyribac-sodium (JinHao et al., 2000; Ferrero and Vidotto, 2004), bentazone and mixtures containing bentazone (Kitano et al., 1999a), cloroacetamides (mefenacet, pretilachlor), molinate, MCPA (Sago et al., 1996) and 2,4-D (Wilson, 1981). No information are available on the effects of some agronomic practices applied in rice cropping on *M. keisak* emergence, growth, competition, seed production and dispersal. As this weed is strongly related to the aquatic environment, the knowledge of the effects of water management, in particular, could be crucial in the definition of integrated control strategies.

The aim of this paper was to study the effect on *M. keisak* of different water management strategies and to investigate the sensitivity of the weed to widely used rice herbicides applied at different growth stage.

Materials and methods

The study was carried out in greenhouse during the year 2002. During the period in which the study was carried out the greenhouse was covered by a shading tissue placed in the outside of the building to prevent excessive temperatures. The shading tissue determined a light intensity reduction of 47%. The resulting natural light was integrated with a series of metal-halide lamps, delivering about 55 mmol s^{-1} m^{-2} (40-770 nm), adjusted to obtain 16-h day-length.

The seeds of *Murdannia keisak* used in the study were obtained in 2001 from plants collected in naturally infested fields of Northern Italy and grown in greenhouse. After harvesting, the seeds were dried in open trays at room temperature for 30 days and then chilled in deionzied water at 4 °C for at least 2 months.

Water management

Pre-germinated seeds of *M .keisak* and rice (*Oryza sativa* L.) cv. Selenio (medium grain *japonica*-type variety) were directly placed on 100 cm^2 pots filled with commercial potting substrate saturated with water. After seeding, six different treatments were applied following a 3x2 factorial design. Water management strategy was the first factor and the following three levels were tested both in *M. keisak* and rice pots: saturation (A), intermittent irrigation (B) and continuous flooding (C). In A the soil was continuously maintained in saturated conditions by placing the pots in trays containing a water layer of about 5 cm, in order to allow the water to completely saturate the substrate through the openings present on the pot's base. In B, the pots were irrigated only when rice started to show visible drought stress (leaves started to fold), by flooding the pots until soil saturation. In C, the pots were placed in flooding conditions, with a water layer of about 10 cm. Timing of application was the second factor: the three water management strategies (A, B and C) were applied either starting immediately after seeding ("early") or ten days later ("late").In the last case, during the first ten days the pots were kept in trays containing water layer of about 1 cm.

Two weeks after the seeding 0.25 g/L of a N-P-K complete fertilizer solution (10-16-25) was added to each pot. A completely randomised design with six replicates was adopted. Each pot was an experimental unit. *M. keisak* and rice aboveground fresh weight were measured 50 days after seeding.

Two-way analysis of variance was performed on fresh weight data. *M. keisak* and rice data were analyzed separately.

Herbicide sensitivity

Seeds of *M. keisak* were placed on 36 cm^2 pots filled with commercial potting substrate. After sowing, the pots were immersed in water in order to maintain the soil in continuously flooded conditions (soil surface about 1 cm above the water level).

One day before herbicide treatment, the seedlings were thinned to 4-5 uniform and equidistantly spaced plants per pot. The plants were sprayed when they reached the stage of 3 or 7 leaves with one of the following herbicides: azimsulfuron, bensulfuron-methyl, cinosulfuron, bispyribac-sodium, bentazone, ethoxysulfuron, glyphosate and triclopyr. The herbicides were applied at the rates of 0 (untreated check), 0.5, 1 or 2 times the recommended field rate (Table 1). A non-ionic adjuvant was added to each herbicide and rate.

Table 1. Herbicides included in the experiment and adopted field rate. Plants were sprayed at 0, 0.5, 1 and 2 times the field rate.

Herbicide	**Mode of action**	**Field rate (g a.i. ha^{-1})**
azimsulfuron	ALS- inhibition	25
bensulfuron-methyl	ALS- inhibition	60
cinosulfuron	ALS- inhibition	80
ethoxysulfuron	ALS- inhibition	60
bispyribac-sodium	ALS- inhibition	31
bentazone	PSII-inhibition	3.5
glyphosate	EPSP synthase-inhibition	2520
triclopyr	IAA action- like	750

The treatments were carried out using a cabinet track sprayer equipped with a single Teejeet DG8002-VS nozzle and adjusted to deliver a spray volume of 400 L ha^{-1} at 203 kPa. Herbicide efficacy was determined by measuring aboveground fresh weight per pot 15 days after spraying. For each stage and herbicide, the fresh weight data were expressed as percent of the untreated check.

Results

Irrigation management

Water management significantly influenced both *M. keisak* and rice growth (Figure 1). In both species, the highest values of aboveground fresh weight (on average, 2.84 and 2.43 g plant^{-1} for *M. keisak* and rice, respectively) were recorded when pots were kept in saturated conditions (water management A). In the case of *M. keisak*, the lower fresh weight values which were corresponding to 9.6% of the values recorded in A, were observed in continuously flooded conditions (C). In rice, the lower values were obtained under intermittent irrigation conditions (B), with a 38.7% of weight reduction in comparison to treatment A (Figure 1).

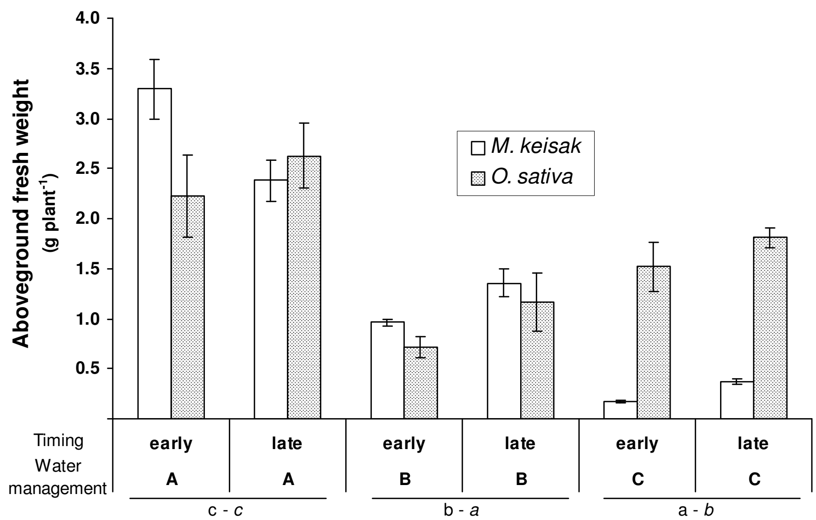

Figure 1 Aboveground fresh weight (g plant^{-1}) of *M. keisak* and rice. Water management strategies not sharing the same small letter are significantly different at *P*< 0.05 (Student-Newman-Keuls test). Regular and Italic small letters refer to *M. keisak* and rice, respectively. Errors bars indicate SE (n=6).

The effect of timing of application of the three water management strategies was not significant, neither for *M. keisak* nor rice (Table 2). Yet, the delayed application of the water management strategies often resulted in higher values of aboveground fresh weight. The opposite behaviour observed in *M. keisak* under saturated conditions (A) can explain the significant interaction between the factors water management and timing highlighted by the analysis of variance for this species.

Table 2 Analysis of variance of *M. keisak* and rice aboveground fresh weight values.

Source of variation	*M. keisak*				*O. sativa*			
	df	**Mean Square**	***F***	**Sig.**	**df**	**Mean Square**	***F***	**Sig.**
Water management (W)	2	20.38	134.99	0.000	2	6.63	15.42	0.000
Timing (T)	1	0.10	0.68	0.416	1	1.32	3.07	0.090
W x T (interaction)	2	1.49	9.86	0.001	2	0.02	0.05	0.952
Error	30	0.15			30	0.43		

Herbicide sensitivity

Bispyribac-sodium, bentazone, triclopyr and glyphosate effectively controlled *M. keisak* already at 0.5x field rate, either when sprayed at 3- or 7-leaf stage plants (Figure 2 and 3). At this rate, the relative growth reduction obtained with these herbicides ranged from 69.5% (triclopyr, 3-leaf stage) to 90.1% (glyphosate, 7-leaf stage). The increase of the rate of application of these herbicides, at both stages of application, resulted in a non significant increase of efficacy. Conversely, the treatment carried out with bispyribac-sodium, triclopyr and glyphosate on older plants gave a significantly higher level of efficacy, regardless the rate of application.

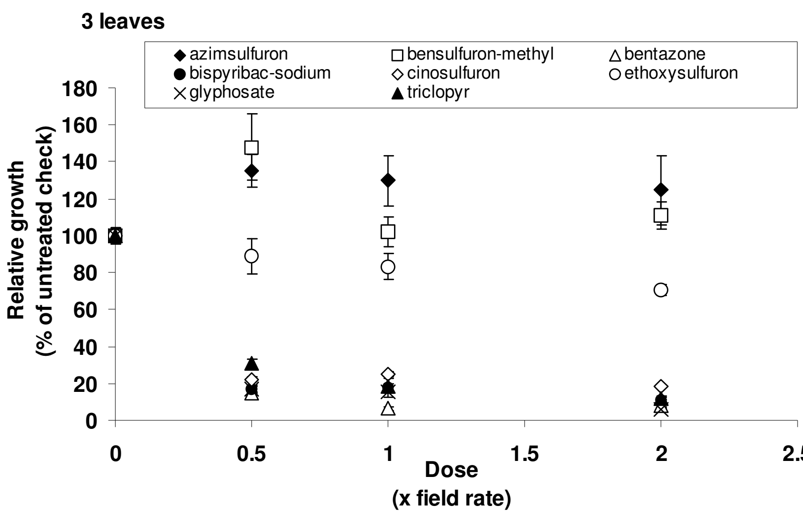

Figure 2 Relative growth as percentage of untreated check of *M. keisak* sprayed at 3-leaf stage. Errors bars indicate SE (n=5).

Both for plants treated at 3- and 7-leaf stage, the aboveground fresh weight was not significantly reduced after spraying with the ALS-inhibitors azimsulfuron, bensulfuron-methyl and ethoxysulfuron. By contrast, application of these herbicides often resulted in an increase of plant biomass (hormetic effect), especially with azimsulfuron and bensulfuron-methyl when plants were sprayed at 3-leaf stage.

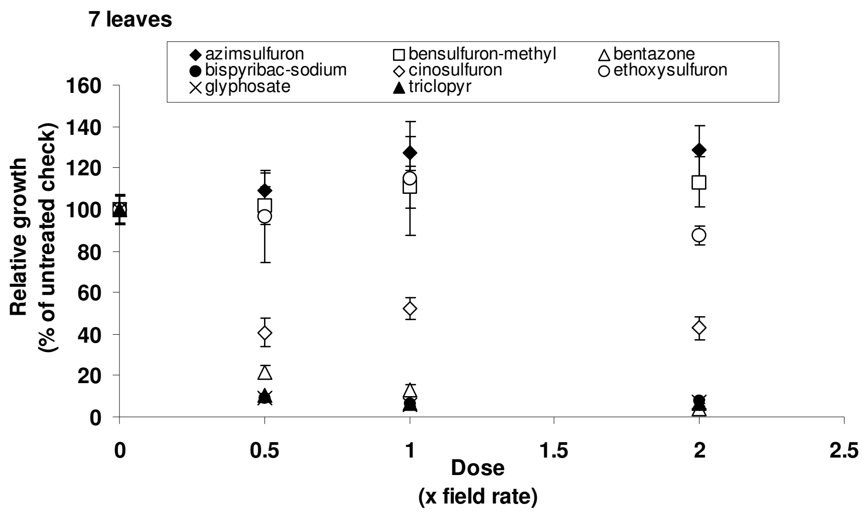

Figure 3 Relative growth as percentage of untreated check of *M. keisak* sprayed at 7-leaf stage. Errors bars indicate SE (n=5).

An intermediate behaviour was observed in the case of cinosulfuron. The efficacy was of 78.2% and 54.7%, on average, on younger and older plants respectively, with no significant differences between 0.5x, 1x and 2x field rate.

Conclusions

Water management had a significant effect on *Murdannia keisak* growth. In particular, the results pointed out that continuous flooding, which is already currently adopted in European rice fields, could be helpful in limiting growth and competitiveness of this weed. The growth of the rice variety used in this study is more affected by intermittent irrigation rather than continuous flooding. It should be noted that the highest values of rice plant biomass recorded under soil saturation conditions could likely be obtained also because of the favourable greenhouse climatic conditions and in particular of the narrow range of temperature variation.
The growth of *M. keisak* was strongly affected already at rates below to the recommended field rate by bentazone, bispyribac sodium, cinosulfuron, glyphosate and triclopyr which are currently adopted in rice cultivation,. In general, plants at 7-leaf stage were more sensitive to herbicides than plants at 3-leaf stage. Azimsulfuron, bensulfuron-methyl and ethoxysulfuron had no effect on this weed; in particular azimsulfuron and bensulfuron-methyl stimulated *M. keisak* growth. When applied at early stage this last behaviour should be investigated in field conditions and for other herbicides used in rice in order to correctly evaluate its practical relevance for *M. keisak* growth and spread.
According to the results obtained in the present study, a successful control strategy of this weed can be achieved with a correct choice of herbicide, stage of application and water management.

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CHEMICALS AND THERMOTERAPY FOR RICE SEED DRESSING

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Abstract

The use of healthy seeds represents the starting point to control the most important seed borne pathogens causing rice diseases: *Fusarium moniliforme* (bakanae), *Pyricularia oryzae* (blast), *Bipolaris oryzae* (brown spot). In consideration of the increasing demand of organic seeds and of the spread of the bakanae disease observed in the last years in Italy, commercial fungicides (available in the Italian market for bakanae control) and thermotherapy were tested. The following active ingredients: carbendazim, fludioxonil, mancozeb, iprodione + triticonazole, carboxin + thiram, prochloraz were assayed. Chemicals were compared to thermotherapy performed either in hot water or by hot-humid air (90% of relative humidity).
Results obtained show a good efficacy achieved through thermal treatments, statistically not different from the most effective fungicides (prochloraz against *F. moniliforme*; carboxin + thiram, fludioxonil, iprodione + triticonazole, mancozeb against *B. oryzae*). Thermal treatments are effective against many different fungal species, while chemical products are more specific in the control of one or few pathogens.

Keywords

Seed-borne diseases; *Oryza sativa*; thermal treatments; fungicides; seed.

Introduction

Rice crop is an economically important culture for the Northern Italy agriculture, cultivated in more than 220 thousand hectares. One of the most important cultural practices is the use of healthy or chemical dressed seeds. In fact, due to many pathogens, seedlings loss is one of the major problem occurring during the first stages of the culture Among the most important diseases bakanae, blast and brown spot are seed borne and seed transmitted and can affect seedlings in the fist stages of the crop. Conventional control against these pathogens is carried out by chemical treatments. In order to avoid an increase in seedborne infection, cereal seed should never be sown for more than two generations without treatment (Maude, 1996). Thermotherapy is A possible alternative to the chemical control , since it has several advantages compared to the chemical treatment, such as a reduced spread of chemicals in the environment; furthermore, thermal treated seeds can be used in organic farming and leftover seeds can be used as feed or food.
Bakanae disease, caused by *Fusarium moniliforme*, was often observed in Italy and its presence is increasing (Moletti, 1997); as the disease is primarily seed-transmitted (Webster, 1992), seed dressing represents the first way to control the spread of the disease.
Conventionally, chemical seed dressing is carried out to treat infected seed lots; however, hot water and dry heat treatments have been known for long time as methods allowing control of seed-borne diseases (Baker, 1962). Due to many disadvantages these methods are not widespread (Forsberg et al., 2003). In order to overcome the obstacles associated with hot water or dry heat methods, some researchers have proposed the use of the aerated steam method, using hot, humid air (Maude, 1996). The idea was to combine the positive effects of the hot water and dry heat treatment methods and to limit their disadvantages. The method has

been further developed through a collaboration between the company Acanova AB and SLU (Swedish University of Agricultural Sciences), both in Uppsala, Sweden.
The efficacy of commercial fungicides available on the Italian market for bakanae control was tested in laboratory and field trials, looking at the side effects of these compounds against seedling blight,. Beside, thermotherapy performed by aerated steam as well as by immersion in hot water were tested and compared to chemicals.

Materials and Methods

In 2002 a sample of cv Poseidone was used; in 2003 two samples belonging to 2 different cultivars, Albatros and Galatxo, were tested. Thermal treatments by hot humid air were carried out by Acanova AB using a laboratory-scale equipment, designed for high precision research use. The treatment system is equipped with modern sensor and computer control technology in order to increase the precision in the control of temperature, exposure time and relative air humidity (Forsberg et al., 2003). Treatments consisted of three phases: 1. seeds were heated to a pre-calculated temperature at a pre-calculated air moisture content; 2. temperature and air humidity were kept constant; 3. seeds were cooled down to room temperature. Seeds were treated at 90% relative humidity; treatment was carried out for 2 minutes (T1: 67°C cv Poseidone, 73°C cv Albatros, 67°C cv Galatxo; T2: 75°C cv Poseidone, 75°C cv Albatros, 71°C cv Galatxo) or for 5 minutes (T3: 64°C cv Poseidone, 68°C cv Albatros, 64°C cv Galatxo; T4: 72°C cv Poseidone, 70°C cv Albatros, 68°C cv Galatxo).
Thermal treatment by immersion in hot water was carried out by Ente Nazionale Risi, following the international method for *Aphelenchoides besseyi* control on rice. Treatment was performed by laboratory-scale equipment; seeds were immersed in water at 60-61 °C for 15 minutes. Then, seeds were dried at room temperature (23-25°C) for 24 h.
Chemical treatments were performed by the Ente Nazionale Sementi Elette, using a seed dressing laboratory equipment. The following active ingredients were tested: carbendazim at 75.0 g/100 kg of seeds (solid formulation) and at 82.6 ml/100 kg (liquid formulation); fludioxonil at 4.8 ml/100 kg; mancozeb at 187.5 ml/100 kg; iprodione + triticonazole at 23.0+2.3 ml/100 kg; carboxin + thiram at 68.8+68.8 ml/100 kg. In 2003 we tested also the efficacy of prochloraz (18.8 ml/100 kg), halved rates of carbendazim (37.5 g/100 kg and 41.3 ml/100 kg), carboxin + thiram (68.8 + 68.8 ml/100 kg of seed) combined with thermal treatments T2 or T4.
Germination test was carried out in Ente Nazionale Sementi Elette laboratories by incubation of 400 seeds, divided in 8 Petri dishes (15 cm diameter), top of layer paper soaked in water; Petri dishes were placed in the dark for 7 days, 16 h at 20°C and 8 h at 30°. Survey was carried out counting the number of normal seedlings, abnormal seedlings due to physiological damages, abnormal seedlings due to fungal damages, ungerminated seeds. Results were expressed as percentage (ISTA, 1996).
Laboratory health tests were performed in Agroinnova laboratories by incubating 400 seeds per sample (top of 3 layer paper soaked in water, in 9 cm Petri plates) and placing 25 seeds/Petri, for 7 days at 20-22°C, 12 h light and 12 h darkness. The survey was carried out by examining seeds under stereoscopic microscope. Results are expressed as percent of infected seeds (Agarwal, 1989). Germination and laboratory health tests were repeated 3 times: 1, 3 and 6 months after seed dressing treatment.
Field trials were carried out in a dry field located in Tavazzano (Lodi province, Lombardia) in 2002 and sited in Garbagna (Novara province, Piedmont) in 2003; plots (10 m^2) were sown in rows by seeder, following a randomized block design. Standard cultural practices (fertilization, herbicide and fungicides treatments) were carried out conforming with local agricultural practices. Infection caused by *F. moniliforme* was detected counting the number

of symptomatic plants in each plot, once a week, starting from the 2nd leaf phase; the number of plants at 3rd-4th leaf phase was counted.
Results were analyzed by Anova and Tukey's test; percentage data were statistically analyzed after transformation of the percentage (P) in angular values: arcsine of square root of P/100.

Results

Here we have summarised results obtained in laboratory tests, partially reported in Titone et al. 2003 and 2004. Both in 2002 and 2003, depending on time, germinability and number of seedlings with pathological damages decreased while ungerminated seeds and seedlings with physiological damages increased. Compared to the control, in 2002, germinability was significantly reduced in seeds treated at 75°C for 2 minutes and, contemporarily, ungerminated seeds were significantly more numerous than in all other treatments. Mancozeb and T3 (64°C, 5 minutes) increased the germinability. In 2003 trials, germination was statistically reduced by treatments performed by thermotherapy in combination with carboxin + thiram and increased by all the other treatments except T1 and carbendazim in solid formulation. Ungerminated seeds were more numerous than in the control when seeds were dressed with the following treatments: thermotherapy + carboxin + thiram, thermotherapy performed by immersion in water, thermotherapy performed by aerated stem at 68° (cv Galatxo) or 70°C (cv Albatros) for 5 minutes. Macroscopically, treatments which were able to better reduce the necrosis occurrence were fludioxonil, iprodione + triticonazole and carboxin + thiram (with or without thermal treatment). Also mancozeb and prochloraz can reduce seedlings showing pathological damages.
Against *F. moniliforme*, generally, thermal treatments, carbendazim, mancozeb and carboxin + thiram provided good control, iprodione + triticonazole in some cases had only a partial effect and fludioxonil had no effect. On the contrary, against *Bipolaris oryzae* and *Alternaria* spp. carbendazim was ineffective and fludioxonil provided a good control. In some surveys prochloraz was not effective against *B. oryzae*. Thermal treatments had a good control of *F. moniliforme* infections, while in few cases did not statistically reduced *B. oryzae* and *Alternaria* spp. occurrence.
In 2002, in contrast with germinability trials carried out in laboratory, the number of emerged plants in field was similar to the control or negatively influenced by thermotherapy; chemical treatments, except iprodione + triticonazole, were able to increase the emergence.
In 2003, the emergence of seedlings in treated plots was similar to the control. The highest number of emerged plants was recorded in plots treated by carboxin + thiram + thermotherapy (75° for 2 minutes, cv Albatros; 68°C for 5 minutes, cv Galatxo) (Table 1).
The number of diseased plant picked-up each week per plot was very variable in different samples (Table 1). Nevertheless, collected plants represented from 2 to 3% of the number of sown seeds and from 17 to 31% of infected seeds observed in laboratory tests (Table 2).
The number of symptomatic plants changed during the growing season and the trend is completely different in different samples. Maximum pick of symptomatic plants was observed during first stages of the crop (until tillering beginning) in cv Poseidone and in cv Galatxo, between end of tillering and booting phase in cv Albatros.
Among tested chemical treatments only mancozeb showed a similar efficacy in both years, reducing the number of infected plants of about 50% compared to the untreated control. Other chemicals (carbendazim, fludioxonil, iprodione + triticonazole, carboxin + thiram) provided a better control in 2002, especially carbendazim, which in 2002 strongly reduced the disease (97% of reduction) and in 2003 halved the number of diseased plant. In 2003 carbendazim was tested also at half strength; reduced rate caused the same level of control of labelled rate in cv Albatros, but not in cv Galatxo. Plots treated by thermotherapy (alone or in combination

with carboxin + thiram) and prochloraz were statistically not different between them. The average reduction of diseased plants was 91% in cv Albatros, 82% in cv Galatxo and 98% in cv Poseidone (Figure 1 and Table 1).

Table 1 Effect of treatments on field emergence (number of plant/m) and on symptomatic plants (number of diseased plant/plot/growing season).

Treatments	Number of plants					
	cv Poseidone, 2002		cv Albatros, 2003		cv Galatxo, 2003	
	Emerged	Diseased	Emerged	Diseased	Emerged	Diseased
Control	13 d*	152 d	28 a	103 d	11 ab	53 f
T1	16 abcd	5 a	37 a	13 a	13 ab	17 abc
T2	6 e	1 a	30 a	8 a	15 ab	7 a
T2+carboxin+thiram	-		48 a	8 a	20 ab	11 ab
T3	15 bcd	5 a	33 a	13 a	15 ab	12 ab
T4	14 cd	3 a	31 a	10 a	17 ab	14 ab
T4+carboxin+thiram	-		39 a	10 a	21 a	9 a
carbendazim 75.0 g/q	20 abc	4 a	32 a	49 b	12 ab	23 abcd
carbendazim 37.5 g/q	-		34 a	47 b	14 ab	38 def
carbendazim 82.6 ml/q	21 ab	4 a	32 a	52 b	14 ab	31 bcde
carbendazim 41.3 ml/q	-		25 a	60 bc	7 b	35 cdef
fludioxonil	21 ab	38 b	38 a	83 cd	18 ab	54 f
mancozeb	19 abc	88 c	32 a	55 b	12 ab	25 abcd
iprodione+triticonazole	18 abcd	42 b	41 a	88 cd	19 ab	49 ef
carboxin + thiram	23 a	58 bc	47 a	60 bc	20 ab	42 def
prochloraz	-		31 a	7 a	16 ab	7 a
61°Cx15 min (in H_2O)			29 a	8 a	13 ab	8 a

**The values of the same column followed by the same letter do not differ statistically between them for Tukey's test (p=0.05).*

Table 2 Percent of symptomatic plant surveyed in control plots and diseased seeds observed in laboratory tests.

Cv	Seeded seeds (n.)	Symptomatic plants (n.)	Symptomatic plants (a) (%)	Diseased seeds (b) (%)	(a)/(b) (%)
Poseidone	6221	152	2.4	14	17.4
Albatros	3320	103	3.1	10	30.9
Galatxo	2350	53	2.3	10	22.6

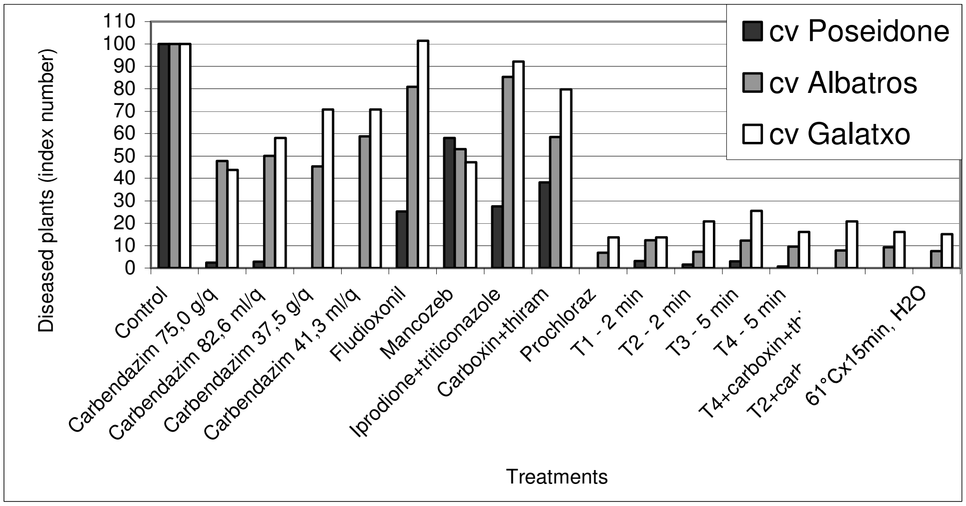

Figure 1 Seed dressing effect on *F. moniliforme* attacks. Result expressed as percent of diseased plants out of diseased plant in untreated control plots.

Conclusion

Field infection of *F. moniliforme* is becoming a problem for seed rice production in Italy and the way to control it at the moment is represented by chemical seed dressing. The most effective active ingredient among those available in the Italian market is prochloraz (although tested only in one year of trials), which in field caused a reduction of 90% in diseased plants. Similar efficacy was obtained by thermal treatments, which unfortunately in Italy do not represent, at the moment, a feasible alternative for seed companies.

While field conditions influence the disease development, laboratory diagnosis can represent a useful method to assess the presence of bakanae symptoms in field. In fact, we observed in field about 1 diseased plant out 4 diseased seeds observed in laboratory test.

Differences in symptoms appearance observed in field during time can not be attributable to soil and climatic conditions, as Galatxo and Albatros samples were cultivated in the same time and in the same location; it maybe due to different plant reactions to the infection or to intrinsic characteristics of the virulent strains.

It is interesting to observe that, among tested treatments, thermotherapy in combination with carboxin + thiram, which caused a reduced germinability *in vitro*, does not cause problems in field in terms of emergence of seedlings. The use of reduced rates of fungicides is not advisable because of the risk of resistance development, especially related to some fungicides, such as carbedazim. In fact, the different effectiveness observed in 2003 trials could be referred to fungal strains with a reduced sensitivity towards carbendazim.

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BASELINE SENSITIVITY OF PENOXSULAM TO *ECHINOCHLOA* SPP. ACCESSIONS FROM ITALIAN RICE FIELDS

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Abstract

Penoxsulam (triazolopyrimidine) is a new selective ALS-inhibitor herbicide for weed control in dry-seeded, water-seeded and transplanted rice, with high efficacy against *Echinochloa* species. The sensitivity to penoxsulam of nine *Echinochloa* populations collected in Italian rice fields was assessed in a dose-response study carried out in the greenhouse. The herbicide was applied at eight rates ranging from 0 to 160 g a.i. ha^{-1}. Fresh weight and visual efficacy were assessed 21 days after treatment. The data of each population were expressed as percent of untreated control plants and fitted to a log-logistic model. The rates of herbicide for 80% and 90% of plant growth inhibition (GR_{80} and GR_{90}, respectively) where calculated from the fitted equations.All tested populations showed to be highly sensitive to penoxsulam, with the highest level of control generally reached already at 20 g a.i. ha^{-1}. The GR_{80} ranged between 2.7 and 9.8 g a.i. ha^{-1}, while GR_{90} ranged between 3.2 and 19.2 g a.i. ha^{-1}.

Keywords

Barnyardgrass; dose-response; ALS-inhibitor.

Introduction

The genus *Echinochloa* accounts for about 50 species, some of them including different subspecies and varieties, which are widely spread in tropical and temperate regions. Several *Echinochloa* species are some of the most important weed species in crops (Costea and Tardif 2002). In most of rice (*Oryza sativa* L.) cropping systems worldwide, in particular, *Echinochloa* species are the main weeds (Ferrero et al. 1999; Holm et al. 1977).

High variability in morphological traits, such as plant size, tillering ability, seed size and germination behaviour can be observed within most species (Barrett and Wilson 1983). This variability led to the development of different taxonomic classifications and makes field identification difficult and uncertain.

Several herbicides, including molinate, thiobencarb, clomazone, propanil, quinclorac, azimsulfuron, cyhalofop-buyl, fenoxaprop, profoxidim and bispyribac-sodium, are widely used to control *Echinochloa* spp. in rice. The sensitivity to the various herbicides varies mainly according to species and stage of application and only few information are available about variability between different populations belonging to the same species or sub-species that can be found in a certain rice growing area.

Cases of lack of control have been reported in several areas for some of these herbicides (bispyribac-sodium, cyhalofop-butyl, molinate, propanil, and quinclorac) as consequence of selection and spreading of herbicide-resistant weed populations (Busi et al. 2002; Fischer et al. 2000; Fischer et al. 1993; Garro et al. 1991).

Penoxsulam is a new selective ALS-inhibitor herbicide for weed control in dry-seeded, water-seeded and transplanted rice. In several field experiments this herbicide provided good control of *Echinochloa* spp. and many broadleaf and annual sedge weeds (Larelle et al. 2003).

This paper reports the results of a preliminary study aimed at determining the rates of penoxsulam (in g a.i. ha^{-1}) able to reduce up to 80% and 90% the vegetative growth (GR_{80} and GR_{90}, respectively) of *Echinochloa* populations collected in Italy.

Materials and methods

Sensitivity to penoxsulam of *Echinochloa* populations was assessed by carrying out dose-response studies in the greenhouse in the period June-September 2003. During the years 2000-2002 a total of nine populations of *Echinochloa* species were singled out in North-west Italian rice fields and the seeds used in this study were collected in summer 2002. After collection the seeds were dried in open trays at room temperature and then stored at +4 °C.

In order to improve germination, the seeds were scarified with concentrated (80%) sulphuric acid. Before the starting of the dose-response studies a preliminary assay was carried out on 20 seeds for each population to determine the optimal duration of scarification. In this assay, these seeds were scarified for either 30, 40, or 50 minutes. Scarification led to a significant improvement of germination percentage, with the exception of two populations (I02E02 and 49), in which germinability was always lower than 30% (Table 1). After scarification and before the transplanting in the pots, seeds were kept for about 24 h in deionised water enriched with air through a bubbler.

Table 1 Label, species, duration of scarification and related germination percentages of *Echinochloa* populations included in the study.

Population	**Species***	**Scarification duration (min)**	**Germination (%)**
I01 E01	ECHsp.	50	88.2
I02 E01	ECHsp.	30	100.0
I02 E02	ECHsp.	50	25.0
15	ECHER	40	77.8
16	ECHCG	40	61.9
25	ECHCG	30	100.0
41	ECHER	30	90.0
45	ECHCG	45	89.5
59	ECHCG	50	15.8

(*): classification according to Pignatti (1982); ECHCG: Echinochloa crus-galli *(L.) Beauv.; ECHER:* E. erecta *(Pollacci) Pign.;ECHsp.:* Echinochloa *sp.*

Seeds were transplanted in 36 cm^2 pots (up to 7 seeds per pot) filled with sandy loam soil when the root tip erupted from the seed coat for about 2-3 mm. Few minutes before transplanting, pots were placed in trays containing a water layer of about 5 cm, in order to allow the water to completely saturate the substrate through the openings present on the pot's base. Natural light was supplemented by metal halide lamps adjusted to obtain 16-h day-length and an average daily photon flux, measured in PAR range (400-770 nm) of 145 $\mu E\ s^{-1}\ m^{-2}$. The average maximum, minimum and mean temperature values recorded during the entire study were 33.5 °C, 22.7 °C, and 27.6 °C, respectively. The average maximum, minimum and mean relative humidity values were 78.3%, 36.1%, and 56.9%, respectively.

When the plants reached the BBCH 13-14 growth stage and about one day before the treatment, the seedlings were thinned to 4-5 uniform and equidistantly spaced plants per pot.

The plants were sprayed at seven growing rates (from 2.5 g a.i. ha^{-1} to 160 g a.i. ha^{-1}) of penoxsulam (formulation GF-657). A single rate of application of the formulation Clincher (cyhalofop), corresponding to the average field rate, and an untreated check were also included as reference (Table 2). Samples of GF-657 and Clincher for carrying out this study were provided by Dow AgroSciences.

All the treatments were foliar and carried out using a cabinet track sprayer delivering a spray volume of 300 L ha^{-1} at 203 kPa with a single Teejeet DG8002-VS nozzle.

All the treatments were arranged according to a randomized complete block design in which each pot was an experimental unit. Three replicate pots were used per treatment for each population. The study was repeated twice.

Immediately after the treatment, each pot was placed in an individual saucer, in order to avoid pot-to-pot interference through water, and arranged in a bench according to the adopted experimental design. After treatment, water was regularly supplied to maintain soil at field capacity until about one day before efficacy assessment.

About 21 days after treatment the plants were assessed for percentage visual control, using a 0-100 linear scale, always attributing 0 to the control plants.

Immediately after visual control plant biomass was assessed by measuring the aboveground fresh weight per pot.

Table 2 Treatments compared in the study.

Treatment	Active ingredient	Formulation	Rate (g a.i. ha^{-1})
1	penoxsulam	Untreated	0
2	penoxsulam	GF-657	2.5
3	penoxsulam	GF-657	5.0
4	penoxsulam	GF-657	10.0
5	penoxsulam	GF-657	20.0
6	penoxsulam	GF-657	40.0
7	penoxsulam	GF-657	80.0
8	penoxsulam	GF-657	160.0
9	cyhalofop-butyl	Clincher*	300.0

(*): Codacide oil added at 1.5 L f.p.- ha^{-1}

Fresh weight data of each population were expressed as percent of untreated control plants (relative growth).

The data of the treatments from 2 to 8 (GF-657 from 2.5 g a.i. ha^{-1} to 160 g a.i. ha^{-1}) were fitted to the following log-logistic regression model:

$$Y = c + \{(d-c)/[1+(x/g)^b]\}$$

where *Y* is percent growth, *c* is the response at very high herbicide rates, *d* is the response when herbicide rates is near to zero, *b* is the slope of the line in the point of inflection, *g* is the herbicide rate at the point of inflection halfway between *c* and *d*, and *x* is the herbicide dose.

Regression analysis was performed using data from all the replicates using the regression utility of the SigmaPlot 2001 version 7.0 software. The rates of herbicide for 80% and 90% plant growth inhibition (GR_{80} and GR_{90}, respectively) were calculated for each population and experiment.

Data of the visual control assessment were fitted to the log-logistic model described above and the rates of herbicide for 80% and 90% of visual growth reduction (VGR_{80} and VGR_{90}, respectively) were calculated for each population and experiment.

Results

The formulation GF-657 of penoxsulam was highly effective against all tested populations. As expected, the variability of response between replicates was inversely related to the rate of application, regardless the population.

In terms of relative growth, in all populations the highest level of control was reached at 20 g a.i. ha^{-1} and no significant improvements were observed with higher dosages. Some differences between populations were observed only for rates below 20 g a.i. ha^{-1}. More than 90% of plant biomass reduction was observed at 10 g a.i. ha^{-1} in the population I02E01, I02E02, 16, 41, and 45 (Figure 1).

In all cases, a good fitting of the data relative growth reduction and rate of application was obtained with the log-logistic model adopted. The calculated GR_{80} and GR_{90} ranged between 2.7 (population I02E02) and 9.8 g a.i. ha^{-1} (population I02E01), and between 3.2 (I02E02) and 19.2 g a.i. ha^{-1} (I01E01), respectively (Table 3).

A plant biomass reduction higher than 90% was obtained with cyhalofop-butyl applied at 300 g a.i. ha^{-1} (treatment 9) .

The visual assessment of efficacy data were in keeping with the data of plant biomass reduction, even though a lower variability was recorded. Also in this case the rate of 20 g a.i. ha^{-1} gave the highest level of efficacy and no significant improvements were observed at higher rates (Figure 2). Except for rates below 10 g a.i. ha^{-1} no differences between populations were observed.

In all cases, the log-logistic model gave a good fitting of the data visual control. The calculated VGR_{80} and VGR_{90} were very similar to the correspondent GR_{80} and GR_{90}, and ranged between 2.6 (population I02E02) and 12.6 g a.i. ha^{-1} (population I01E01), and between 3.3 (I02E02) and 19.4 g a.i. ha^{-1} (I01E01), respectively (Table 4).

Also in the case of visual control, very high levels of efficacy were obtained with cyhalofop-butyl applied at 300 g a.i. ha^{-1} (treatment 9).

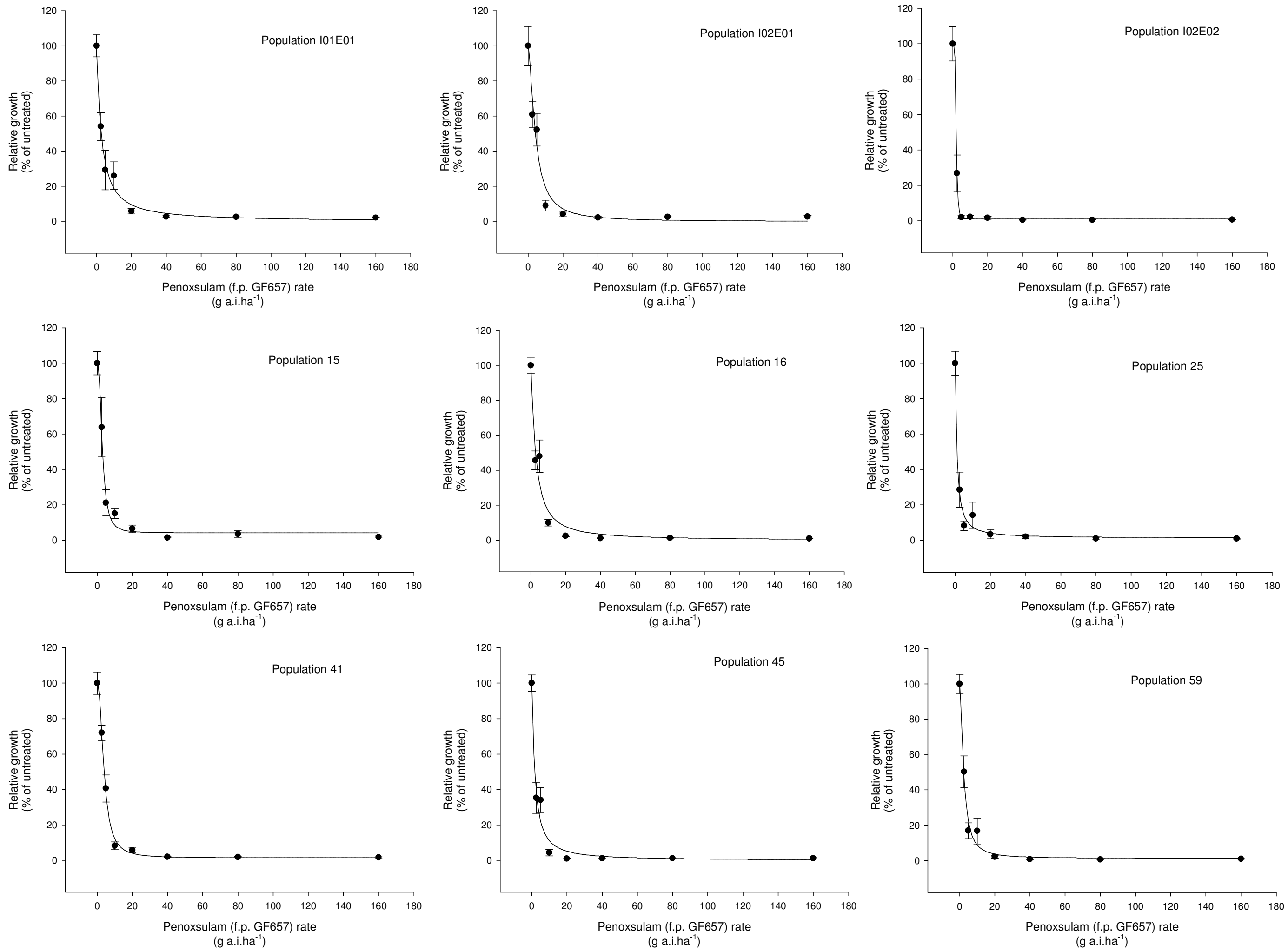

Figure 1 Relative growth (% of fresh weight of untreated plants) as response to growing rates of penoxsulam (GF-657, treatments 1-8). Curves represent the fitted log-logistic model. Error bars are SE.

Table 3 Parameters, adjusted r^2, GR_{80} and GR_{90} of the log-logistic curve describing the relative growth in response to increasing rates of GF-657 (treatments 2-8), and relative growth after treatment with GF-1076 (treatment 9) and cyhalofop + oil (treatment 10).

Treatment	**Formulation**	Population.	Curve parameters				Adj. r^2	GR_{80}	GR_{90}
			c	d	b	g			
2-8	GF-657	I01 E01	0.00	100.0	1.13	2.77	0.83	9.4	19.2
		I02 E01	0.00	100.0	1.64	4.23	0.82	9.8	16.1
		I02 E02	1.13	100.0	5.09	2.04	0.88	2.7	3.2
		15	4.20	100.0	2.52	2.99	0.90	5.7	8.9
		16	0.00	100.0	1.25	2.80	0.94	8.5	16.2
		25	1.32	100.0	1.20	1.04	0.93	3.5	7.3
		41	1.42	100.0	2.23	3.92	0.94	7.6	11.3
		45	0.00	100.0	1.20	1.78	0.89	5.6	11.1
		59	1.24	100.0	1.73	2.37	0.88	5.5	9.2
			Relative growth (%±SE)						
9	Clincher	I01 E01	2.9 ± 0.51						
		I02 E01	2.7 ± 0.51						
		I02 E02	2.8 ± 1.54						
		15	4.7 ± 2.50						
		16	4.9 ± 3.10						
		25	1.4 ± 0.55						
		41	2.3 ± 0.41						
		45	1.3 ± 0.26						
		59	1.6 ± 0.28						

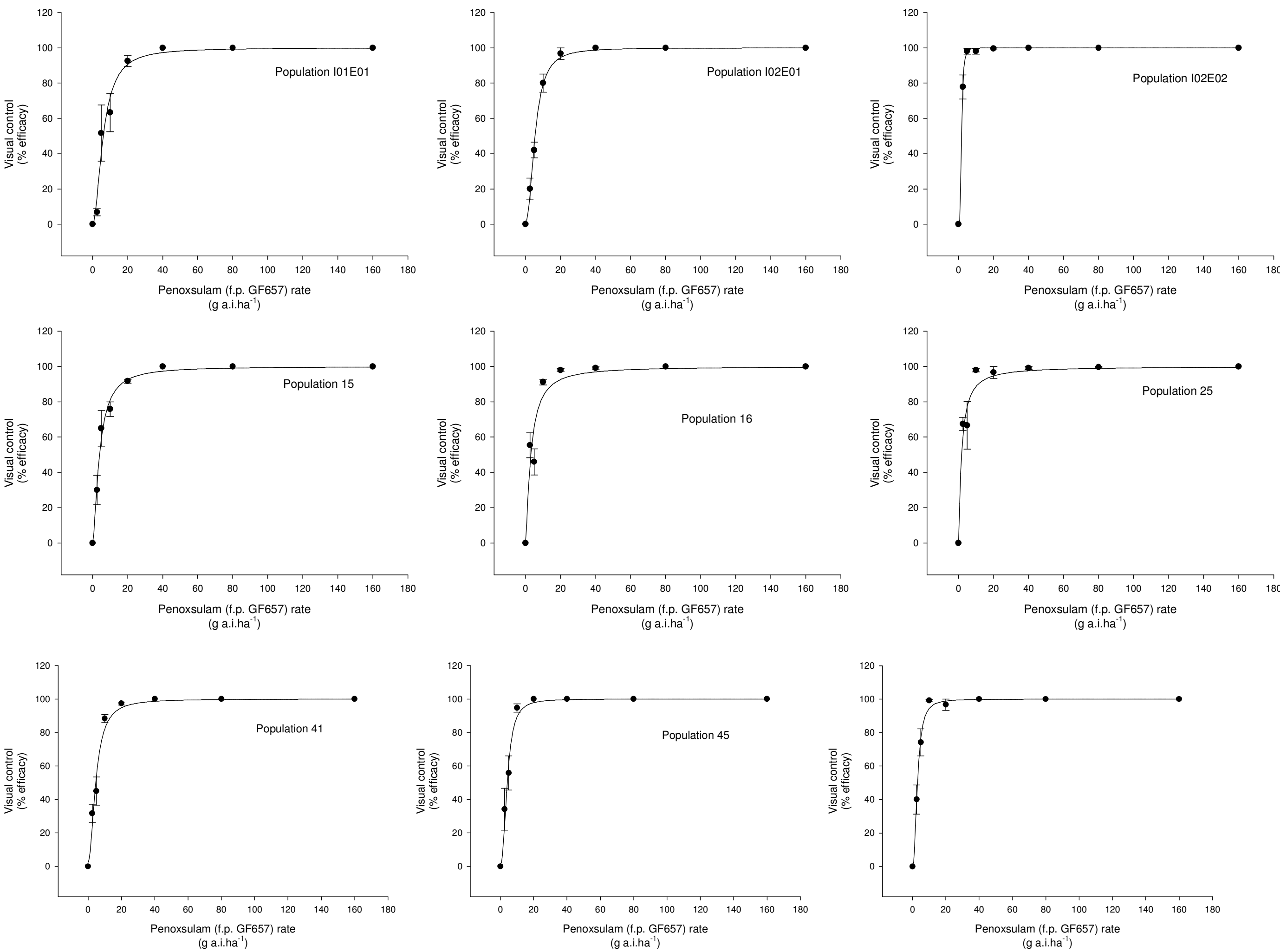

Figure 2 Visual control (% of efficacy) as response to growing rates of penoxsulam (GF-657, treatments 1-8). Curves represent the fitted log-logistic model. Error bars are SE.

Table 4 Parameters, adjusted r^2, VGR_{80} and VGR_{90} of the log-logistic curve describing the visual control in response to increasing rates of GF-657 (treatments 2-8), and visual control after treatment with GF-1076 (treatment 9) and cyhalofop + oil (treatment 10).

Treatment	Formulation	Population.	Curve parameters				Adj r^2	VGR_{80}	VGR_{90}
			c	d	b	g			
2-8	GF-657	I01 E01	100.0	0.00	1.90	6.09	0.83	12.6	19.4
		I02 E01	100.0	1.67	2.19	5.51	0.96	10.3	14.9
		I02 E02	100.0	0.00	3.57	1.76	0.97	2.6	3.3
		15	100.0	0.00	1.55	3.95	0.88	9.6	16.2
		16	100.0	1.22	1.35	3.10	0.91	8.6	15.7
		25	100.0	0.24	1.19	1.79	0.86	5.7	11.3
		41	100.0	2.58	1.97	4.70	0.92	9.3	14.1
		45	100.0	1.48	2.41	4.18	0.95	7.4	10.4
		59	100.0	0.21	2.35	3.02	0.91	5.4	7.7
			Visual control (%±SE)						
9	Cyhalofop	I01 E01	98.3 ± 1.67						
		I02 E01	95.0 ± 2.24						
		I02 E02	97.7 ± 1.67						
		15	100.0 ± 0.00						
		16	99.3 ± 0.42						
		25	99.7 ± 0.33						
		41	99.8 ± 0.17						
		45	100.0 ± 0.00						
		59	98.8 ± 0.83						

Conclusions

All tested populations showed to be highly sensitive to penoxsulam, with the highest level of control generally reached already at 20 g a.i. ha^{-1}. In all experiments the log-logistic model used to describe the dose-response behavior fitted significantly both relative growth and visual efficacy data.

The results of the visual assessment of efficacy were, in general, in good keeping with the data of fresh weight measurements.

The calculated rates corresponding to 80% or 90% of biomass reduction or visual efficacy were always below 20 g a.i. ha^{-1} but varied of up to 6-fold between the *Echinochloa* populations.

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DYNAMICS OF WEEDS IN KUBAN RICE FIELDS

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Abstract

Rice cultivation in Krasnodar territory began in 30's of the 20-th century from reclamation of Priazovsky flooded areas. In first decades of rice growing at irrigation engineering systems weed strains were very diverse. The main rice weeds were representatives of *Echinochloa* Beauv., *Alisma* L., *Bolboschoenus* Palla, *Cyperus* L., *Typha* L., *Phragmites* Adans. and *Oryza sativa* (red rice).

In 70-80's in Kuban rice intensification technology was widely used by application of big quantity of fertilizers and chemicals for plant protection. It reduced the biodiversity. Some weeds of local flora totally disappeared from agrophytocoenosis of rice fields (*Cyperus rotundus*, *C. fuscus*, *Najas* L.), some of them decreased their population and areal (*Spargamium polyedrum*, *Alisma lanceolatum*). At the same time, some species of *Polygonum* L., *Scirpus* L., not mentioned among weeds earlier, widely spread in rice fields of the territory.

In 90's economic problems in the country influenced on the development of rice growing. Red rice was widely spread because of breaking the crop rotation and lowering the demands to seed stock quality. Plants of new biotype of red rice (low, non-shattering, deawned) were added to the known biotype (tall, shattered, awned, with long seed dormancy). Red rice control is difficult because its morphological and biological traits resemble the cultivated varieties.

In the late 90's the researchers developed and introduced new nonpesticide technology of rice cultivation and changed the varieties partially. New varieties overcame water layer well at the beginning of growth stage. As a result, *Echinochloa crus-galli* plants, not able to overcome water layer at sprouting stage decreased. At the same time algae of the family Cladophoraceae and *Monochoria korsakowii* became widely spread in all rice growing zone. *Monochoria korsakowii* was brought to Kuban from the Far East, with rice seeds in 1957. At present, it infests 35% of sawn rice areas.

The study of the causes of weeds dissemination and their peculiarities in rice fields is necessary for development of effective means to control them.

Introduction

Rice cultivation in Krasnodar territory began with reclamation of Priazov flooded areas in 30's, 20^{th} century. In first decades of rice growing in irrigation systems there was a wide spectrum of weeds. I.S. Kosenko (1949) noted 144 weed species, growing in rice field. The presence of water layer (15-20 cm) for 100-120 days in rice field creates more difficult ecological conditions than in upland fields. Besides that, rice field is very complicated irrigation system; it creates unusual places for inhabitants in upland fields. This is the reason of original weed species, their ecological diversity and dynamics of rice agricultural phytocoenosis.

Weed Dynamics

The following species were brought from the Far East to Kuban during the first years of rice growing: *Echinochloa phyllopogon* (Stapf.) Koss., *Elatine oryzetorum* Kom., *Lindernia pyxidaria* All. Local weed species, ecologically corresponding to rice, were included into agricultural phytocoenosis. First of all, they are: *Typha* L., *Alisma* L., *Cyperus* L., *Phragmites* Adans. During the Second World War, part of the rice fields in the lower Kuban were abandoned and occupied by Bolboschoenus compactus (Hoffm.) Drob. At present, it is one of the most harmful weeds (Table 1).

Table 1 The main weeds of Kuban rice fields (Kosenko, 1949)

Family	Species
Alismataceae	*Alisma lanceolatum* With.
	Alisma plantago-aquatica L.
Cyperaceae	*Bolboschoenus compactus* (Hoffm.) Drob.
	Cyperus difformis L.
Elatinaceae	*Elatine oryzetorum* Kom.
Marsileaceae	*Marsilea quadrifolia* L.
Poaceae	*Echinochloa coarctata* (Stev.) Kossenko
	Echinochloa crus galli (L.) R. et Sch.
	Echinochloa phyllopogon (Stapf.) Kossenko
	Oryza sativa L.
	Phragmites communis (L.) Trin.
Typhaceae	*Typha latifolia* L.

In 70's – 80's in Kuban intensive rice cultivation technology with fertilizer and chemical application (in a big quantity) was widely used. It decreased the biological diversity. Some species of local flora fully disappeared from agricultural phytocoenosis of rice fields (*Cyperus rotundus* L., *C. fuscus* L., *Najas* L.), and some species decreased the population and area (*Spargamium polyedrum* Asch. et Graebn., *Sagittaria trifolia* L.). At the same time, some species of *Polygonum* L., *Scirpus* L., not mentioned as weeds, were widely spread in rice fields of the territory. In the opinion of G.V. Yefimova and B.A. Kryzhko (1982) wide-leaved water-boggy plants, suppressed by herbicides, were removed by *Scirpus* L. These species also as widely spread *Cyperus difformis* L. have vegetation period up to the half summer under water layer and there is no chemical control of these weeds. *Polygonum* L. is indicator of water regime in check-plots. *Polygonum persicaria* L. grows in water layer not more than 15-20 cm; *Polygonum amphibium* L. grows in the water layer higher than 40 cm (in drainage, along the check edge). *Polygonum hydropiper* L. occupies the intermediate position and grows in check-plots, uneven as the result of bad land leveling.

In 90's the development of rice growing was under influence of economic problem in country. Rice sawn areas decreased two times. It caused the spreading of boggy plants in flooded areas of non-used rice systems and ruderal plants in steppe zone. *Echinochloa* Beauv. and *Bolboschoenus* Palla as formerly prevailed among weeds in rice fields. Also *Alisma plantago-aquatica* L., *Butomus umbellatus* L., *Scirpus supinus* L., *Cyperus difformis* L., *Phragmites* *communis* (L.) Trin. grew very often. *Salvinia natans* (L.) All., *Potamogeton natans* L., *Polygonum amphibium* L. were wide spread at the edges of check-plots and in discharging channels.

End of the 20th century was marked by the addition of phytocoenosis of rice fields by the objects, competitive to rice. We can note new red rice biotypes, algae and *Monochoria korsakowii* Regel et Maack. among them.

Breaking of crop rotation and decrease of demands to sowing material quality caused the wide spreading of red rice forms (*Oryza sativa* L.) in rice fields. Plans of new biotype (low, deawned, non-shattering) were added to the known biotype (high, awned, shattering, with long seed dormancy). Control of new red rice forms is difficult because their morphological and biological forms resemble the cultivated varieties. For example, in 2001 infestation level by new red rice forms of Rapan and Liman rice varieties was 12%, in 2002 after reseeding the level was 35% (Zelenskaya, 2004).

To the late 90's the researchers developed and introduced new non-pesticide technology of rice cultivation and carried out partially change of varieties. New varieties, resistant to several diseases, overcame water layer at the beginning of growth stage very well. It excluded the application of grassy herbicides. As the result of it, the priorities among weeds changed. The quantity of *Echinochloa* plants, non-able to overcome water layer at sprouting stage, decreased. At the same time, such algae as Cladophoraceae family and *Monochoria korsakowii* Regel et Maack. were widely spread in all zones of rice growing.

Cladophoraceae family made the most damage: *Cladophora fracta* (Mull. ex Vahl) Kutz., *Cladophora glomerata* (L.) Kutz., *Pithophora oedogonica* (Mont.) Wittr., *Rhizoclonium hieroglyphicum* (Ag.) Kutz. Water optimum temperature for their growth under Kuban conditions started in April and May. Thanks to early beginning of vegetation period, these algae are more intensively growing in years with cool spring or when there are rice sprouts from deep-water layer. As investigation showed, during increase of water layer in check-plot from 10 to 30 cm, water temperature decreased, quantity of rice sprouts decreased 6 times (up to 23 p/m^2), and algae quantity increased 3,5 times (up to 126 g/m^2, in calculation for dry weight) (G.G. Fanyan et al., 2001). Control of algae family Cladophoraceae in rice fields is difficult because of high viability and non-advisability of the application of agro technical method of temporary water drainage from check-plots during introduction of non-pesticide technology.

Monochoria korsakowii Regel et Maack. was first found in rice fields in Kuban in 1957. The main cause of its appearance is considered to be the seed material, taken from Primorsky region because of total elimination of rice plants in 1956, because of early frosts. Next 15-20 years, in spite of increase of rice areas by flooded area reclamation, intensive spreading of weed was not observed. In 90's the exclusion of grassy herbicides of contact activity and introduction of new method of rice sprouting from water layer of 30 cm influenced on wide spread of this weed. Such agrotechnical method creates favourable conditions for growth and development of *Monochoria korsakowii* Regel et Maack., because the sowing are spare, especially at bad leveling of check-plots. According to the data of E.V. Prikhodko (2002), 17% of rice areas in Krasnodar territory have weak infestation of *Monochoria korsakowii* Regel et Maack. (less than 3 plants/m^2), there are 14% of middle infestation, 6% of strong infestation (more than 7 plants/m^2). To decrease harmful influence of *Monochoria korsakowii* Regel et Maack. on rice plants, it is necessary to combine phytocoenotic, agro technical and chemical methods of weed control.

Conclusions

Weeds are included into agricultural phytocoenosis. They are closely interrelated in genetic correlation with wild and cultural species. Thus, total elimination of weeds is not good. It is necessary to regulate their big quantity in agricultural coenosis at the level, lower than harmful level, taking into consideration the possible maximum safety for environment. Biocoenotic monitoring of weeds in rice field helps to make prognosis of weed species, their spreadiness in the fields, to evaluate possible yield losses and to determine the strategy and tactics of control methods.

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Session 1

AGRONOMY

Abstracts

GENOTYPIC RESPONSE OF RICE CULTIVARS TO DROUGHT STRESS

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Climate global changes and water shortage due to increasing use for human consumption and industry may strongly affect rice cultural systems traditionally grown in permanent submersion. A typical case is rice cultivation in the North-West regions of the Po Valley, in Italy, were almost the complete rice area is located and cultivated with no water supplement limits. In order to explore the genotypic response of Italian rice cultivars to a different water regime, an experimental design was set up considering 16 rice varieties, either cultivated in conventional submergence either with a strongly reduced water regime, considering three water supplements at definite plant stages: tillering, just before heading, and flowering. As a whole, the water supplement was 93,6 m^3 ha^{-1}, having been the water supplement derived from rain of 2.614 m^3 ha^{-1}, for a total supplement of 2.707,6 m^3 ha^{-1}. This value is accounting up to 52% recovery of the loss due to evapotranspiration.
As a mean yield, during 2003, the value of 4,42 t ha^{-1} yield was obtained. Besides this, genotypic differential responses to drought stress were recorded, thus allowing the identification of cultivars which better adapt to the particular water regime. Cv.Panda yielded highest, with 5,48 t ha^{-1} production and 60% intere grain yield to milling; cv. Vialone Nano yielded 5,43 t ha^{-1}, and cv. Koral 5,14 t ha^{-1}. Some selected lines derived from the ISC breeding programmes yielded interesting values, such as Armonia x Giada (5,31 t ha^{-1}), and Upla 61 x Thaibonnet with 5,24 t ha^{-1}.

IMPOSED DROUGHT STRESS ENHANCES THE COMPETITIVE ABILITY OF RICE WITH RICEFIELD BULRUSH

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Field experiments were established at the Rice Research Experiment Station (RRES) in Biggs, California to quantify the efficacy of induced drought stress to reduce the competitive ability of ricefield bulrush (*Schoenoplectus mucronatus* L. [Palla]) in rice. Treatments consisted of mixed communities of bulrush and rice that were drained until bulrush necrosis reached 30, 60, or 90%. After the desired level of bulrush necrosis was obtained, plots were re-flooded until rice maturity. Results indicated that the canopy photosynthetic rates (P_c) of both species declined rapidly soon after leaf necrosis was initiated. Bulrush necrosis was generally 3-fold greater than neighboring rice plants among the dry-down treatments, indicating that imposed drought stress had much greater effects on bulrush P_c than that of rice. Consequently, each dry-down treatment effectively reduced the competitive ability of bulrush with rice. However, rice yield declined as drought stress increased. Drought stress to 30% bulrush necrosis resulted in average weed-free rice yields for that location, yet drought stress to 60 or 90% bulrush necrosis resulted in approximately 20% less rice yield. Therefore, imposed drought stress resulting in 30% bulrush necrosis may be adequate to effectively suppress bulrush growth and prevent excessive rice yield loss due to drought stress.

EFFECT OF FRACTIONATED NITROGEN FERTILIZATION ON RICE YIELD

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Nitrogen fertilization and NUE (Nitrogen Use Efficiency) are key factors during the rice cultural cycle in determining yield. Moreover, it is well know that, due to the particular cultural conditions in paddy fields, N availability is greatly reduced due to various negative transformations (denitrification, NH_3 production, leackage) strongly affecting the plant response and causing environmental concern. The optimization of N fertilization practices are of great value in integrated crop management; namely, NUE seems to be positively influenced when fractionation of N fertilization during the plant life cycle is performed. Four Italian cultivars (Roma, Koral, Perla and Thaibonnet) were subjected to an experimental design evaluating the effect of N fertilization with Urea (46%) in four dosages (55; 100; 150 e 200 kg N ha^{-1}) against non-fertilized counterparts either as complete dosage before sowing, either as two fractions, one before sowing and the rest just before the heading stage. Results obtained indicated that higher yield (8,36 t ha^{-1}) was obtained when the lowest dosage (55 kg ha^{-1}) was distributed completely before heading. Slightly lower - but statistically not different - yield levels (8,27 e 8,18 t ha^{-1}), were obtained with fractionated N supplement with combinations of 55 + 55 kg N ha^{-1} and 100 + 55 kg N ha^{-1}, respectively. Complete N fertilization supplemented before sowing was, on the other had, yielding the lowest values, for all cultivars.

LUNDBERG FAMILY FARMS: CALIFORNIA PIONEERS IN SUSTAINABLE AND ORGANIC RICE FARMING

Lundberg J

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Lundberg Family Farms is a family-owned and operated business that is committed to producing the finest quality rice and rice products. The Lundberg family has farmed in Northern California since 1937. The four founding Lundberg brothers (Eldon, Wendell, Harlan, and Homer) were deeply influenced by their late father, Albert. Albert and his wife, Frances, moved to California from Nebraska with their four young sons during the Dust Bowl years. Albert believed in ecological farming long before it was fashionable. His wish was to leave the soil in better condition than he found it. In addition to the family farm, the operation consists of a dryer, mill, rice cake production, packaged entrée production, storage and other facilities in Richvale, California. Today the Lundbergs package their rice themselves in over 100 different products and sell directly under the family name. These products range from whole grain rices and gourmet blends to rice cakes and rice chips. About 140 individuals are employed in support of the operation. Lundberg Family Farms utilizes both Nutra-Farmed and certified organic methods in growing rice. Products are certified organic by U.S.D.A. and international organizations including CCOF, OCIA, OMIC, and Skal International.

TOXIGENIC AND GENETIC CHARACTERIZATION OF *FUSARIUM* SPECIES ASSOCIATED WITH RICE IN SARDINIA

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Rice plants are often contaminated by toxigenic fungal species belonging to *Fusarium* genus, among which those belonging to *Liseola* and *Elegans* section are often predominant. The identification of species within these two sections is difficult and their taxonomy has been recently revisited. Moreover, the range of toxins that could be produced by these species is wide and their toxic effects cause serious diseases toward humans and animals. Each species possess a specific toxigenic profile and therefore the correct identification of the main *Fusarium* species isolated from rice is important to elucidate the toxicological risks related to the *Fusarium* contamination.

We report here the toxin production and the genetic characterization of strains belonging to the main species isolated from rice plants in several fields cultivated in Sardinia. In particular, the production of fumonisins, beauvericin, enniatins and fusaproliferin by *F. verticillioides*, *F. proliferatum*, *F. nygamai*, *F. fujikuroi*, and *F. oxysporum* has been investigated. Moreover, mating populations of strains isolated by performing *in vitro* fertility tests has been assessed, showing that a high ratio of fertility among the strains analyzed. Finally, AFLP analysis and calmodulin gene sequences were performed in order to develop molecular tools for the identification of the different species.

EFFECT OF CALCIUM SILICON SLAG ON YIELD, DISEASE RESPONSE AND SILICON CONCENTRATION IN RICE IN ITALY

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Rice is a silicon (Si) accumulator and the plant benefits from Si nutrition. Silicon application increases rice yield and has direct and indirect beneficial effects on the rice grown.
Different Si sources are available as fertilizer but some characteristics as high content of available Si, low cost and low content of heavy metal reduce the number of products able to be used.
This work was aimed to analyze the effects of calcium silicon slag on rice production and on Brown spot, a disease strictly linked with Akiochi incidence, that is largely diffuse in some Italian sandy soils.
An experimental trial was set up with treatments including no amendment and calcium silicon slag at different rates up to 1 ton/ha
Calcium silicon slag was fully characterized, soil samples were analyzed for Si extractable in water and in 1 M sodium acetate buffered at pH 4. Y leaves, straw and rice husks were analysed for total Si content. Yield, Brown spot disease incidence and were also determined.
The soil and the plant Si content resulted lower than the deficiency limit. So the large incidence of Brown spot can be attributed to Si deficiency. Yield, Si extractable in water and Si content of plants was not increased by the fertilizer addition while Si extractable in sodium acetate increased significantly. Identification of more efficient fertilizers seem to be a priority.

Keywords
rice; silicon; slag

RICE GROWING IN RUSSIA

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Rice in Russia is grown according to scientifically based technologies including large rice irrigation engineering systems with 2.5 - 4.0 ha average area of a rice field and automated flooding. Application of highly productive mechanisms for main technological operations, use of chemicals both for mineral nutrition and disease, pests and weed control are also important features of the system. These technologies provide ecologically safe agrocoenosis and possibility to grow other crops in rice rotation. They are also developed for different variants of pesticide- and herbicide-free programmes.

Application of herbicides in rice growing in Russia began in 1966. Earlier the only used methods of weed control were cultivation and crop rotation. Rice yield in this case reached only 3.5 - 4.0 t/ha, labour expenses reached 160 - 180 man hours/ha, self cost of rice was rather high.

Chemical disease, pest and weed control allowed to increase rice yields to 4.3 - 4.8 t/ha, reduce labour consumption to 60 - 120 man hours/ha. But herbicide application in rice growing in Russia endangers environment. This is due to the fact that irrigation water is taken from water sources and then returned to reservoirs playing an important role in fishing. Herbicides are applied to rice fields flooded after sowing (systemic herbicides) or covered with water layer (contact products). Violation of herbicide application rates results in excess accumulation of chemicals in water reservoirs, causing harm to fish and other water inhabitants.

Existing recommendations are rather strict about parameters and terms of application of pesticides. They also include application of modern pesticides with ultra low volumes. Their observation provides ecologically safe rice growing technologies, safe environment and high quality of white rice.

Further improvement of rice growing should follow the trend of reduced or possible complete refusal to apply pesticides by plane, new methods of soil cultivation, breeding new rice varieties with high competing ability, resistant to pests and diseases.

DECISION OF ECOLOGICAL QUESTIONS IN RICE PRODUCTION

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In Ukraine the rice systems are located basically along the Black sea, that is coastal resort zone, in this connection the special urgency have scientifically are proved systems of agriculture and water, which must provide favorable phytosanitary conditions in ricegrowing zone. In Institute of rice of UAAS the irrigation system with the closed cycle by water distribution and repeated separate use of drainage-escape waters is developed.The author of system is leading scientific employee of Institute of rice Vitaliy Makovskyy. On the area of 450 hectares its skilled sample is entered into action. The construction of this system has allowed deciding the basic questions of ecological reliability. The factor of soil use has increased with 0,81 up to 0,94 in comparison with open rice irrigation systems; efficiency has increased with 0,72-0,96, average irrigation norm gross has decreased with 34,0 up to 14,0 thousand м^3 ha^{-1}.For 10 years of operation of system mineralization of drainage waters was stabilized at a level 0,8-0,10 gram litre^{-1}; salinization of soil in aeration zone and layer 1-2 meters is not revealed; toxicity soil is not marked. The level of profitability of rice production on system has made 113,9 % against 72 % on open system.

Keywords
ecology; irrigation system; water.

ECHINOCHLOA POLYSTACHYA MANAGEMENT IN LOUISIANA RICE

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Echinochloa polystachya (Kunth) Hitchc. is a perennial grass native to South America that has been found in Florida, Louisiana, Texas, and Puerto Rico. Found mainly in the Amazon flood plain, it is well adapted to flooded conditions found in rice. A glasshouse study was established to evaluate emergence with respect to depth of burial of *E. polystachya* vegetative segments. The design was a randomized complete block with four replications. Planting depths evaluated were: 0, 1.3, 2.5, 5, 10, and 20-cm. The glasshouse was maintained at a day-night temperature of 30:25 ± 5 C and relative humidity of 60 ± 10%. Soil was a Commerce silt loam with less than 0.1% organic matter, 80.3% sand, 5.8 % silt, 13.9% clay, and pH 7.0. Emergence was evaluated daily for 28 days. Emergence was defined as a shoot emerging from the soil line. Fifty percent of the *E. polystachya* stem segments planted at 1.3 to 2.5-cm emerged within 28 days; however, segments planted below 10 cm resulted in no emergence. When the non-emerged segments were recovered and replanted at 1.3-cm, 19% of the replanted segments emerged. These results indicate that deep tillage may be used as a management option for *E. polystachya*.

Keywords
Echinochloa polystachya; deep tillage.

Session 2

QUALITY AND NUTRITION

Full papers

ROLE OF AGRONOMIC PRACTICES IN IMPROVING QUALITY AND NUTRITIONAL VALUE OF RICE GRAINS

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Abstract

Rice is one of the major field crops in Egypt not only for the local consumption but also it considers as a very important crop for exportation. Efforts of Egyptian rice breeders can be realized through developing of highly improved rice varieties characterizes with high yield, resistant to pests and diseases. They achieved the high yield per hectare. Grain quality and nutritional value of rice has now become a research priority.

Quality characters and nutritional values of rice grains was found not only controlled by the genetic background of the variety, but also significantly affected by the agronomic characters such as fertilization, date of sowing and harvesting, post harvest treatments and storage conditions. Grain quality of some rice varieties have been studied under different levels of nitrogen and potassium, different, different sowing and harvesting dates, and different water regimes at the Rice Research and Training Center, Sakha, Kafr El-Sheikh, Egypt. Main results of these studies revealed that the different rice cultivars were varied in their grain shape, hulling, milling and head rice%, amylose content, gelatinization temperature, gel consistency, elongation ratio and water absorption ratio and protein content were significantly increased by increasing both nitrogen and potassium levels. Data revealed also that significant differences between short grain and long grain varieties were observed in response to harvesting time. Harvesting long grain must be done 35-40 days after heading. Regarding the effect of irrigation water quality, varieties showed a significant variation, Giza 178 rice cultivar was less affected while, Sakha 102 was the most affected variety. Data showed also that milling output, head rice % and protein content were higher with wider spacing (15 x 30 cm) than that of narrow spacing (15 x 10 cm). Generally grain quality characters were found to be controlled not only by the genetic background but also affected by the different cultural practices.

Introduction

Rice is one of the most important cereal crop in Egypt. It occupies annually about 0.60 million hectares, produces about 6.0 million tons of paddy rice with national average of 9.75 ton/ha. Since the cultivated land area is limited in Egypt and there other summer crops are competed with rice, a lot of efforts have to be done in order to increase the productivity per unit area. This can be reached through developing high yielding varieties with improved cultural practices and good management to weed, insects and diseases control and improve grain quality to meet the requirement of both local consumption and export. The high productivity came from parietal improvement, optimization of cultural practices, integrated weed, best, and diseases management and improved grain quality. Grain quality depends mainly on the growth of the plants through its three growth stages (vegetative, reproductive and repining). Egyptian scientists have been working on developing high yielding varieties and the productivity per unit area reached its maximum. Now the main target for the Egyptian scientist is to develop varieties characterized with high yield and good grain quality and acceptable cooking quality by the Egyptian consumers.

Cooking and eating quality of rice became a research priority and ranked parallel to yield. All efforts were done for the sake of high production with good grain quality to meet the needs of both consumption and export.
The newly released rice varieties (Giza 177, Giza 178, Sakha 101, Sakha 102, Sakha 103 and Sakha 104) mostly have low amylose content (~ 18%) with acceptable cooking and eating quality for local consumers.

Grain quality and cultural practices:
Amylose content, gel consistency, gelatinization temperature and grain elongation are the main factors affecting directly on cooking and eating quality.

Sowing date:
Grain quality characters are found to be very much affected by the cultural practices and fertilization. Significant effect was found due to sowing date on grain protein, protein yield and gel consistency (A. Basuony, 1996). Late sowing produced the highest amount of protein yield while, early sowing produced the lowest protein yield (Table 1). Rice varieties varied in their protein content and protein yield as well as gel consistency.

Table 1 Protein yield, protein content and gel consistency of three rice varieties planted in different dates.

Sowing dates	Variety	Characters		
		Protein yield kg/ha	Protein %	Gel Consistency
May 15th	Giza 176	513.88	5.66	92.60
	Giza 171	458.45	6.03	93.50
	Giza 177	384.53	6.35	92.15
May 30th	Giza 176	368.57	6.22	91.17
	Giza 171	349.50	6.29	88.46
	Giza 177	368.08	6.59	91.21
June 1st	Giza 176	332.48	6.76	91.42
	Giza 171	289.90	6.83	91.84
	Giza 177	321.30	6.69	91.77
LSD 5%		19.25	0.15	1.72

Hulling, milling and head rice percentages of three rice varieties under different sowing dates are presented in Table 2. Data showed that sowing rice in 30th May significantly increased hulling percentage followed by sowing rice in 15th of May. On the other hand late Jun 15th decreased hulling % significantly. This could be attributed mainly to the fact that sowing rice in the proper time all growth stages particularly the vegetative stage takes its time without any overlapping with other stages which found when plants planted late sine the day length decreases so plants forced to shorten negative stage to start the reproductive stage early.

Table 2 Hulling, milling and head rice percentage of some rice varieties as affected by sowing dates.

Sowing dates	Variety	Characters		
		Hulling %	Milling %	Head rice %
May 15th	Giza 176	80.00	72.05	66.61
	Giza 171	80.65	73.51	67.73
	Giza 177	81.43	74.06	68.35
May 30th	Giza 176	80.72	73.10	68.45
	Giza 171	8089	73.90	69.55
	Giza 177	81.66	74.45	66.94
June 1st	Giza 176	79.68	71.76	67.14
	Giza 171	80.15	70.76	66.45
	Giza 177	81.17	74.24	68.97
LSD 5%		0.29	1.17	0.61

Hulling, milling and head rice of some rice varieties as affected by sowing dates are presented in Table 2. Data indicated that both early and late sowing caused a significant reduction in hulling, milling and head rice percentage, and maximum values of these characters were found when rice was planted in May 30th (Basuony, 1996).

Plant Spacing

Hulling , milling , head rice and protein content are presented in Table 3. Data indicated that hulling percentage did not affected by the plant population, while milling output significantly affected by plant spacing since highest milling output was found when plant spacing was 20 x 15 cm. The other spacing either narrow (10 x 15) or wider 30 x 15) gave lower milling output then medium spacing (20 x 15). Data in Table 3 indicated also that head rice percentage and protein content was higher with wider spacing than with narrow or medium spacing. Similar conclusion was reported by El-Gohary (1998) and Abd El-Hamed (2002).

Table 3 Hulling Milling , head rice as protein content as affected by plant spacing.

Plant spacing cm	Quality characters			
	Hulling %	Milling %	Head rice%	Protein %
10 x15	83.88	71.18	62.73	7.70
20 x 15	83.31	72.96	62.94	7.82
30 x 15	83.13	71.14	63.45	7.98
LSD 5%	NS	0.43	0.20	0.10

Harvest date

Hulling, milling, head rice and protein content as affected by different harvest date are presented in Table 4. Data indicated that these quality characters did not show any significant variation due to the harvest date except head rice percentage which decreased significantly when plants were harvested late 40 days after heading. This mainly due to the fact that delaying harvesting increased the exposure period to the sun this in turn increased crakes in the grains so this increases the percent of broken grains. Similar findings were reported by Roy et al., (1989).

Table 4 Some grain quality characters as affected by days after heading.

Harvest date (days after heading)	Quality characters			
	Hulling %	**Milling %**	**Head rice%**	**Protein %**
30	**83.47**	**72.03**	**63.17**	**7.81**
35	**83.52**	**72.11**	**63.45**	**7.83**
40	**83.46**	**72.09**	**62.46**	**7.84**
LSD 5%	**NS**	**NS**	**0.20**	**NS**

Nitrogen levels

Data of hulling, milling, head rice and protein content of Sakha 101 rice variety are presented in Table 5. Data showed that the abovementioned characters are significantly affected by the nitrogen levels up to 192 kg N/ha. The highest values were obtained when nitrogen fertilizer was applied at the rate of 192 kg N/ha with no significant difference between these values and values obtained with 144 kg N/ha, while the lowest values were obtained when no nitrogen fertilizer was applied. These findings are in agreement with those reported by El-Rewainy (1996), Mazid Miah (1998) El-Kady and Abdel Wahab (1999) and El-Rewainy (2002).

Table 5 Hulling, milling, head rice and protein content of Sakha 101 rice variety as affected by nitrogen levels.

Harvest date (days after heading)	Quality characters			
	Hulling %	**Milling %**	**Head rice%**	**Protein %**
0	**81.78**	**69.79**	**60.30**	**7.40**
48	**83.08**	**70.94**	**61.29**	**7.61**
96	**83.78**	**72.03**	**62.59**	**7.78**
144	**84.25**	**72.80**	**64.11**	**7.99**
192	**84.52**	**72.90**	**64.41**	**8.37**
LSD 5%	**0.24**	**0.31**	**0.45**	**0.09**

Source RRTC, 2002.

Data in Table 6 presents the effect of nitrogen levels on hulling % of some rice varieties. Data showed that varieties were differ significantly in hulling percentage. Giza 177 gave the highest value but it was not significantly differ than other varieties except Giza 176. Data showed also that increasing nitrogen level from 0 to 48 significantly increased hulling percentage and the further increase caused significant reduction and there was no significant difference between 96 and 144 kg N/ha.

Table 6 Hulling percentage of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	45	96	144	
Giza 176	**79.68**	**80.18**	**80.60**	**80.07**	**80.13 b**
Giza 177	**82.03**	**82.88**	**82.80**	**82.72**	**82.61 a**
Giza 178	**80.68**	**80.65**	**80.75**	**79.50**	**80.39 ab**
Giza 182	**79.63**	**84.45**	**80.70**	**80.83**	**81.40 ab**
Sakha 101	**80.07**	**80.90**	**81.03**	**82.23**	**81.06 ab**
Sakha 102	**82.3**	**82.65**	**82.50**	**82.52**	**82.43 ab**
Sakha 103	**80.5**	**80.68**	**81.40**	**81.78**	**80.97 ab**
Sakha 104	**80.88**	**85.30**	**81.75**	**80.38**	**82.08 ab**
	80.63 c	**82.21 a**	**81.44 b**	**81.25 b**	

Adapted from Shymaa A.Tantawi (2002)

Data in Table 7 showed significant varietal differences in their milling output. Giza 177 showed higher milling percentage than Giza 176, Giza 178, Giza 182 , and Sakha 101. Data showed also that highest milling out put value was found when nitrogen was applied at the rate of 96 kg N/ha

Table 7. Milling percentage of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	45	96	144	
Giza 176	**69.43**	**69.68**	**70.32**	**68.65**	**69.52 d**
Giza 177	**71.70**	**72.28**	**74.60**	**72.60**	**72.79 a**
Giza 178	**69.38**	**70.43**	**70.15**	**69.20**	**69.79 d**
Giza 182	**69.40**	**70.93**	**71.38**	**69.33**	**70.26 cd**
Sakha 101	**70.88**	**71.10**	**72.10**	**70.90**	**70.99 bcd**
Sakha 102	**70.95**	**72.43**	**74.08**	**72.78**	**72.56 ab**
Sakha 103	**71.22**	**71.33**	**72.90**	**71.85**	**71.83 abc**
Sakha 104	**70.38**	**71.40**	**72.25**	**71.10**	**71.28 a-d**
	70.42 d	**71.70 b**	**72.22 a**	**70.80 c**	

Adapted from Shymaa A.Tantawi (2002)

Followed by 48 kg N/ha while, the higher rate of nitrogen gave less milling than the other two rates. Results indicated that milling out put not only controlled by genetic background but also by the environment such as nitrogen. These finding are found by Gorgy (1995) and El-Kady and Abd El-Wahab (1999).

Table 8. Head rice percentage of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	45	96	144	
Giza 176	**61.05**	**61.90**	**62.90**	**62.28**	**62.03 bc**
Giza 177	**64.50**	**65.58**	**66.15**	**65.47**	**65.43 a**
Giza 178	**59.15**	**61.95**	**63.50**	**62.63**	**61.81 cd**
Giza 182	**60.40**	**60.90**	**61.75**	**56.18**	**59.81 d**
Sakha 101	**64.38**	**63.80**	**63.10**	**63.60**	**63.72 abc**
Sakha 102	**62.50**	**62.90**	**63.50**	**63.15**	**63.01 bc**
Sakha 103	**63.03**	**63.80**	**64.78**	**63.08**	**63.67 abc**
Sakha 104	**61.58**	**63.50**	**66.30**	**65.40**	**64.19 ab**
	62.07 d	**63.04 b**	**64.00 a**	**62.72 c**	

Adapted from Shymaa A.Tantawi (2002)

Data given in Table 8 show that the different rice varieties differed significantly in head rice percentage. Giza 177 significantly produced more head rice than Giza 176, Giza 178, Giza 182 and Sakha 102**.**

Regarding nitrogen levels, highest head rice values was obtained when nitrogen was applied at the rate of 96 kg N/ha followed by 48 kg N/ha and 144 kg N/ha and the lowest value of head rice was obtained when no nitrogen fertilizer was applied. Trend was obtained by Gorgy (1959) and El-Kady and Abd El-Wahab (1999).

Amylose content of some rice varieties are presented in Table 9. Data indicated that amylose content is a genetic character of the variety and it rarely affected by the environment. So data showed significant variation in amylose content among the different varieties. Giza 177 has higher amylose content while, Giza 182 has the lowest amylose content. The difference in amylose content among the varieties attributed mainly to their genetic background. Generally all tested varieties were having amylose content less than 20% and this is one of the major consideration of breeders when they select such variety. The application of nitrogen fertilizer caused significant increase in amylose content but there was no significant difference between the rates from 48 up to 144 kg N/ha. According to William (1958), amylose content is the major factor influencing water absorption and volume expansion during cooking and its affect also on eating quality.

Table 9. Amylose content of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	45	96	144	
Giza 176	**19.63**	**18.85**	**19.73**	**19.60**	**19.45 a**
Giza 177	**19.18**	**19.18**	**19.23**	**19.03**	**19.15 ab**
Giza 178	**17.28**	**17.52**	**17.33**	**17.13**	**17.31 e**
Giza 182	**19.65**	**19.30**	**19.20**	**19.80**	**19.49 a**
Sakha 101	**18.70**	**18.58**	**18.90**	**17.83**	**18.50 bc**
Sakha 102	**16.30**	**17.53**	**17.93**	**18.43**	**17.54 e**
Sakha 103	**17.63**	**18.38**	**18.35**	**18.88**	**18.31 cd**
Sakha 104	**17.15**	**18.25**	**17.48**	**18.08**	**17.74 de**
Mean	**17.19 b**	**18.45 a**	**18.52 a**	**18.59 a**	

Adapted from Shymaa A.Tantawi (2002)

Data in Table 10 present the protein content values of some rice varieties as affected by nitrogen fertilizer levels. Data showed that there was no significant difference in protein content among the different varieties. Generally the Egyptian rice varieties having protein content ranged between 6 to 10% according to the different practices during the growing season..

Concerning the nitrogen levels, the first addition of nitrogen resulted in a significant increase in grain protein content . Increasing nitrogen levels up to 144 kg N/ha increased protein content gradually. However, the differences between 96 and 48 kg N/ha and between 144 and 96 kg N/ha was not significant. Additional nitrogen fertilizer almost always increases the protein content of rice grain. Similar findings were reported by Youssif et al., (1980), El-Kalla et al., (1992), Zhong et al., (1992) Mohamed (1994) and Abou Khadrah et al., (1999). Data concluded also that varieties varied in their response to the application of nitrogen as it clearly shown in Table 10.

Table 10. Protein content of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	48	96	144	
Giza 176	**5.14 j**	**6.38 gh**	**6.27 hi**	**6.98 c**	**6.19 a**
Giza 177	**5.20 j**	**6.15 I**	**6.96 ef**	**7.14 bc**	**6.28 a**
Giza 178	**4.41 m**	**6.64 ef**	**7.13 bc**	**7.27 b**	**6.36 a**
Giza 182	**4.95 k**	**6.22 hi**	**6.46 cd**	**7.08 bc**	**6.30 a**
Sakha 101	**4.05 n**	**6.09 I**	**6.79 fg**	**7.09 bc**	**5.93 a**
Sakha 102	**5.22 j**	**6.24 hi**	**6.63 ef**	**7.03 c**	**6.28 a**
Sakha 103	**4.74 l**	**6.40 gh**	**6.50 fg**	**7.69 a**	**6.33 a**
Sakha 104	**4.81 kl**	**6.18 I**	**6.50 fg**	**7.02 c**	**6.20 a**
Mean	**4.82 c**	**6.29 b**	**6.67 ab**	**7.16 a**	

Adapted from Shymaa A.Tantawi (2002)

Gelatinization temperature as affected by nitrogen levels are presented in Table 11. Data showed that varieties exposed significant variation in their gelatinization temperature. Giza 176, Giza 182, Sakha 101 and Sakha 104 had low gelatinization temperature. On the other hand, Giza 177, Giza 178, Sakha 102 and Sakha 103 had intermediate gelatinization temperature. It is note worthily to mention that the low gelatinization temperature varieties had a soft cores (Little et al., 1958) and require less water and shorter time for cooking.

Data indicated also that there was no significant effect due to the application of nitrogen fertilizer.

Table 11. Gelatinization temperature of some rice varieties as affected by nitrogen levels.

Varieties	Nitrogen levels kg/ha				Mean
	0	45	96	144	
Giza 176	**6.00**	**6.00**	**6.00**	**6.00**	**6.00 ab**
Giza 177	**5.25**	**5.25**	**6.00**	**5.25**	**5.44 b**
Giza 178	**6.00**	**5.75**	**6.00**	**6.00**	**5.94 ab**
Giza 182	**6.75**	**5.75**	**6.00**	**6.75**	**6.31aa**
Sakha 101	**5.75**	**6.25**	**6.25**	**6.00**	**6.06 ab**
Sakha 102	**5.75**	**5.75**	**6.00**	**5.75**	**5.81ab**
Sakha 103	**5.75**	**6.00**	**6.00**	**6.00**	**5.94 ab**
Sakha 104	**5.75**	**6.00**	**6.25**	**6.00**	**6.00 ab**
	5.88ab	**5.84b**	**6.06 a**	**5.97 ab**	

Adapted from Shymaa A. Tantawi (2002)

Data presented in Table 12 showed significant variation between varieties in their gel consistency, Sakha 102 and Sakha 104 gave higher values than other cultivars and the lowest values were found with Giza 176 and Giza 178 rice varieties, while the other varieties showed intermediate values of gel consistency.

Table 12 Gel consistency of some rice varieties as affected by nitrogen levels.

Varieties	**Nitrogen levels kg/ha**				**Mean**
	0	**45**	**96**	**144**	
Giza 176	**96.25**	**97.25**	**86.50**	**87.00**	**91.75 f**
Giza 177	**92.00**	**92.25**	**93.50**	**94.50**	**93.06 d**
Giza 178	**91.50**	**90.25**	**98.25**	**88.50**	**92.13 e**
Giza 182	**91.50**	**96.00**	**95.75**	**95.50**	**94.75 bc**
Sakha 101	**97.75**	**91.75**	**94.25**	**96.75**	**95.13 b**
Sakha 102	**95.25**	**96.25**	**98.75**	**96.75**	**96.75 a**
Sakha 103	**97.25**	**90.75**	**91.50**	**98.25**	**94.44 c**
Sakha 104	**94.50**	**100.00**	**96.00**	**97.00**	**96.88 a**
Mean	**94.50 a**	**94.31 a**	**94.31 a**	**94.31 a**	

Nitrogen application had no significant effect on gel consistency as shown in Table 12. So data concluded that this trait is controlled mainly by the genetic background

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PHYSICO-CHEMICAL CHARACTERIZATION OF VARIETIES OF RICE OF DIFFERENT COOKING AND EATING QUALITIES

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Abstract

To access different Portuguese cooking and eating qualities of rice, thirteen varieties including Indica and Japonica grain types and covering the commercial range, were characterized by protein, amylose contents and pasting parameters. The correspondent starch was isolated and the granules observed in scanning electron microscopy. The cooked rice was evaluated by time of gelatinization and by texture analyser to determine firmness.
Varietals differences in amylose content and pasting properties were detected and related with texture and sensory attributes of cooked rice.

Keywords

Rice quality; amylase; amylograph; texture analyser.

Introduction

Portuguese paddy rice production is about 160 000 tons (ANIA, 2003) with important impact in the economy and environment of the regions (25 500 ha) around the rivers of Mondego, Tagus and Sado.
Rice is very important in the traditional cooking recipes and Portuguese consumers prefer grains that absorb sauce, Japonica variety type "Ariète" prevails (about 95 % of the total production). Per capita consume of 15,2 kg (ANIA, 2003) is the highest value of European Union.
As a consequence, the objectives of the rice breeding programme at Portuguese Agronomic Station are to obtain germplasm with yield and quality potential. Cooking and eating qualities have recently been considered and are largely determined by physico-chemical and rheological properties of rice (Juliano, 1998).
The purpose of this study was to measure the differences of varieties of rice representatives of commercial market, using chemical and rheological analysis and relate these data with Portuguese recipes.

Materials and methods

Eight Portuguese sample varieties of rice (five Japonica types: Ariète, Koral, Zeus, Bravo, Alório, and three Indica: Gládio, Albatroz, Thaibonnet) and five quality eating references (Arborio-Italy; Bahia-Spain, Basmati-India; Jasmin-Thayland; Surinam-Suriname) were analysed.
Grain rice was ground through a Retsch ZM100 sample mill using 0,5mm aperture to produce rice flours for physico-chemical analyses. Protein (%N x 5,95) content was determined by Kjeldhal method with Tecator Kjeltec apparatus. Amylose content was determined on undefatted (AM und.) and defatted (AM def.) rice flour by a colorimetric standard method (ISO/CD 6647-1,2 2004).

The viscosity of flour was evaluated using an amylograph (AACC Method 61-01:1999). Curves were obtained with 10% rice flour, peak (P), end of holding (Vh, hot-paste) and end of cooling (Vc, cold-paste) viscosities, setback (SB, cooled minus peak viscosity) and breakdown (BD, peak minus hot-past viscosity) were parameters calculated in Brabender units.

Starch was extracted (Wang & Wang, 2003), samples were coated with gold and scanning electron micrographs (SEM) were taken at 3500x magnification.

Rice was cooked and time of gelatinization (TG) was evaluated by ISO 14864 (1998).

Firmness was evaluated with a Texture Analyser (Stable Micro Systems, TAHDi) using a modified method derived from U.E. Regulation nº. 2580/88. Cooked rice samples (25 g) were put into an Ottawa Texture Measuring System Cell with a 4,9 x 4,9 cm plunger. Firmness was defined as the average force in kg/cm^2 needed to extrude rice through the cell with perforated base at a crosshead speed of 10 cm/min. For each sample, 3 cooking's were carried out and each analysis was determined in duplicate.

Textural properties were assessed also by sensory analysis (Champagne *et al*, 1999), eight subjects were used and three traditional Portuguese recipes were tested.

Analysis of variance was done (SAS Software Institute, Cary, NC) to detect significant differences in protein, amylose contents and firmness scores, among rice samples. Significantly different means were identified by Duncan's multiple range test at $P<0,05$.

Results and discussion

As is expected and shown in Table 1 the undefatted milled rice gave mean amylose values lower than defatted due to the interference of lipids in the iodine-amylose colour.

Table 1 Mean values of protein, amylose contents, amylograph parameters and firmness for Portuguese sample varieties and references

Variety	PROT (% d.b.)	AM und. (% d.b.)	AM def. (% d.b.)	GT (min)	Vp (BU)	Vh (BU)	Vc (BU)	SB	BD	FIRM (Kg/cm^2)
Ariète	8.5^{f}	18.4^{def}	20.6^{f}	18.8	440	400	740	300	40	1.08^{def}
Koral	8.5^{ef}	18.2^{ef}	20.7^{f}	17.6	370	330	610	240	40	1.01^{f}
Zeus	8.1^{g}	18.2^{ef}	19.5^{g}	16.9	450	380	680	230	70	1.04^{ef}
Bravo	8.0^{g}	18.2^{ef}	20.4^{f}	17.4	500	370	690	190	130	1.02^{f}
Alório	7.7^{h}	19.5^{de}	20.0^{fg}	17.7	450	420	800	350	30	1.18^{cd}
Gládio	9.5^{b}	26.0^{b}	29.7^{b}	19.0	224	224	567	344	0	1.49^{a}
Albatroz	8.4^{f}	19.9^{d}	22.2^{e}	16.8	440	380	770	330	60	1.14^{cde}
Thaibonnet	8.8^{d}	24.6^{bc}	28.0^{c}	20.0	240	240	920	680	0	1.47^{a}
Arbório	8.3^{fg}	17.7^{f}	18.2^{h}	19.0	580	520	960	380	60	1.20^{c}
Bahia	8.8^{de}	19.4^{de}	20.4^{f}	19.6	390	340	780	390	50	1.13^{cdef}
Basmati	10.3^{a}	23.4^{c}	23.4^{d}	14.7	710	670	1080	370	40	1.31^{b}
Jasmin	9.1^{c}	15.0^{g}	17.0^{i}	15.6	850	600	970	120	250	1.18^{cd}
Suriname	9.3^{bc}	30.1^{a}	31.7^{a}	17.6	400	400	1002	602	0	1.49^{a}

PROT=protein content (%d.b.), AM und.= amylose content of undefatted flour (%d.b.), AM def.= amylose content of defatted flour (%d.b.), GT= gelatinization time (min), Vp= peak viscosity (BU), Vh= hot-paste viscosity (BU), Vc= cold-paste viscosity (BU), SB= setback , BD= breakdown, FIRM= Firmness (kg/ cm^2).

The Portuguese Japonica rice samples had no significant differences in amylose contents and firmness scores measured by texture analysis. The Indica varieties exhibit more diversity and "Gládio" had the highest amylose content, excluding the reference rice "Suriname".

The amylograph Brabender viscosities curves from Japonic Portuguese rice samples (Figure 1, "Ariète" sample variety), exhibits moderate pasting peak, moderate decrease in viscosity during prolonged cooking and a high setback or increase in viscosity on cooling due to retrogradation. The Indica varieties "Gládio" and "Thaibonnet" exhibits very low peak viscosities and "Thaibonnet" shows the highest setback, consequently a considerable retrogradation.
Significant (P<0,01) positive correlation between amylose content and setback was obtained (results not presented).

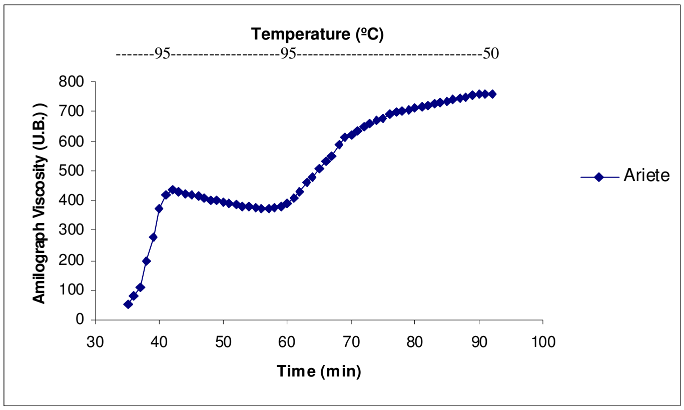

Figure 1 Amylograph Brabender viscosity curve of rice flour from sample variety "Ariète"

Rice starch granules showed polygonal distribution (Figure 2). No obvious morphological differences were observed in the starch granules of different varieties.

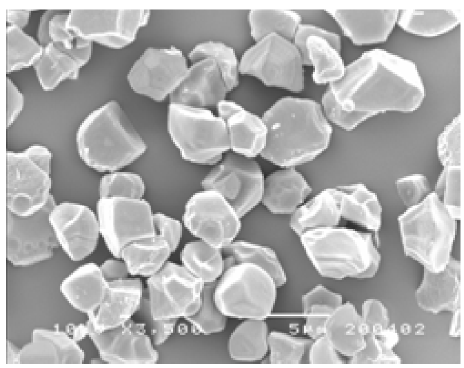

Figure 2 Scanning electron micrograph of rice starch from sample variety "Ariète"

The gelatinization time according ISO 14864 not differentiate the rice samples and is five to six minutes longer than the disable cooking time.
The cooked rice firmness of samples varieties "Gládio", "Thaibonnet" and "Suriname" are no significant different, Japonic varieties shows texture curve similar to the sample variety "Ariète" (Figure 3).

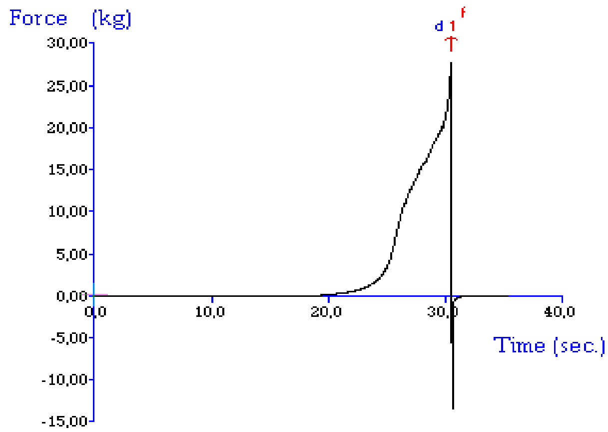

Figure 3 Cooked rice firmness measured by texture analysis of sample variety "Ariète".

No consistent data were obtained by sensory analysis, consequently more accuracy to define the sensory profile is needed with assessors with experience in rice tasting. Portuguese sample variety "Ariète" was selected when compared with the reference Japonic varieties for traditional Portuguese tomato rice recipe. "Alório" rice sample with lower protein content was elected for Portuguese sweet rice. However, for traditional oven duck rice the reference variety "Suriname" was preferred.

Conclusions

Portuguese rice production presents good ability for some traditional recipes but missing varieties with quality for others. So, the knowledge of biochemical basis of the cooking and eating quality is in mind specially the contribution of starch and their genetic control.
The next step could be to define the sensory attributes to discriminate rice types, especially the most indicate for traditional Portuguese cooking.

Acknowledgements

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INFLUENCE OF SOME AGRICULTURAL PRACTICES ON CD CONTENT IN RICE GRAIN

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Abstract
Cadmium is a toxic heavy metals for humans, for animals and for plants. The European limit for its concentration in rice grain is 0.2 mg kg^{-1} (EU Regulation 466/2001), but a lower presence in foodstuff (in baby food especially) is suggested.
Cadmium content in rice grain may be reduced by a specific agronomic management, consisting of i.e. soil dressing, soil amendment and paddy field flooding. A field trial was performed in 2003 to assess the origin of this metal in paddy field (geological, irrigation waters or fertilizers) and the influence of some agricultural practices (rice sowing and soil treatment) on Cd uptake in plants.
Soil pH and Cd (total and extractable amount), soil solution pH and total and Cd, plant and brown rice grain Cd content were monitored during the growing season (6 replicates in each subplot of the experimental split plot). The irrigation water, the used fertilizers and the metal distribution along the soil profile were also investigated.
Results of this study will be very helpful for rice growers and in general for the rice quality and safety.
Keywords
Paddy soil; cadmium; water management; fertilizers

Introduction
Cadmium is known as one of the most toxic heavy metals (Adriano, 2001), without essential function for plants. Hence, while the European limit for its concentration in rice grain is 0.2 mg kg^{-1} (UE Regulation 472/2002), a lower presence in foodstuff –in particular in baby food- is suggested. Cadmium origin in soils may be geological, agricultural (wastewaters, pesticides, fertilizers (Gimeno-Garcia et al., 1996) and sewage sludges utilization (Tichý et al., 1997) or atmospherical. According to Juste and Tauzin (1992) and Kashem and Singh (2001), some agronomic practices, i.e. soil dressing, soil amendment and paddy fields flooding time, may reduce or increase Cd level in grains. In 2003, a field trial was performed in order to evaluate the origin of this metal in paddy field and the influence of some agricultural practices on Cd uptake in plants.

Materials and methods
A field of 2 ha in the locality Rosate, 25 km south-westerly from Milan, Italy, was selected for this study, on account of the brown grain Cd content (0.4 mg kg^{-1}).
The experimental design consisted of a split plot, with two different rice (*Oryza sativa*, var. Baldo, a long-grain rice for risotto) sowing type (dry seeded rice, followed by flooding at

tillering stage, and water seeded rice) in the main plots and four treatments (compost, compost+lime, lime, test) in the subplots.

In each subplot six replicates (Figure 1, grey squares) of soil pH (three times during the experiment), soil solution Cd and pH (twice), plant (three times) and brown grain (subplot means) Cd content were measured. Cd concentration in soil (total and extractable amount), in irrigation water and in used fertilizers were also investigated. In order to evaluate the possible geological origin, the metal distribution along the profile was also analyzed, after removal of the different layers, to a depth of 1.20 meters.

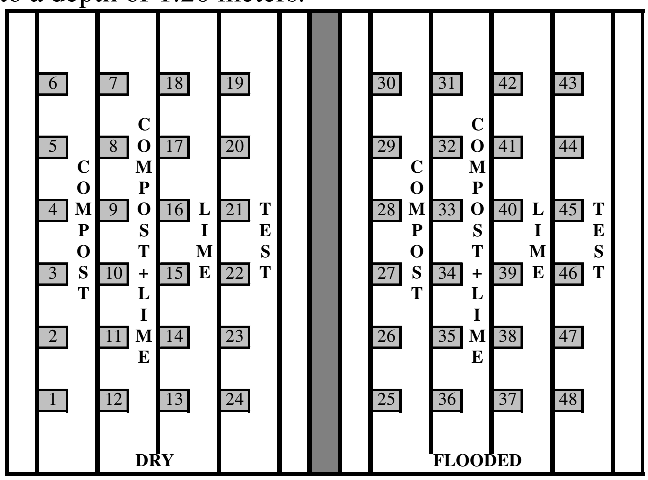

Figure 1 The experimental split plot

Results and discussion

Cd concentration in soil, in irrigation water and in fertilizers are shown in Table 1. Soil characteristics and the metal distribution along the profile are reported in Table 2 and Table 3 respectively.

The contribution of water and fertilizers is negligible. As the cadmium content is often higher in the deeper layers, the geological origin of this element may be seems to be plausible.

Table 1 Cd content in soil, irrigation water and fertilizers (mg kg^{-1}).

Fertilizer	N	Cd (mg kg^{-1})
Compost	1	0.24
Lime	1	<0.31
KCl	1	<0.31
Urea	1	<0.31
Water (mean)	6	<0.0012
Soil (mean)	20	0.96

Table 2 Soil characteristics.

N=20	pH	Total Cd (mg kg^{-1})	DTPA-Cd (mg kg^{-1})	O.M. (%)	C.E.C. (meq/100g)	Silt (%)	Clay (%)	Sand (%)
Mean	6.21	0.96	0.15	2.59	14.04	37.67	11.35	50.98
Std. Dev.	0.16	0.16	0.03	0.19	2.57	10.97	3.24	9.33

Table 3 Cd distribution along the soil profile (means, mg kg^{-1}).

Layer (m)	Flooded	Dry
0 - 0.14	1.13	1.13
0.14 - 0.24	1.19	1.17
0.24 - 0.54	1.57	1.00
0.54 - 0.84	1.43	1.40
0.84 - 1.20	1.16	1.47

The obtained results of plant and brown rice Cd content show a significant dependence ($P<0.01$) on both the sowing technique and the treatments, with lower amount (below the UE trigger limit) in the case of flooded rice and lime application (Table 4 and 5).

Table 4 Comparison of plant and brown rice Cd content resulting from the different treatments.

	Plant Cd (mg kg^{-1})						Brown rice Cd (mg kg^{-1})	
Fertilizer	July		August		September		September	
Compost	0.31	A	0.48	A	0.80	A	0.19	A
Test	0.28	A	0.47	A	0.73	A	0.17	B
Lime	0.16	B	0.33	AB	0.58	B	0.12	BC
Compost/lime	0.16	B	0.30	B	0.47	B	0.09	C

Means with the same letters are not significantly different (P<0.01) according to the Duncan test.

Table 5 Comparison of plant and brown rice Cd content resulting from the different rice sowing.

	Plant Cd (mg kg^{-1})						Brown rice Cd (mg kg^{-1})	
Rice sowing	July		August		September		September	
Dry	0.29	A	0.51	A	0.87	A	0.20	A
Flooded	0.17	B	0.28	B	0.42	B	0.09	B

Means with the same letters are not significantly different (P<0.01) according to the Duncan test.

Compost seems to produce an opposite effect, probably by causing a soil acidification (Table 6 and 7), a dissolved organic carbon (DOC) increase and a soil organic matter solubilization (Antoniadis and Alloway, 2002), resulting in a mobility increase. However, the association of compost with lime attenuates or cancels this phenomenon.

Table 6 and 7 Comparison of soil pH, soil solution pH and Cd content resulting from the different treatments (mean of the dates). Means with the same letters are not significantly different ($P<0.01$) according to the Duncan test.

Table 6 Soil pH

Fertilizer	Soil pH	
Lime	7.47	A
Compost/lime	7.35	AB
Test	7.32	AB
Compost	7.27	B

Table 7 Soil solution pH and Cd content

	Soil solution			
Fertilizer	pH		Cd (μg kg^{-1})	
Compost/lime	6.46	A	3.79	AB
Lime	6.24	B	2.98	B
Compost	6.12	B	3.58	AB
Test	6.08	B	4.22	A

Although soil and soil solution pH demonstrated the effects of the different fertilizers on brown rice content (Table 6-7), the differences were important only in dry condition (Table 8). Table 8 Comparison of soil pH, soil solution pH (mean of the dates) and brown rice Cd content resulting from the interaction treatment x rice sowing.

Fertilizer x rice sowing	Soil pH		Soil solution pH		Brown rice Cd (mg kg^{-1})	
Compost x dry	7.28	B	6.06	C	0.35	A
Test x dry	7.37	AB	6.03	C	0.31	B
Lime x dry	7.51	A	6.24	BC	0.23	C
Compost/lime x dry	7.43	AB	6.40	AB	0.21	C
Compost x flooded	7.26	B	6.18	BC	0.13	D
Test x flooded	7.28	B	6.13	C	0.11	D
Compost/lime x flooded	7.28	B	6.51	A	0.11	D
Lime x flooded	7.44	AB	6.25	BC	0.10	D

Means with the same letters are not significantly different (P<0.01) according to the Duncan test.

Conclusions

According to our results and to many authors (Adriano et al., 2001, Basta et al., 2001, Bolan et al., 2003), liming and flooding are no doubt positively effective on Cd uptake by rice plants. The low solubility of this metal in the reduced soils is explained by the presence of sulphides (sulphate ions reduction, complexation and immobilization as sulphide salt (Van Den Bergh et al., 1998)), while in the oxidized soils is explained by high pH and organic matter content (Tack et al., 1996). The compost effect on Cd mobility (acidification, dissolved organic carbon (DOC) increase and soil organic matter solubilization (Antoniadis and Alloway, 2002)), need to be further investigated. The atmospherical supply of this element need to be measured too, in order to confirm the geological origin.

Differently from what is reported in bibliography (Kusaka et al., 1972), we found that the subplot mean brown rice Cd content is still affected by paddy field conditions (e.g. flooding) during the vegetative stage of the cultivation. The measurement of the real biovailable concentration of Cd in soil solution will enable us to understand better this phenomenon, as well as the observed interactions with rice sowing and treatment.

As the irrigation water and fertilizers Cd concentrations are lower than the analytical detectable limits, we can state that soil contents below 1 mg kg^{-1} are already dangerous for consumer health.

Results of this study will be very helpful to create some guidelines for rice growers, in order to improve the European rice quality and safety.

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METABOLIC PROFILING OF ITALIAN AROMATIC RICE VARIETIES

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Abstract

Aromatic rice varieties are very popular in Asia and have recently gained wider acceptance in Europe and in the U.S.A. Because of high prices in rice markets, methods for the detection of adulteration of aromatic rice by other long-grain varieties are desirable. Additionally, rapid methods focused on aromatic rice quality are needed to develop new aromatic rice cultivars. Conventionally aroma constituents of rice are extracted by steam distillation, concurrent-distillation, supercritical extraction, extracts are concentrated and compounds identified by GC-MS analysis.

This study describes results obtained using a Headspace-SPME/GC-MS method. In a previous paper this technique has been applied to quantify 2-acetyl-1-pyrroline, a key odorant in fragrant rice. The aim of this work was to evaluate and compare the total aroma composition of different rice cultivars. Results obtained show that volatile fraction is composed of aldehydes, alcohols, hydrocarbons, ketones, heterocycles, phenols, terpenes; 2-acetyl-1-pyrroline is present just in fragrant rice cultivars. Headspace-SPME/GC-MS gives data comparable to data obtained with conventional extraction methods but produces samples with high compound concentration, low level contamination and shorter sample preparation time.

Keywords

Solid phase microextraction (SPME); Italian rice; volatiles; 2-acetyl-1-pyrroline; headspace; gas-chromatography-mass spectrometry.

Introduction

Scented rices (*Oryza sativa* L.), characterised by an aroma flavour similar to popcorn, characteristically appreciated in India, Pakistan and the Middle East, are becoming more and more popular in the U.S.A. and Europe. Recent evaluations at the level of European market (data Ente Nazionale Risi, Italy, 2003), have highlighted the dramatic import of scented rices, namely from India and Pakistan, by member states of North Europe, such as The Netherlands, Sweden, Germany, Belgium and, at a greater extent, United Kingdom. The European import of aromatic rices – namely Basmati – is thought to be increased in such a way due to the new ethnic market and to an increasingly discriminating demand of the consumers. Market and therefore consumers are, in fact, becoming more and more discriminating and, as a result, sensory quality has become an important issue. Most rice breeding programmes in U.S.A. and Europe now incorporate sensory evaluations of breeding lines into their evaluation protocols.

More than hundred volatile compounds contribute to the aroma of scented rice (Tsugita 1986; Widjaja et al, 1996): some of them are determinants to constitute the real appreciated aroma, whereas others are responsible of the opposite effect and are detrimental to the rice quality, thus determining consumer rejection. Moreover, the aroma of a given variety appears to be conditioned by the influence of pedoclimatic situations and agronomical practices. It is therefore of major importance for the breeder, the acquisition of analytic methodologies providing a valuable tool for the evaluation of rice lines under selection. This will contribute to the acquisition of a trait which represents an added value for the European varieties which, in this way, can compete in the world rice trade.

The aromatic compound 2-acetyl-1-pyrroline (2-AP) is the primary component responsible of the rice aroma (Buttery et al., 1983, 1988; Bergman et al., 2000), whereas the aldehyde hexanal has been indicated to be the major responsible of the negative smell and product rejection. A detailed study on the volatile compounds of Italian aromatic rice lines derived from breeding programmes and Basmati have confirmed the presence of 2-AP in the scented lines and a vast array of volatile compounds (Tava and Bocchi, 1999), and a more recent paper (Bergman et al., 2000) has afforded the problem of 2-AP quantification in order to discriminate between the scented and non-scented rices. Because of their nature, volatile compounds that elicit aroma are easily degraded and standard methods of extraction and analysis have to be properly set up in order not to disrupt their integrity and give to the researcher the possibility to perform an accurate quantification. Recent advances in analytical instrumentation and methodology have approached the threshold of selectivity and sensitivity demonstrated by the human nose. The methodology includes a solid phase microextraction (SPME) for collecting and concentrates the compounds in the headspace of rice analysis, coupled with GC/MS (Grimm et al., 2001). The new technology has proven to be a rapid, simple and sensitive method for the scented rice aroma analysis.
The present research is aimed at the characterisation of the volatile metabolic profile of the Italian varieties of aromatic rices, for their evaluation against the conventional scented rices such as Basmati, and to provide a specific “metabolic profile” for their valorisation on the marketplace.

Materials and methods
Five aromatic Italian rice cultivars, belonging to the Long B grain type were analysed (Table 1). Their metabolic volatile profile was compared to the non-aromatic Long B type rice Gladio.
Each rice sample was analysed before and after milling, and therefore the metabolic volatile profiles were compared at the brown and white rice stages. All the samples were stored at 0-4°C before analysis.

Table1 Italian rice cultivars analysed.

Cultivar	type
APOLLO	Long B aromatic
ASIA	Long B aromatic
FRAGRANCE	Long B aromatic
GANGE	Long B aromatic
GIANO	Long B aromatic
GLADIO	Long B non-aromatic, control

Samples were preparared placing 1.5 g of rice directly into a 20 ml vial. Water was added to the sample by spraying 500µl of water (HPLC grade Riedel-de-Haen) onto the top of the rice kernels. Vials were placed in the autosampler tray (CombiPAL CTC Analytics SPME autosampler) maintained at room temperature until analyzed and preheated at 80°C for 25 min prior to sampling. Collection of volatile compounds in the headspace was accomplished using a 15 min adsorption period at 80° C. The selected volatile compounds were extracted using the 50/30 µm divinylbenzene/carboxen on poly(dimethylsiloxane) StableFlex fiber

(DVB/CAR/PDMS Supelco) which showed the best efficiency in extraction compared to other commercially available tools.
Compounds were desorbed for 5 min. on a DSQ-TRACE GC-MS system. The injector temperature was held constant at 270°C and maintained in the splitless mode for 5 min. The GC oven temperature was held for 1 min. at 50°C and then ramped to 250°C at 10°C/min. A 30 m, 0.25µm, RTX5-MS capillary column was used with helium as the carrier gas under a constant flow of 1.2 ml/min. The mass spectrometer was operated in full scan mode from 35 to 350 Da. At the end of desorption phase the fiber was kept at 250°C for 15 min for cleaning.
Compounds were identified by comparing their mass spectra (NIST and PBM algorithms) with the mass spectral data of the standard compounds in the NIST02, Wiley7 and Adams libraries (Adams 1989).

Results and discussion

The olfactory and sensory sensations perceived from volatile molecules are a difficult issue to handle with analytical measurements. Research correlating analytical and sensory measurements is limited for different reasons: conventional volatiles isolation methods (steam distillation, concurrent-distillation, supercritical extraction) are solvent and time consuming, no single compound could be judged as responsible for any observed rice aroma then number of compounds to be quantified is considerable. The solid-phase micro-extraction (SPME), developed by Pawliszyn in the past decade (Belardi et al 1989; Arthur et al. 1990; Zhang et al. 1993), is a rapid, direct, inexpensive and efficient technique for sampling liquid or gaseous matrixes. SPME is a multi-analyte extraction technique that requires no solvents and provides linear results over a wide range of analytical concentration in a large range of matrices.
The aim of this work was to evaluate and compare the total aroma composition of different rice cultivars by a SPME-GC/MS method. Chromatograms obtained are shown in Figure 1 and results are summarized in Table 2.

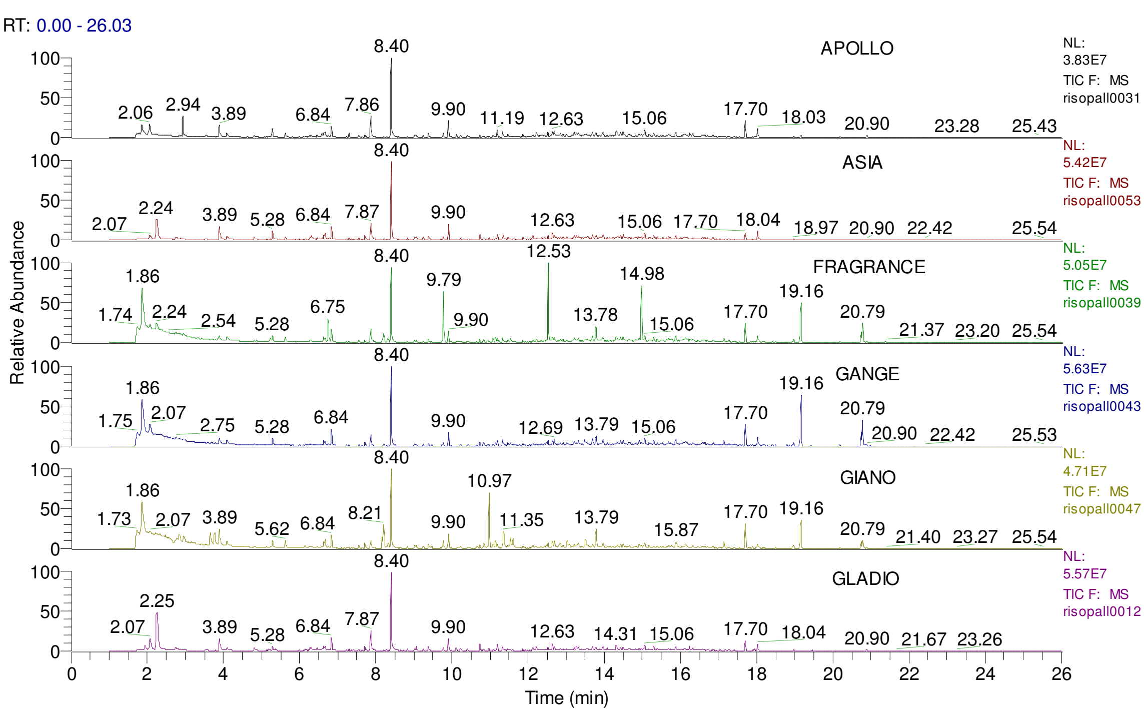

Figure 1 SPME/GC-MS TIC profiles of Apollo, Asia, Fragrance, Gange, Giano and Gladio cultivars.

Table 2 Volatile compounds in rice.

Compounds	Rt
Aldehydes	
Hexanal	3.89
Heptanal	5.29
2-Heptenal (E)	6.14
Benzaldehyde	6.27
Octanal	6.84
Benzene acetaldehyde	7.56
2-Octenal	7.71
Nonanal	8.40
2-Nonenal, (E)-	9.25
Decanal	9.90
2,4-Nonadienal, (E,E)-	10.07
2-Decenal, (E)-	10.72
Undecanal	11.33
2-Undecenal	12.13
Alcohols	
Ethanol	1.86
1-Pentanol	3.51
1-Hexanol	4.81
Ethanol, 2-butoxy	5.36
Heptanol	6.31
1-Octen-3-ol	6.46
1-Octanol	7.87
2-Nonanol	8.33
Phenylethyl Alcohol	8.63
Hydrocarbons	
Hexane	2.25
Toluene	3.57
p- Xilene	4.87
Decane	6.75
Dodecane	9.78
Tridecane	11.19
Tetradecane	12.52
Pentadecane	13.79
Ketones	
Acetone	1.94
2-Heptanone	5.13
5-Hepten-2-one, 6-methyl	6.59
3-Octen-2-one	7.41
Acetophenone	7.93
2-Nonanone	8.21
2-Decanone	9.70
2-Undecanone	11.14
2-Tridecanone	13.78
2-Pentadecanone, 6,10,14-trimethyl-	17.70
Heterocyclic compounds	
Pyridine	3.43
2-Acetyl-1-pyrroline	5.63
Furan-2-pentyl	6.66
Indole	11.31
Phenols	
Phenol, 2-(1-methylpropyl)-	10.96
4-vynilguaiacoloinylphenol	11.54
Terpenes	
Limonene	7.30

Aldehydes, both saturated and unsaturated, are the most abundant class of compounds, the major peak in brown rices chromatograms is nonanal, reported as having a characteristic floral-fruity aroma (Widjaja et al.1996).

Alcohols seem to be more abundant in white than in brown rices, 1-octanol and 1-nonanol (fruity-flavour aroma (Widjaja et al.1996)) are most abundant components of this class of compounds. Polished white rice of cv.Fragrance and especially cv. Giano white rice, are characterized by the presence of 2-nonanol.

Hydrocarbons, that are components of rice grain waxes, are presents in particular in cv.Fragrance brown rice. However, data in literature do not ascribe any action in the resultant arome due to this class of compounds. Polshed white rice, derived from all cultivars, appears rich in ketones: Fragrance, Gange, Giano and Asia white rices show 2-nonanone, responsible of the fruity-herbaceous aroma (Widjaja et al.1996)) as major compounds instead of nonanal. Another significant difference, very likely involved in the cultivar specific aroma, is that Fragrance, Gange, Giano and Asia contain 4-vinylguaiacol reported as having a smoky unpleasant aroma (Widjaja et al.1996).

Among the heterocyclic compounds, as expected, 2-acetyl-pyrroline, the major responsible of popcorn-like aroma, is present only in aromatic rices and its concentration is comparable in brown and white rices, thus assuring its presence at significant levels also in the endosperm. Considering that the original aromatic rices such as Basmati, Jasmine and others, are true ssp.*indica*, typically grown in the tropical and sub-tropical areas of the world, it is of major importance the evaluation and characterisation of the volatile aromatic compounds of the Italian varieties which belong to the true ssp. *japonica* and therefore suitable for cultivation in the temperate areas.

Comparing the chromatographyc profiles obtained from white and brown rices of the same variety, it was clear that that brown rices are generally richer in nonanal and ester of palmitic acid: these compounds are metabolites derived from the oxidation of lipids, that are concentrated in the aleurone layer, commonly removed during milling. The presence of phthalates and antioxidants such as 2-(1-methylpropyl) phenol in white rices could be attributed to contamination during milling.

Conclusions

SPME/GC-MS provides an excellent methodology for the qualitative determination of volatile aroma in scented rice, and further investigation is underway to quantify the volatiles compounds identified during this preliminary study. Scented rice aroma are provided by several molecules, which all together contribute to the specific scent of a given genotype. Moreover, the nature and the composition of the aroma appears to be under the influence of several factors non directly genetically determined such as: the pedoclimatic conditions of the region where the rice is grown, the culture management, the soil texture and composition, the type of nitrogen fertilisation and so on. In order to take into account as many parameters as possible for each variety, a great number of sampling is generally needed, and therefore low analysis costs and short analysis times are required. SPME/GC-MS method a is multi-analyte extraction technique that requires no solvents and provides linear results over a wide range of analyte concentration in a large range of matrixes. It is rapid, direct, inexpensive and efficient, all characteristics are indispensable for the evaluation of a large number of samples. In order to render this analytical methodology suitable for routine analysis and with a real efficiency, powerful statistical softwares should be made available. They would allow to accumulate and integrate the huge number of values relative to the nature and the composition of the main aroma constituents, and to reduce the multivariate data obtained into a two or three-dimensional system simple enough for display on graphs.

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COMPARATIVE CHARACTERISTIC OF GROATS QUALITY OF NEW RICE VARIETIES

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Abstract

The practice of world rice-growing testifies that today it is necessary to production new varieties with high ready production competitiveness in the consumer market. It is possible to solve this task only by creating and introduction in production new high-yielding varieties of rice of domestic plant breeding with high parameters of quality of grain and groats. In the Institute of rice of the Ukrainian academy of agrarian sciences the new varieties of rice adequate these created requirements.

In comparison with the standards existing in Ukraine, the parameters of chemical structure and food value of rice groats of varieties Zubets and Slavutich and groats of rice imported from Egypt are determined.

Under the contents of proteins, lipids and vitamins the domestic varieties do not concede to import rice; the quantity monosaccharides and disaccharides exceeds parameters characteristic for rice groats. The power value of groats of domestic selection exceeds meanings of average sizes on 24 %, and at the Egyptian rice - on 23 %. Samples of rice groats differ from the standard under the contents essential amino acids on 103-104 %.

Thus, the groats of new of rice created on Ukraine of food and biological value does not concede to groats of import rice and standards of rice groats, and according to some parameters that exceed them, for example, contents of proteins, including essential amino acids, monosaccharides and disaccharides exceeds these parameters.

Keywords

rice; varieties; groats; quality.

The practice of world ricegrowing testifies that today it is necessary to production new varieties with high ready production competitiveness in the consumer market. It is possible to solve this task only by creating and introduction in production new high-yielding varieties of rice of domestic plant breeding with high parameters of quality of grain and groats.

For the producer of rice the quality is a high productivity, simultaneity of maturing, complex resistance to the pests, diseases and lodging, good responsiveness on fertilizers, ease thrashing. For purveyor the quality is moisture, contents of weed and grain impurity, red grains, grains with yellowed endosperm. For produce processer quality are important technological parameters of traits of quality, such, as filminess, translucency, cracking, sizes and form of a grain, husking factor, that is the parameters, on which define a total milled rice and it variety structure. For the consumer of rice quality are such advantages of groats, as colour, consistence of endosperm, form of a grain, culinary properties (colour, taste, boiled rice consistence, boiling to pulp). The large importance there is a food value of rice described by the contents of proteins, separate essential amino acids, fat acids, mineral compounds, vitamins and other substances determining as common calorie content of rice, and ease of its mastering.

Last years Ukrainian ricegrowing has replenished new high-yielding and high-quality varieties of rice, such as, Ukraine - 96, Slavutich, Antey, Zubets, Memory of Gichkin. These varieties have high translucency 92-98 %, low cracking 4-10 %, high total milled rice 68-69 % and head rice yield 90-92 %. Table 1 shows the qualitative indexes of a rice grain.

Table 1 Quality of a grain of new rice varieties

Variety	1000 grains weight	Trans-lucency %	Cracking %	Total milled rice %	Head rice yield %	L/b
Slavutich	31.5	96	4.0	67.7	91.3	1.9
Ukraine - 96	30.0	98.0	4.0	69.6	95.1	2.0
Antey	29.5	98.0	10.0	69.1	92.8	2.0
Memory of Gichkin	32.5	96.0	10.0	68.4	93.0	2.0
Zubets	29.5	98.0	6.0	69.0	94.2	2.0

The basic traits of groat qualities of rice grain have variety character, though can change depending on conditions of growing, ways of harvesting, storage and processing. Contents of total milled rice and head rice yield are complex parameters of technological qualities of rice. Head rice yield at these varieties is provided at the expense of high translucency and low cracking of a grain and due to high 1000 grains weight, the groats has a good packaging.
In Institute of rice together with the Ukrainian Food Research Institute (Kiev) in comparison with the standards existing in Ukraine, the parameters of chemical structure and food value of rice groats of varieties Zubets and Slavutich and groats of rice imported from Egypt are determined.

Table 2 Qualitative indices of rice groats

Indices	Samples of rice groat		
	Zubets	Slavutich	Egyptian rice
Proteins, %	6.3	7.2	7.3
Essential amino acids, mg	2250	2571	2607
Valine	378	432	438
Isoleucine	297	339	344
Leucine	558	638	647
Lysine	234	267	271
Methionine	144	165	167
Treonine	216	247	250
Tryptophan	90	103	104
Phenylalanine	333	380	386
Lipids, %	0.019	0.023	0.022
Carbohydrates, % :			
Monosaccharides and disaccharides	3.68	3.35	3.01
Starch	90.3	89.8	89.0
Vitamins, mg:			
B1	0.08	0.08	0.08
B2	0.04	0.04	0.04
PP	1.60	1.60	1.60
Calorie content, Cal	410	410	406
α-amylases complex activity, sec	443	444	447

The contents of proteins in groats of a variety Zubets makes 90 %, in a variety Slavutich - 103 %, in the Egyptian rice 104 %. The contents of lipids in the given samples makes 2 % from average sizes. The quantity of simple carbohydrates exceeds parameters, characteristic for rice groats. So, in a variety Zubets it makes 3,68 g/100 g of a product, that in 5,3 is higher average parameters (Table 2). Concerning other samples, it is necessary to note, that the contents of monosaccharides and disaccharides was little bit lower. The given literatures testify, that the contents of the specified parameter happens much above (grain in a film, g/100g of a product - 0,1-4,5; the hulled rice - 2,1-4,8).
The quantity of starch in the submitted samples has no a statistical divergence, however is little bit higher than average parameters. The content of vitamins at the submitted samples does not differ from parameters of average sizes for rice groats. The power value differs from meaning of average sizes. So, at groats of varieties Zubets and Slavutich it exceeds meanings of average sizes on 24 %, at the Egyptian rice - on 23 %.
In Table 3 the contents of the basic nutritious compounds and calorie content of some groats and samples of rice groats of varieties Zubets and Slavutich and groats imported from Egypt is given.

Table3. Contents of the basic nutritious compounds and calorie content of some groats in 100g of a product

Groats	Proteins g	Lipids g	Mono- and disaccharides g	Starch g	Calorie content, Cal	Vitamins mg		
						B1	B2	PP
Buckwheat	12.6	3.3	1.4	60.7	335	0.43	0.20	4.19
Oatmeal	11.0	6.1	0.9	48.8	303	0.49	0.11	1.10
Millet	11.5	3.3	1.7	64.8	348	0.42	0.04	1.55
Pearl-barley	9.3	1.1	0.9	65.6	320	0.12	0.06	2.00
Rice	7.0	1.0	0.7	70.7	330	0.08	0.04	1.60
Samples of rice groat								
Zubets	6.3	0.019	3.68	90.3	410	0.08	0.04	1.60ù
Slavutich	7.2	0.023	3.35	89.8	410	0.08	004	1.60
Egyptian rice	7.3	0.022	3.01	89.0	406	0.08	0.04	1.60

In Table 4 the contents of essential amino acids in some groats with samples of rice groats are given.

Table 4 Contents of essential amino acids in some groats with samples of rice groats in 100g of a product.

Indices	Groats				Samples of rice groats		
	Millet	Buckwheat	Oatmeal	Rice	Variety Zubets	Variety Slavutich	Egyptian rice
Essential amino acids	4228	3817	3151	2500	2250	2571	2607
Valine	470	590	473	420	378	432	438
Isoleucine	430	460	398	330	297	339	344
Leucine	1534	745	700	620	558	638	647
Lysine	288	530	420	260	234	267	271
Methionine	296	320	140	160	144	165	167
Treonine	400	400	350	240	216	247	250
Tryptophan	180	180	170	100	90	103	104
Phenylalanine	580	592	500	370	333	380	386

Thus, the groats of new of rice created on Ukraine of food and biological value does not concede to groats of import rice and standards of rice groats, and according to some parameters that exceed them, for example, contents of fiber, including irreplaceable amino acids, monosaccharides and disaccharides exceeds these parameters.

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Протокол сетифікаційних випробувань харчової продукції №78 від 08.07.2000р. Центр випробувань продовольчої сировини та харчових продуктів ВАТ«УНДХ» Державне підпрємство«Державний науково-дослідний центр з проблем гігієни харчування» -м.Київ.

Session 2

QUALITY AND NUTRITION

Abstracts

EVALUATION OF NUTRITIONAL CHARACTERISTICS OF SOME ITALIAN RICE VARIETIES

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Rice (Ory*za sativa*) is high in complex carbohydrates, contains almost no fat and it is cholesterol free. It is low in sodium and represents a good source of vitamins, minerals, fibre and a fair source of protein. Due to protein characteristics, this cereal is suitable for people suffering from metabolic disease such as the celiac disease. Rice starch is one of the most easily digestible starches: this characteristic together with the fact that it is totally hypoallergenic, is one of the main reasons why rice is used so widely in baby food and other special dietary foods.
Italy is the major European rice producer, exporting about 60 percent of the production. This cereal is grown in the Po valley where is often considered more important than pasta. According to the Italian regulation, rice is divided into four different categories (*Comune, Semifino, Fino, Superfino*). In consideration of the important role played by this cereal in human diet, a study concerning the evaluation of nutritional properties of some Italian rice varieties, belonging to different varieties, was assessed. In addition to the main chemical parameters (moisture, protein, ash, lipid and starch), the amino acid composition and protein digestibility as well as amylose-amylopectin content and starch digestibility were determined in all the samples under investigation. To have a comparison, a parboiled and a long grain rice (Thaibonnet) were also considered.

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ANTIOXIDANT CHARACTERIZATION OF "RED RICE"

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With 'red rice' it is meant a rice whose kernel is characterised by a brick-red (reddish brown) colour, visible after removing the hull (floral bracts) which cover the caryopsis. With this term it is usually meant either wild rice varieties or cultivated varieties. They have been shown to have antioxidant and hepato-protective capacity due to the high level of antioxidant compounds. However, their antioxidant chemical composition is poorly known.
Aim of this work was to establish the antioxidant chemical composition (tocols, oryzanols and polyphenols) and the total antioxidant capacity of a red kernel rice variety (S. Eusebio), compared to white kernels rice varieties cultivated in Italy in the same farm. Furthermore, we investigated the effects of commercial processing and cooking on levels of antioxidants in both white and pigmented rices.
The results achieved showed that dehulled red kernel rice possess a significant higher antioxidant capacity (about five times higher) if compared either with white rice (either milled or dehulled). Both white and kernel rice have similar tocols, oryzanol and phenolic acid composition. Moreover in red kernel rice differs for the presence of proanthocyanidins, polymeric polyphenols which are known to possess strong antioxidant capacity.

Session 3

GENETICS AND BREEDING

Full papers

HYBRID RICE VARIETIES IN SOUTHERN SPAIN

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Abstract
In Southern Spain first trials on Indica type hybrids were carried out in 2002 by evaluating a wide hybrid collection from China. The majority never reached flowering stage, and only three of them developed a vegetative cycle similar to that of the two local control varieties (Indica type varieties L-202 and Puntal).
During 2003, besides screening new material, some trials about agronomic performance (grain yield, seeding and N-fertilization rates) were carried out on the selected hybrids of 2002. The best ones exceeded on about 1,5 Tm/ha the average yield of the control varieties. The panicle size and the number of grains per panicle were bigger too.
The optimum seeding rate stands between 60 and 90 Kg./ha, significantly lower than the traditional rate, as there is a higher tillering. No significant differences on yield were found among the three tested nitrogen rates (175–220-265 Kg./ha), so lower rates must be tested for each hybrid. Milling yield was slightly lower in hybrids and the grain width was bigger.
As these are early results, it is necessary to keep on testing new hybrid material and carry out more agronomical trials, and further studies on phytopathology and grain quality.

Key words
Hybrid rice; yield; Nitrogen and sowing rates

Introduction

Hybrids are produced by crossing two inbred (genetically fixed) varieties of a particular crop (Devlin Kuyek, 2000). Many hybrids display a phenomenon called heterosis (or hybrid vigour) that is responsible for higher vigour in comparison to their respective parents (Balasubramanian et al., 2003).
Hybrid rice has about a 30 percent yield advantage over conventional pure line varieties. From 1976 to 1995 hybrid rice technology had helped China to increase rice production by nearly 300 millions tonnes. A well-established package of the technology for hybrid rice seed production would greatly boost the expansion of hybrid rice. The technology has been well developed in China to give and average seed yield of 2.3 tonnes ha^{-1} nationwide (Yuan, 1998). Hybrid rice varieties yielded 1 Mt ha^{-1} higher than inbred varieties that had a mean yield of 6.5 Mt ha^{-1} for middle and late season rice (He et al., 1987; Lin, 1990).
More productive tillers and larger panicle size contribute to higher yield of hybrid rice in temperate regions. Planting at lower density and promoting early crop growth is the key to early and rapid tiller production. Early tiller emergence also lead to larger and stronger tillers that produce large panicles. This is the reason why Chinese researchers emphasized the importance of seedling health and seedling age at planting, plant spacing, and fertilizer management to promote early crop growth and biomass development (Balasubramanian et al., 2003).

However, there are other opinions about rice hybrids. The stubborn equation of hybrid rice with progress must be questioned. First, the technology has demonstrated minimal impact to improve yields. Significant increases in yield are rare if not site-specific; there are no cost-effective methods for seed production; and studies show that hybrids require more pesticides because they are more susceptible to disease and pests. The technology is also severely limited by the fact that it is impractical for most tropical countries in Asia. China's hybrid varieties are almost entirely *indica* varieties planted early in the year. The early *indica* crop is China's lowest quality crop and consumers do not like the hybrid varieties. In fact, much of the crop is purchased by the state and a large part of the harvest is used in the state programs to feed the urban poor, stored in the country's rice stocks, or used as animal feed. Besides its low quality, China's hybrid rice has poor disease and pest resistance (Devlin Kuyek, 2000).
Hybrid rice cultivation is well established in temperate China, covering 50% of the irrigated rice area (14.82 million of ha) (Balasubramanian et al., 2003).

Materials and methods

The trials located in Isla Mayor (Sevilla) and Casas de Hito (Badajoz) were carried out by Koipesol, S.A. The trials placed at *La Abundancia* (Sevilla) and *Sartenejales* (Sevilla) were carried out by the Rice and Corn Department (IFAPA).
The hybrid varieties SYCR 89, SYCR 90 and SYCR 91, from the Schuan Academy of Agricultural Science of China, and the conventional cultivars Puntal and Thaibonnet, were used.
In the Koipesol trials, the agronomical value of the hybrid cultivars was evaluated through a trial design (randomized block with three replications, elemental plot 50 m^2 sized). The grain biometry was carried out with samples of 25 g. per elemental plot. Concerning seeding rate, a trial was established with factorial design and three replications. The size of the elemental plot was 20 m^2. The tested seeding rates were 45 Kg. ha^{-1}, 90 Kg. ha^{-1} and 135 Kg ha^{-1}, and the hybrid cultivars SYCR 89 and SYCR 90. Concerning N-fertilization, a trial consisting of a randomized block with four replications (elemental plot 20 m^2 sized) was set. The tested fertilizer doses were 175, 220 and 265 Kg. ha^{-1}. The chosen cultivar was SYCR89.
The Rice and Corn Department carried out two trials (randomized block with three replications, elemental plot 6 x 10 m).
Agricultural practices (fertilising, irrigation, pest treatments...) were those usually made in the area. The whole Nitrogen fertiliser was applied before seeding as traditionally. The seeding was carried out by hand and the harvest of every elemental plot with conventional harvester.
The requested data per elemental plot were:

- Days to heading (50% panicles).
- Plant height (between the ground and the upper top of the panicle).
- Lodging (just before harvest).
- Grain yield (Kg. ha-1- 14% moisture).
- Milling yields (head and total).
- Yield components: grains per panicle (40 sampled panicles per elemental plot, considering the filled grains), blank grains percentage and 1000 grain weight.

Statistics analysis (ANOVA) and Duncan test were calculated.

Results and Discussion

An increase of yield was observed in these hybrids compared with the control cultivars (Table 1 and 2).

Table 1 Agro-physiological results. Rice and Corn Department (IFAPA). Sevilla, 2003.

		CULTIVAR				
		SYCR-89	SYCR-90	SYCR-91	PUNTAL	THAIBONNET
LA ABUNDANCIA	Height (cm)	101	105	100	97	83
	Lodging (%)	10	21	2	1	10
	Days to heading	88	81	86	85	78
	Days to maturity (21% moisture)	141	136	150	139	137
	Panicles / m^2	362	431	317	481	681
	Grains / panicle	89	77	98	88	52
	Blank grains (%)	1,1	1,4	4,3	2,2	4,2
	Weight (g) of 1000 grains	33,5	33,5	31,0	25,5	26,5
	Kg ha^{-1} (14% moisture)	10781 ab	11080 a	9598 ab	10838 ab	9347 b
SARTENEJALES	Height (cm)	101	96	90	88	80
	Lodging (%)	4	20	0	0	2
	Days to heading	84	82	86	83	75
	Days to maturity (21% moisture)	138	137	150	137	135
	Panicles / m^2	442	361	321	391	568
	Grains / panicle	74	84	90	85	54
	Blank grains (%)	1,2	2,4	2,0	1,4	1,3
	Weight (g) of 1000 grains	35,0	30,0	34,5	26,5	25,0
	Kg ha^{-1} (14% moisture)	11403 a	9100 ab	10027 ab	8853 b	7682 b

Table 2 Yield results . Koipesol (2002, 2003)

Cultivar	Isla Mayor (2002)				Badajoz (2003)			
	Grain Yield Kg. ha^{-1} (14% moisture)		Head rice yield (%)	Total rice yield (%)	Grain Yield Kg. ha^{-1} (14% moisture)		Head rice yield (%)	Total rice yield (%)
SYCR 90	10618	a	**60,6**	**70,2**	6747	ab	**68,0**	**72,6**
SYCR 89	7743	b	**55,8**	**72,1**	7578	a	**58,5**	**69,8**
SYCR 91	9956	a	**62,6**	**69,9**	5606	c	**68,5**	**71,5**
Puntal	7512	b	**65,1**	**71,2**	6142	bc	**68,8**	**73,8**
Thaibonet	7226	b	**57,5**	**69,1**	5952	bc	**67,4**	**72,5**

Hybrids produced longer and thicker grains than long-grain conventional cultivars. Nevertheless (Table 3), their industrial yields were a bit lower, although it has to be evaluated case by case.

Table 3 Grain biometrics. Isla Mayor (Sevilla). Koipesol 2002.

Cultivar	Weight of 1000 filled grains (g)	Caliber (cm)		
		Length	Height	Width
SYCR 90	28,5	9,2±0,23	2,4±0,13	1,93±0,08
SYCR 89	30,6	9,4±0,26	2,6±0,01	2,3±0,04
SYCR 91	25,6	9,3±0,34	2,4±0,10	1,9±0,13
Puntal	23,9	8.9±0,19	2,4±0,15	2,0±0,01

Seeding rate is the determinant factor of the hybrids implantation. Three seeding rates have been evaluated: 45, 90 and 135 kg. ha^{-1}. The optimum plant density ranged between 60 and 90 Kg. ha^{-1}, depending on the hybrid, which represents a 33-50% of the seeding dose of conventional cultivars (Table 4). With doses over 90 Kg. ha^{-1} a high level of lodging was observed. Among hybrid cultivars, the seeding dose can vary from one variety to another, so seeding rate for each variety has to be recommended.

Table 4 Seeding rate. Isla Mayor (Sevilla). Koipesol 2003.

SEEDING RATE	Grain Yield Kg/ha (14% moisture)
45 Kg ha^{-1}	8524 b
90 Kg ha^{-1}	9254 a
135 Kg ha^{-1}	8448 b

Regarding Nitrogen fertilisation, the applied doses (175, 220 and 265 kg. ha^{-1}) did not seem to have a positive influence on yield (Table 5). Lower rates have to be tested.

Table 5 N-fertilizer results. Isla Mayor (Sevilla) Koipesol 2003.

N-fertilizer dose (Kg. ha^{-1})	Kg ha^{-1} 14% moisture
175	8525 a
220	8589 a
265	8467 a

These results must be considered as preliminary, being necessary to continue essaying in order to get a more accurate information about the rice hybrid varieties performance in the conditions of Southern Spain.

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THE PERFORMANCES OF NEW RICE VARIETIES AND GENOTYPES IN THE AEGEAN REGION OF TURKEY

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Abstract

Rice production area ranges between 40 000 ha and 70 000 ha in the last years in Turkey. Rice is grown in every part of Turkey, however, Marmara especially Europe part of Marmara (Thrace) and Black sea region are the main rice production areas respectively. Total annual milled rice production changed between 150 000 and 250 000 tons.

But some rice cultivars and genotypes are released by Thrace Agricultural Research Institute during last decade. This rice cultivars as Toag92 and Osmancık-97 were released respectively in 1992, 1995 and 1997. Toag92 has suitable second crop condition under Aegean climate condition. Growing area of Osmancık-1997 is increasing very sharply in all rice growing regions of Turkey. It is also very popular variety in rice industry and market. Three new rice varieties (Kral, Demir, Yavuz) were again registered by Thrace Agricultural Research Institute in 2000. These three varieties are also very high yielding varieties. It has been reported that some farmers are getting more than 10 ton/ha paddy yield with these new varieties.

The Aegean Region Ecological Condition is affected by Mediterranean Climate condition. On this paper, the research was conducted at Izmir/ Menemen Experimental Farm in the area of Field Crops Department, Faculty of Agriculture, Ege University, during the year 2002-2003. It will give the some agronomic and quality performances of some releasing new varieties and genotypes at Izmir on Ege Region Ecological Condition in Turkey. Those modern genotypes are promising for high yield, short height and flowering period in the Mediterranean region so that it should cooperation between countries for using the developed new rice varieties and genotypes in Mediterranean countries.

Keywords

Rice; variety; genotype; quality

Introduction

Rice production area ranges between 40 000 ha and 70 000 ha depending on the water ability and Government policies in the last 50 years (Gaytancıoğlu, 2001).

Rice is grown in every part of Turkey, however, Marmara especially Europe part of Marmara (Thrace) and Black Sea region are the main rice production areas respectively (Gaytancıoğlu, 2001).

Total milled rice production change between 150 000 and 200 000 tons, and this production is not sufficient for domestic consumption. Rice yield increased 2.7 ton/ha in 1927 to more than 5 ton/ha in 2002, but this yield increase not sufficient to meet increasing in consumption. But some farmers can get more than 10 ton/ha paddy yields. Thus, we can say that there is potential to increase rice paddy yield more than 10 ton/ha in near future (Beşer, 2000).

Before 1960's mostly local varieties were grown in Turkey. Some Italian varieties were introduced and begun to grown after that time.

Baldo, Rocca and Ribe are still grown, but their percentages are getting down after releasing new varieties during last decade.
Toag92 and Osmancık-97 were released respectively in 1992 and 1997. Growing area of Osmancık-1997 is increasing very sharply in all rice growing regions of Turkey. It is also very popular variety in rice industry and market.
Three new rice varieties (Kral, Demir, Yavuz) were registered by Thrace Agricultural Research Institute in 2000. These three varieties are also very high yielding varieties (Beşer, 2000).
All varieties released in the last decade are semi dwarf, resistant to logging and suitable for combine harvesting. Yield and quality are also higher than traditional rice varieties, it has been reported that some farmers are getting more than 10 ton/ha paddy yield with these new varieties (Beşer, 2000).
As a result of growing these rice varieties and genotypes, farmers get high quality and yield of paddy. We can say that widening of these varieties Turkey rice production and yield will increase sharply.
In Aegean region that is a typical Mediterranean climate and lies on the west coast of Turkey shows a distinctive climatic patterns which are different from any other area in the country. In this region, lowermost and the uppermost limits of temperature are 15°C-35°C respectively from the beginning of April to the first week of September. Average rainfall is 500-600 mm. Summer is hot and dry while winter is mildly and rainy. Average humidity is 57%. This climatic patterns gives us 140 days available from sowing to flowering for rice cultivation in Aegean region.
On this study, the research was conducted at Izmir/ Menemen Experimental Farm in the area of Field Crops Department, Faculty of Agriculture, Ege University, during the year 2002-2003. It was intended to obtain the some agronomic and quality performances of some releasing new varieties and genotypes that it was especially realized new varieties and genotypes by Thrace Agricultural Research Institute at Izmir on Ege Region Ecological Condition and indirectly Mediterranean climate.

Materials and Methods

This study was conducted at Menemen / Izmir experimental farm in the area of Field Crops Department, Faculty of Agriculture, Ege University, during the year 2002-2003.
In this study, 6 rice varieties and 6 rice genotypes were used as experimental material. All of the varieties used in this study are given in Table1 (Açıkgöz N; Gevrek M N, 1996 and Beşer N, 2000). They are given in Tables of Results and Discussion section.
Tables 2 show average and the average of highest temperature, the average of daily solar energy and average relative humidity at at Menemen / Izmir location, during the year 2002-2003.
It was given some physical and chemical properties of soil samples of the research fields at Table 3.
The experiment was laid out in a randomized complete blocks design with 3 replications under continuous flood irrigation two years (2002 and 2003). 450 pregerminated seed/m² were broadcasted in the standing water. Plot size was 20 m² and fertilizer rate was 180 kg N/ha and 80 kg P/ha. Varieties and genotypes were sown on May 20, 2001 in the first year and May 20 2002 in the second year. Some important phenologic, morfologic, agronomic and quality characters were recorded.
Results were analyzed statistically by the analysis of variance using the Statistical Analysis System (SAS) computer package (SAS Institute 1980).

Table 1 The list of experimental materials.

Varieties/ Genotypes	Some characters
Demir (Plovdiv x lido)	* earlier, resistant to lodging, suitable machine harvest
Yavuz	*
Osmancık (Rocca x Europa)	* resistant to lodging, suitable machine harvest, resistant to *Fusarium moniliforme*
Kral (Gritna x Ballia)	* earlier, resistant to lodging, suitable machine harvest, resistant to *Fusarium moniliforme*
TR-848	**
TR-851	**
TR-1047	**
Baldo X Sarıkılçık X Kros-424	***
ToyhikaryXArborioX Nucleoryza	***
Toyhikary X Solnecnyj	***
Toag92 (Nucleoryzax Labella)	****earlier, low sterility, medium height, resistant to lodging and leaf blast
Baldo (control)	Italy

**It was released at Thrace Agricultural Research Institute-Edirne,Türkiye*
***It was developed at Thrace Agricultural Research Institute-Edirn, Türkiye*
****It was developed at Ege University, Agricultural Faculty-Izmir, Türkiye*
*****It was released at Ege University, Agricultural Faculty-Izmir, Türkiye*

Table 2 Meteorological data of Menemen / Izmir location in July, August, and September.

	July		August		September	
	2002	2003	2002	2003	2002	2003
Average temperature ^oC	27.1	26.8	26.0	27.2	21.5	21.7
The average of highest temperature ^oC	34.0	33.6	32.6	34.6	28.1	28.4
The average of daily solar energy cal/cm^2	637.3	662.2	579.9	574.2	427.7	467.5
Average relative humidity (%)	62.2	52.4	64.7	55.0	75.7	66.3

Table 3 Selected physical and chemical properties of soil samples of the research fields at Menemen/ Izmir

Soil properties	2002	2003
PH	7.0	6.9
Total salt (%)	0.10	0.15
Lime (%)	9.20	8.00
Texture	Silty loam	Silty loam
Organic matter (%)	1.40	1.65
Total N (%)	0.06	0.04
P (ppm)	3.70	3.90
K (ppm)	290	310.00

Results and Discussion

It was given the analysis of variance F values for paddy yield and some important agronomic, phenologic, and morfologic characters on Table 4. On the contrary to this year and variety interaction were found statistically no significant.

Table 4 Analysis of variance F values for paddy yield, spikelets, main productive, tillers 1.000 grain weight, plant height, flowering time and main panicle length of rice varieties.

Variance sources	Paddy yield plant^{-1} (g)	Spikelets main panicle^{-1} (no.)	Productive tillers plant^{-1} (no.)	1000 grain weight (g)	Plant height (cm)	Flowering time (day)	Main panicle length (cm)	$F_{0.001}$
Replicaton	0.123 [1]	3.450[3]	0.410 [1]	0.159[1]	0.832 [1]	2.802 [1]	1.000 [1]	5.120
Variety	4.935 [3]	66.068[3]	2.871 [3]	55.666[3]	123.245[3]	32.769 [3]	6.188 [3]	2.745
Year	1.089 [1]	1.110[1]	20.763 [3]	0.951 [1]	0.287 [1]	0.000[1]	0.000 [1]	4.056
Year x variety	0.176 [1]	1.631[1]	0.169 [1]	0.544 [1]	0.292 [1]	0.000[1]	0.000 [1]	2.745

1 =no significant, 2= significant at 0.01 level, 3=significant at 0.05 level

According to the average figures of two years the highest yield (18.3 g per plant) was produced by Osmancık variety. It was followed by TR-851 (18.2 g), Baldo X Sarıkılçık X Kros-424 (16.5 g), Yavuz 16.3 g) and Toyhikary X Arborio X Nucleoryza (14.4 g). Osmancık has the highest spikelets per plant (563). It was followed by Yavuz (b) variety whereas TR-851 was also found in the different statistical group (c). Demir has the highest productive tillers per plant (9.6). It was followed by Kral variety (ab) whereas Toyhikary X Solnecnyj, Toag92, Baldo, and Yavuz were also found in the same statistical group (bc).

Baldo has the highest 1.000-grain weight (38 g). It was followed by TR-848 genotyp (36.6 g) whereas TR-851 (36.5 g) and TR-1047 (36.2 g) were also found in the same statistical group.

The price of Baldo is the highest than other commercial varieties, because Turkish people prefer bold rice. Thus Baldo production is more than other commercial varieties. As a conclusion, TR-848, TR-851 and TR-1047 genotypes will have been preferred by the farmers to be high yield and 1000 grain weight in near future.

Toag92 has the shorter plant height (63 cm) and than Baldo X Sarıkılçık X Kros-424 and Toyhikary X Solnecnyj genotypes were found the shorter plant in the same statistical group (f). This character is very important that resistant logging and combine harvesting.

Again, Toag92 variety has the shorter flowering time (63 days) (days to 50 % flowering). Toag92 matures 2 wk earlier than Baldo variety. Toag92 is the most suitable variety among the materials on the second crop conditions in this study.

Toyhikary X Arborio X Nucleoryza and Toyhikary X Solnecnyj genotypes were found the longest main panicle length (16 cm). It was followed by TR-851, Baldo X Sarıkılçık X Kros-424 genotypes and Baldo variety in the same statistical group (ab).

It has been given below the quality characteristics of varieties and genotypes (Table 5). According to those figures, TR 848 has highest grain length and grain breadth 6.95 mm and 2.98 mm, respectively. TR-848 and TR-851 has lowest grain length/breadth as 2.33. As Toyhikary X Arborio X Nucleoryza has highest grain length/breadth as 2.76. % Amylose content is chancing between 15.6 and 22.2. Demir has highest amylose content (22.2 %). Again, % protein content is chancing between 9.43 and 12.41. Toag92 has highest % protein content (12.41 %).

Baldo and Osmancık varieties and TR-848, Baldo X Sarıkılçık.X Kros-424 and Toyhikary X Arborio X Nucleoryza genotypes have no belly seed.

Table 4 Comparison of some agronomic characters of varieties/ genotypes in the second crop condition in Menemen, Izmir/ Turkey. 2002-2003.

Varieties/ genotypes	Paddy yield plant^{-1} (g)	Spikelets main panicle^{-1} (no.)	Productive tillers plant^{-1} (no.)	1.000 grain weight (g)	Plant height (cm)	Flowering time (day)[1]	Main Panicle length (cm)
Demir	13.2 de	445.0 d	9.7 a	29.0 f	79.6 de	83.0 abc	12.0 cd
Yavuz	16.3 abcd	515.0 b	7.5 bc	30.3 ef	78.5 e	83.0 abc	11.0d
Osmancık	18.3 a	562.0 a	7.3 c	31.4 de	82.3 d	85.0 b	13.0bcd
Kral	14.1 cde	395.0 e	9.0 ab	34.5 c	78.3 e	91.0 a	12.0cd
TR-848	13.3 cde	372.0 ef	7.5 bc	36.6 ab	96.6 b	83.0 abc	13.0bcd
TR-851	18.2 ab	475.0 c	7.5 bc	36.5 b	90.0 c	83.0 abc	15.0ab
TR-1047	14.7 cde	397.0 e	8.2 abc	36.2 b	101.1 a	83.0 abc	13.0bcd
Bal X Sarıkıl X Kros-424	16.5 abc	472.0 cd	8.0 bc	33.2 bc	70.0 f	80.0 bc	15.0ab
Toy.XArb.X Nuc.	15.4 abcde	467.0 cd	7.5 bc	32.4 cd	79.6 de	84.0 ab	16.0a
Toy. X Sol.	14.4 cde	467.0 cd	8.8 abc	30.1 ef	70.0 f	83.0 abc	16.0a
Toag92	12.4 e	355.0 f	8.5 abc	33.2 bc	63.0 g	63.0 d	14.0abc
Baldo	14.9 bcde	387.0 e	8.5 abc	38.0 a	89.1 c	79.0 c	15.0ab
Approx.	**15.1**	**442.4**	**8.1**	**33.4**	**81.3**	**82.0**	**14.0**
LSD (1 %)	3.241	29.153	1.657	1.500	3.755	4.351	2.533

[1]*:Days of 50% flowering*

Table 5 Quality characteristics of some varieties/ genotypes, Menemen/ Izmir, Turkey, 2002 and 2003.

Varieties/ genotypes	**Grain** length (mm)	**Grain** length (mm)	Grain length-breadth	Amylose content (%)	Protein Content (%)	White belly[a]
Demir	6.48	2.60	2.49	22.2	-	3
Yavuz	6.28	2.56	2.45	20.4	-	4
Osmancık	6.37	2.74	2.33	15.6	9.43	0
Kral	6.78	2.83	2.40	16.5	-	2
TR-848	6.95	2.98	2.33	16.8	10.66	0
TR-851	6.76	2.90	2.33	18.7	10.13	1
TR-1047	6.69	2.85	2.35	18.3	8.74	2
BalXSarkıl.X Kros-424	6.41	2.61	2.58	18.2	10.48	0
Toy.XArb.X Nuc.	6.74	2.54	2.76	20.1	10.83	0
Toy. X Sol.	6.72	2.53	2.64	21.9	10.31	3
Toag92	6.41	2.68	2.39	20.4	12.41	4
Baldo	6.74	2.79	2.41	19.6	9.43	0

[a] *Scored using the Standard Evaluation System (SES) 0-9 scale where 0= no belly, 9= more than 20% of kernel area*

Conclusions

Those modern varieties and genotypes are promising for high yield, short height and flowering period in the Mediterranean region. Developed by crossing new varieties will transmit to 10 ton ha^{-1} national paddy yield in near future. It proves that rice varieties, improved for a region, can be grown in such a broad climatically area like these materials, which are improved in Izmir and Edirne and than grown perfectly Medrice countries.

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THE DEVELOPMENT AND INTROGRESSION OF CLEARFIELD TECHNOLOGY INTO COMMERCIAL RICE PRODUCTION

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Abstract

The use of Clearfield rice technology allows the control of red rice (*Oryza sativa*) and other weeds in commercial rice production. This technology incorporates rice varieties that are resistant to imidazolinone herbicides into the use of those herbicides in rice production. The varietal resistance is a result of induced mutations that were selected for this resistance. Two separate mutations have been used in commercialization of this technology. The genetic resistance in these mutations has been incorporated into agronomically viable varieties by conventional plant breeding techniques. Three commercial rice varieties are being used with this system in the southern United States rice growing region. The first two varieties released (CL121 and CL141) were developed from the first mutant discovered to be resistant to the imidazolinones. The level of resistance with this mutant was moderate at best. The most recent variety being used in this growing region (CL161) was developed from the second mutant and has a much higher level of resistance to the herbicides. All future varieties will be developed using the second, more resistant mutant. This technology will be used on an estimated 15% of the planted area in the southern United States during the 2004 growing season. The commercialization of Clearfield rice technology is well underway in a number of other rice producing areas of the world. The specifics of the development of this technology and plans for future research will be explored.

Keywords

Red rice; Clearfield; imazethapyr; cultivars; weed control; mutation breeding.

Introduction

Rice (*Oryza sativa*) has been in large scale commercial production in the southern United States since the latter part of the 19^{th} century. Mechanized rice production began in the southwestern part of the state of Louisiana in 1880 and soon spread into Texas and Arkansas. In the middle part of the 20^{th} century, rice production expanded into Mississippi and Missouri. Since the beginning of this rice production era, red rice (a weedy relative of commercial rice) has been a major constraint to rice production. Red rice was introduced with the first rice seed that was used in commercial rice production and because of the characteristics of shattering and dormancy, has been very difficult to eradicate. Since commercial rice and red rice are so closely related, it has been impossible to develop traditional herbicides that will selectively eliminate red rice from a commercial rice field without harming the commercial rice.

Materials and Methods

Research was begun by the Rice Biotechnology Project at the LSU AgCenter's Rice Research Station in the early 1980s to develop rice mutations with resistance to non-rice herbicides. This work was conducted under the direction of Dr. Timothy Croughan. This work was initially conducted using a number of herbicides, such as glyphosate and metolachor. Later work was conducted with emphasis on imazethapyr, which at that time was primarily used as a soybean herbicide. Rice seed from a number of varieties were subjected to ethyl methyl sulfonate (EMS) to induce mutations.

Results

In 1993, a single rice plant was identified as displaying resistance to imazethapyr. This plant was derived from the commercial variety AS3510, which had been developed by a private seed company a number of years before. The line was given the designation 93AS3510. The line itself did not have good potential as a commercial rice variety because of inherent low yield and disease susceptibility. An accelerated conventional breeding program was undertaken to develop commercial rice varieties with the resistance gene. Two varieties were released in 1999 (CL121 and CL141) from these efforts. In 1998, the LSU AgCenter signed a commercialization agreement with American Cyanamid (now BASF) to develop and commercialize this technology based on the use of the resistant rice line and the herbicide imazethapyr. Imazethapyr has excellent activity on red rice (as well as conventional rice). A provisional label was obtained from the Environmental Protection Agency for use of imazethapyr on rice with this technology in 2001. A limited amount of commercial production occurred in that year with CL121 and CL141.

A second mutation (CFX18) with a much higher level of resistance to the herbicide was identified by this program in 1997. This mutant was derived from the cultivar Cypress, which was released by the LSU AgCenter in 1992. This line was extensively tested and purified by the Rice Breeding Program at the Rice Research Station. The resulting cultivar (CL161) was released by the LSU AgCenter to BASF in 2001 and was made available for commercial production with the Clearfield system in 2003. A full Federal label for imazethapyr (Newpath) was obtained for use with this system in 2002.

Work continues to develop improved cultivars with the gene for resistance to imazethapyr. All breeding work today is done using the CFX18 source of resistance. This breeding work is conducted using a modified pedigree method of breeding and selection. All breeding and initial selection are conducted at the Rice Research Station in Crowley, Louisiana, USA, as well as at a winter nursery facility on the University of Puerto Rico – Mayaguez Research Center near Lajas. Yield, milling, and agronomic characteristic evaluations are also conducted at the Rice Research Station, as well as at nine off-station locations throughout the rice growing areas of Louisiana. A second CFX18-based cultivar (CL131) has been identified and is currently being increased for possible commercial release in 2006.

Discussion

The Clearfield rice production system was used on 160,000 hectares in the southern United States in 2004 (40,000 hectares in Louisiana). The system is providing good to excellent control of red rice, as well as a number of other important rice weeds. In addition, this system is allowing many producers to change production systems. Prior to the introduction of this technology, water seeding was the predominant seeding method in Louisiana. Water seeding and the maintenance of soil saturation were used as a means of cultural control of red rice. This system could be successful but often necessitated draining of fields shortly after seeding. The water drained from these fields often had a high level of suspended sediments, which caused environmental problems in receiving streams, as well as soil loss from the rice fields.

This technology has allowed many producers to dry seed (drill or dry broadcast) instead, which has reduced soil losses, as well as led to more uniform seedling establishment.

Forecasts called for the area seeded to Clearfield varieties to possibly double from 2004 to 2005 in the southern United States.

In addition, this technology has been commercialized in several other countries, including Costa Rica, Columbia, and Brazil. Commercial production should be forthcoming in a number of additional countries within the next few years.

Conclusions

The development of Clearfield rice technology was the result of the work of a number of people at several public institutions in cooperation with private industry. The successful introduction of the technology can serve as a model for future similar endeavors in the international rice industry.

ANTIFUNGAL GENES IN RICE AS A STRATEGY FOR CROP PROTECTION

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Abstract

The Eu-funded Project EURICE (QLK5-1999-1484) aimed at improving and evaluating the use of transgenes in the control of rice blast (*Magnaporthe grisea*) as major pests for rice in Europe. This approach aimed to create new rice genotypes enhanced for resistance and therefore modified cultivars protected against the pathogens, as well as to an evaluation of the genetically modified genotypes in the environment. Three genes of different origin were chosen as potential source of resistance because of their different characteristics and modality of action: the maize gene *b32*, the fungal gene *AFP*, and the insect gene *cecA*. Their constitutive expression in the rice plant showed that *AFP* and partly *b32* were effective in their defence action. The AFP gene placed under the inducible promoter derived from maize, p-ZmPR4, showed to be activated in response to fungal attack and proved to be effective. The details of the strategy will be illustrated and discussed.

Keywords

Antifungal genes; rice (*Oryza sativa* L.); biotechnology

The concrete possibility to contrast the heavy damage caused by fungal pathogens to a crop is an important issue (Oerke *et al.*, 1994). In Europe, rice is mainly cultivated in environmentally fragile areas, such as in Spain at the delta of river Ebre, the plain of river Guadalquivir, the *albufera* of Valencia; in France at the delta of river Rhone in Camargue; in Italy, in the west part of the Po Valley and coastal plains of Oristano (Sardegna). In most cases, rice cultivated fields are near or even integrated into National or Regional Wildlife Reserves. The environment and ecosystems in most of these regions are deeply influenced by rice cropping cycles, and massive use of pesticides and fungicides applied for crop protection have a dramatic impact. Plant biotechnology has become a source of agricultural innovation, providing new solutions to age-old problems. This technology has led in some cases to the commercial introduction of valuable transgenic crops for farmers, such as the *bt* cotton and maize (De Maagd et al., 1999), or herbicide resistance systems in soybean and canola (De Greef et al., 1989). The advances in plant biotechnology are presenting new opportunities for modifying crop plants in a novel way: to provide genes for disease and pest resistance, product quality, tolerance to environmental stress, and plant architecture. Among these targets, programmes of crop protection will likely receive a significant improvement by the advent of transgenic crops correctly expressing pathogen and pest defence genes (Takken and Joosten, 2000). This goal is directed towards the obtention of new genotypes with improved resistance against biotic diseases, with the ultimate scope to reduce the chemical impact of the current agricultural practices on the environment.

The rice blast disease, caused by the Ascomycete pathogenic fungus *Magnaporthe grisea* (anamorph *Pyricularia grisea*) on rice, represents the most important and widespread disease on rice in Europe. The fungus is a pathogen of a large number of grass species but it is best known as the causal agent of rice blast disease, the most serious disease of cultivated rice (Asuyama, 1965). The fungus causes two types of attacks: one early during the vegetative phase, known as leaf blast, and a second later during maturation, known as neck blast. This second attack is much more important than the first one in its effect on the crop, because of the serious yield reduction suffered by the attacked plants (Ou, 1985). Historically in Europe, the rice blast caused severe attacks, which determined an almost complete loss of harvest.

Conventional breeding constantly produces new cultivars characterized by high resistance to the rice blast, but even the most resistant ones, after few years, suffer of a strong reduction in resistance, due to the appearance of new pathotypes and more virulent blast strains (De Datta, 1981; Grayson et al., 1990). Creating varieties with improved and durable level of resistance to blast and stem rots is thus an important goal that does not seem to be reached by classical breeding methods. The availability of new tools for controlling pathogen attacks are offered by biotechnology (Tyagi and Mohanty, 2000; Tu et al., 2000a: Tu et al., 2000b), although contrasting criteria rise a general concern about the use of gene transfer technology and the commercialisation of its products.

However, it is believed that use of gene resistance to control these diseases would be of lower cost and less damaging to the environment than spraying fungicides. Farmers will need less expensive inputs and therefore will be able to lower their production costs and compete more effectively in world markets. This will lead to a greater environmental acceptability and at the same time increase the competitiveness of European agriculture by reducing production costs and/or increasing product quality.

Therefore, as an aid to conventional breeding, a biotechnological approach was afforded, in order to supply the rice plant with new tools suited to contrast the pathogen invasion. An integrated approach to control rice blast by means of a transgenic methodology was proposed within the Vth Framework Program of the European Communities, Quality of Life and Management of Living Resources. The general objective of the project EURICE (QLK5-1999-1484) was to improve and evaluate the use of potential anti-fungal genes in controlling rice blast disease. This approach aimed to improve our knowledge on the use of resistance genes to tailor new rice cultivars enhanced in resistance, for stable and reliable use of transgene technology in wider programmes for rice crop protection, related to a reduced release of fungicides in the environment and a safer end-product.

Once the due safety and environmental evaluations of the new genotypes would guarantee their use, they could be incorporated into breeding programmes, leading to new tailor-made crops.

The major goal was reached throughout actions targeted to specific objectives, focused on: 1) the identification of anti-fungal candidate genes for genetic engineering of important European rice cultivars; 2) Production of transgenic events in which the antifungal genes are constitutively expressed and characterisation of the events; 3) Pathogenicity assays to test for acquired resistance of the transgenic plants; 4) Effect of gene pyramiding in crop protection, 5) Construction of chloroplast-targeted plants in order to contain gene flow, and to evaluate potential of the approach; 6) Characterisation and functional analysis of two putative fungal-inducible promoters from maize and barley, suitable for anti-fungal gene expression under pathogen attack; 7) Evaluation of the transgenic events under field conditions; 8) Evaluation of spontaneous gene flow from transgenic plants toward cultivated and weedy rice (red rice); 9) Evaluation of the experimental conditions for safe field experimental designs devoted to test transgenic events preventing gene flow and environment contamination.

The project was carried out from march 2000 up to the end of 2003, and benefited from the cooperative collaboration of nine partners belonging to seven European countries: Italy, Spain, Greece, France, Germany, Denmark and United Kingdom. The project was exploited with the expertises of each partner giving integrative results derived from agronomy, genetics, plant pathology, molecular biology, tissue culture and transformation, and plant/pathogen investigations by means of advanced techniques in microscopy.

The results obtained are clearly indicative that the biotechnological approach was in some instances successful, although a clear cut complete resistance – such as in the case of the *bt* transgenic crops – was not observed. This is very likely due either to the complexity of the plant/pathogen interaction, process, difficult to hamper in all steps of development, or to the numerous tools available to the pathogen for overcoming the expression of toxic proteins, or finally to a non adeguate expression level of the transgenic protein conferring resistance.

The results obtained in the various areas of research can be summarised as follows.

Three genes of different origin were considered for genetic transformation of selected rice cultivars widely cultivated in the Mediterranean area: the maize gene *b-32* (reviewed in Motto and Lupotto, 2004), the *afp* gene of *Aspergillus giganteous* (Martinez-Ruiz et al., 1997), and the *cecA* gene from the insect *Cecropia* (Cavallarin et al., 1998). All three gene display anti-fungal properties by means of various mechanisms. They were introduced by itself or after a proper transformation with specific sequences. A synthetic *afp* agene was created by recursive PCR and implementation of the G/C content for monocot plant species. The nucleotide sequence for the signal peptide of the AP24 protein of tobacco was also added for specific secretion of the gene product in the extracellular space. The same strategy was adopted for creating a synthetic *cecA* gene, plus a synthetic trimer of cecA. All genes and derivatives were introduced into rice via biolistic transformation as well as via *Agrobacterium* transformation. Various events of transformation, constitutively expressing the three anti-fungal genes *afp, CecA* and *b-32* were produced in European rice cultivars (Senia, Selenio and Maratelli). Plants engineered with the native and synthetic *afp* gene showed the highest degree of fungal resistance (Coca et al., 2004). Plants engineered with gene *CecA* also showed resistance, but some sterility problems occurred. Plants engineered with gene *b32* were normal and fully fertile. Gene pyramiding was therefore afforded with the two genes *afp* and *b-32*, engineered in the tester variety Maratelli. Gene pyramiding was performed in cv.Maratelli for the two genes *b32* and *afp*, but the strategy did not improve the resistance. A transient gene expression assay including pathogenicity tetsts was set up and made available in rice, with the aim to help in studying promoters and defence gene action. This test, coupled to a deep investigation of the interaction plant/fungal pathogen, by means of fluorescence microscopic histology, allowed a complete description of the action of each molecular constructs at the cellular level (Schaffrath et al., 1995). Vector construction for expressing the transgenes into the chloroplast demonstrated that the *b32* gene can be expressed in the plastid, thus preventing the transgene flow from the GM rice to cultivated and weedy rice.

The characterisation and functional analysis of two putative fungal-inducible promoters from maize (Raventos et al., 1995) and barley (Gregersen et al., 1997), suitable for anti-fungal gene expression under pathogen attack were performed with transient assay studies and by means of stably transgenic rice lines carrying the promoters and parts of them, as drivers for the *gus* marker gene. Pathogenicity assays for studies of the interaction plant/pathogen (rice/*Magnaporthe*) for attesting the properties of fungal-inducible promoters attested the validity of the BH6-12 promoter as wounding inducible promoter. More in detail, the maize *ZmPR4* promoter was shown to drive expression at the sites of pathogen invasion, as well as at the wounding site. Moreover, *gus* expression showed that *ZmPR4* confines gene expression at the spore germination area. Most importantly, *ZmPR4* promoter also confers elicitor responsiveness to the *gus* gene, thus resulting an optimal fungal inducible plant promoter candidate. Experimental designs to be adopted in the field for a reliable evaluation of leaf and neck blast resistance have been performed: the contiguous design and honeycomb design with artificial inoculation resulted the best for evaluation: in a field trial, the Ubi1-b32 engineered plants resulted the most promising events. Evaluation of grain quality traits related to the disease effect have been accomplished in order to verify beneficial effects of he engineered genes. Evaluation of spontaneous gene flow from transgenic plants toward cultivated and weedy rice (red rice) was performed in field experiments conducted in two years, demonstrating that direct and reverse gene flow occurs, and that the flow strongly depends from the climatic conditions. The *bar* herbicide resistance marker was chosen to follow gene flow and the trait confirmed to be stably expressed in the weedy rice as dominant trait. A containment-planned experimental field condition was also studied in order to establish a reliable protocol for testing transgenic events without gene flow occurring. Safety of the strategies adopted for conduction of GM-field trial respect to gene flow, has been attested, showing the need of distances and the help of a border protective variety which completely protected normal rice from being contaminated by GM pollen (dedicated paper in these Proceedings, Messeguer et al.).

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TEMPORAL TRENDS IN ITALIAN RICE VARIETIES

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Abstract
Rice has a great importance for European economy and Italy is the main rice producer in Europe. Since its introduction in Italy in the 15 th century, the breeding programs, conducted both by public and private sector, produced a large number of varieties. The relative small rice surface and the introduction of rice from abroad make Italian rice cultivars a good model to study changes in genetic diversity over the time. We used SSRs in order to analyse genetic diversity among the most cultivated Italian rice cultivars and to assess temporal trends in genepool.

Keywords
Rice; SSR; Genetic diversityM; Germoplasm

Introduction

Italy is the main rice grower in Europe with a paddy production of 11.359.826 t and grown area of about 220,000 ha (FAO database 2003). Rice was introduced in Italy by Spaniards or Arabs (Piacco 1959) in the 15 th century. Its cultivation started with a variety called Nostrale. Since 1800 many other varieties have been introduced from different countries, thanks to their resistance to biotic and abiotic stress: resistance to *Pyricularia grisea* was the main force driving the breeding effort. Cultivar Bertone was introduced in Italy 1872, Originario Chinese in 1904, Lady Wright in 1925, and Thaibonnet in 1992 (Ente Nazionale Risi 1966). Mass selection was the main method to improve existing cultivars since 1925, when the first cross was obtained. Nowadays, 139 varieties are listed in the National Register in order to satisfy the evolving market needs.

In order to study the genetic relationships by molecular markers and pedigrees information of Italian rice varieties we have recently analysed 96 rice accessions; 88 of these were a comprehensive selection of Italian accessions while eight were originated in foreign countries (Spada et al 2004). Ours results showed that these cultivars can be split in two main groups: a small one, comprising exotic accessions and a larger one, encompassing historical and recent grown cultivars. The latter can be further split in four subgroups, although no information about temporal trends in Italian germoplasm is available so far.

The introduction of cultivated rice from foreign countries and the absence of wild rice in the Italian country make this species a good model for studying genomic relationships as well as temporal trends.

Unfortunately, little is known about pedigree of the Italian cultivars. Their origin is frequently covered by patents or missing or incorrectly recorded in particularly for ancient cultivars.

It’s a matter of public concern that breeding programs may lead to reduction of genetic diversity within the cultivated gene pool. This erosion could reduce the success of plant breeding. Reduction of gene pool drives to dramatic consequences, such as genetic vulnerability of crops to novel pests or to climatic changes (Russel et al. 2000; Macaferri et al. 2003).

In this work we analyse the DNA of 123 rice cultivars, including both ancient and modern selections by mean of microsatellite (SSR) in order to asses the temporal trends in diversity and to check if genetic erosion occurred in diversity since its introduction in Italy.

Table 1 The 123 rice accessions, divided in temporal intervals, used for DNA extraction and SSR analysis.

1884-1899	
OSTIGLIA	1850
BERTONE	1872
LENCINO	1890

1900-1915	
RANGHINO	1900
VIALONENERO	1904
ORIGINARIOCINESE	1904
ALLORIOPRECOCE	1915

1915-1930	
PRECOCE6	1917
MARATELLI	1919
AMERICANO1600	1923
BALILLA	1924
ARDIZZONE	1925
LADYWRIGHT IP6	1925
VERCELLI	1926
PIERROT	1927

1930-1945	
ROMA	1931
AGOSTANO	1933
NOVARA	1933
SEN.NOVELLI	1935
BALZARETTI	1936
GIGANTENOV	1937
STIRPE136	1937
VIALONE NANO	1937
OLDENICO	1939
R.B.	1943
ADELAIDECHIAP.	1944
CARNAROLI	1945

1945-1960	
ARBORIO	1946
RONCAROLO	1947
RECORD253	1948
RIZZOTTO	1950
CORBETTA	1952
PRECOCERO	1953
ROVERBELLA	1955
LAFERLA	1956
BARAGGIA	1957
ORIGINARIOLEN	1959
ITALPATNA	1960

1960-1975	
RIBE	1961
RENO	1962
RAFFAELLO	1963
ARBORIOPRECOCE	1963
ROSAMARCHETTI	1963
REDI	1964
BALDO	1964
ROMEO	1968
PADANO	1973
S.ANDREA	1974

1975-1990	
LIDO	1976
ARGO	1978
ALFA	1980
CRIPTO	1980
STRELLA	1980
VOLANO	1983
BELGIOIOSO	1984
ELIO	1985
ARIETE	1985
KORAL	1986
SELENIO	1987
LOTO	1988
MIARA	1988
CERVO	1989
MEDUSA	1989
PANDA	1989
PREVER	1989
ARTIGLIO	1990
DEDALO	1990
IDRA	1990
MIDA	1990

1990-2001	
ELBA	1991
THAIBONNET	1991
PORTO	1992
ROVA	1992
ALPE	1993
DRAGO	1993
LAGO	1993
ASSO	1994
DIANA	1994
LAMPO	1994
SARA	1994
ARTICO	1995
SAVIO	1995
CISTELLA	1995
COBRA	1995
CONDOR	1995
DORELLA	1995
FENIS	1995
GANGE	1995
GARDA	1995
BASTIA	1995
ARCO	1996
EOLO	1996
ALICE	1997
FLIPPER	1997
GIOVE	1997
GLADIO	1997
PERSEO	1997
VENERE	1997
S.PIETRO	1997
GHIBLI	1998
PERLA	1998
SANTERNO	1998
SATURNO	1998
ARES	1998
EBRO	1998
TEJO	1998
NEMBO	1998
MERCURIO	1998
TEA	1999
CHIMERA	1999
AMBRA	1999
PONY	2000
ALBATROS	2000
STRESA	2000
FRAGRANCE	2000
ADRIANO	2001
DELFINO	2001
TICINO	2001
POSEIDONE	2001
BIANCA	2001
KARNAK	2001
ASIA	2001
ARELATE	2001

We chose SSR molecular markers for their multiallelic nature and co-dominant transmission. This makes them a suitable tool to differentiate closely relate genome, to study family structure and to follow genetic changes.

Materials and methods

Plant materials

The 123 accessions of rice*, Oryza sativa* L. (Tab 1) were chosen to represent the germoplasm in the years ranging from 1800 to 2001, and divided in 9 periods as shown in Tab 1 All the plant material was obtained from "Ente Nazionale Risi" (Rice Research Centre, Castello d'Agogna, Italy). The seedlings were grown in transparent boxes, on moist filter paper, at the constant temperature of 26°C, under 12 h light photoperiod.

DNA isolation

Up to 100 mg of leaf material was harvested from each seedling, frozen in liquid nitrogen and ground for DNA extraction. Genomic DNA was isolated using the GenElute Plant Genomic DNA Kit (Sigma-Aldrich) in accordance with supplier's instruction. The DNA concentration and quality were determined by spectrophotometer and agarose gel analysis.

SSR analysis

Twelve microsatellite loci were analyzed (Tab. 2). The reaction mixture (20 µl) contained: 2 µl 10x Sigma Red Taq PCR buffer, 10 ng of genomic DNA, 400 µmol dNTPs, 25 ng of each primer, and 0.4 units of Sigma Red Taq DNA polymerase. The PCR was performed in a Perkin-Elmer 9600 thermocycler programmed as follows: 94°C, 1 min; Ta (annealing temperature, as reported in Tab.2), 1 min; 72°C, 1 min, during 35 cycles. The PCR products were separated in 8-10% polyacrylamide gels and visualized by silver staining, according to the Promega silver staining protocol.

Data analysis

Only the unambiguous bands were scored on the polyacrylamide gels as present (1) or absent (0). Genetic similarity was estimated from binary matrices using Jaccard's coefficient (Sneath and Sokal 1973). The principal coordinate analysis (PCO) was performed using the NTSYS-PC options: d-center and Eighten algorithms.

The rice varieties were grouped in temporal interval from 1800 to 2001 as shown in (Tab 1) among this groups genetic diversity (H) was computed according to Lynch and Milligan (1994) using AFLP-SURV (http://www.ulb.ac.be/sciences/lagev/software.html; Vekemans et al. 2002).

Results

Seventy-four alleles were identified at the twelve analyzed SSR loci (Table 2). The total numbers of alleles per locus ranged from 9 (for locus RM 249) to 4 alleles (for locus RM 258 and RM 234).

The average number of alleles (Figure 1), which measures genetic diversity at gene locus, was 2.1 for ancient group (1800-1900 class, Table 1) and 4.2 for the 2001 group, revealing their constant increase.

The study of genetic diversity (H_J) among the nine groups, as computed by the Lynch and Milligan method (Table 3), showed that modern varieties maintained the genetic diversity at a constant level.

Genetic relationships among the 123 rice cultivar were evaluated by means of Principal Coordinate Analysis (Figure 1). The first and second Principal Coordinate explain 12.4% and 8.6% of the total variation respectively (the third accounted for 5.25%).

As shown in Figure 2, the lines joint together, in PCO space, the extreme of the variation for 2001 group varieties (referred as hull) encompasses much of the genetic diversity in the earlier varieties.

Table 2 SSRs used for the study of genetic similarity. Primers name and sequence are those described by Cheng et al. (1997).

Primers	Sequence	Chromosome number	Annealing temperature	Alleles number
RM 235f	5'-AGAAGCTAGGGCTAACGAAC-3'	12	58°C	6
RM 235r	5'-TCACCTGGTCAGCCTCTTTC-3'			
RM 253f	5'-TCCTTCAAGAGTGCAAAACC-3'	6	56°C	6
RM 253r	5'-GCATTGTCATGTCGAAGCC-3'			
RM 263f	5'-CCCAGGCTAGCTCATGAACC-3'	2	60°C	6
RM 263r	5'-GCTACGTTTGAGCTACCACG-3'			
RM 24f	5'-GAAGTGTGATCACTGTAACC-3'	1	56°C	8
RM 24r	5'-TACAGTGGACGGCGAAGTCG-3'			
RM 220f	5'-GGAAGGTAACTGTTTCCAAC-3'	1	56°C	9
RM 220r	5'-GAAATGCTTCCCACATGTCT-3'			
RM 241f	5'-GAGCCAAATAAGATCGCTGA-3'	4	56°C	7
RM 241r	5'-TGCAAGCAGCAGATTTAGTG-3'			
RM 218f	5'-TGGTCAAACCAAGGTCCTTC-3'	3	58°C	8
RM 218r	5'-GACATACATTCTACCCCCGG-3'			
RM 234f	5'-ACAGTATCCAAGGCCCTGG-3'	7	58°C	5
RM 234r	5'-CACGTGAGACAAAGACGGAG-3'			
RM 38f	5'-ACGAGCTCTCGATCAGCCTA-3'	8	56°C	5
RM 38r	5'-TCGGTCTCCATGTCCCAC-3'			
RM 258f	5'-TGCTGTCTGTAGCTCGCACC-3'	10	56°C	5
RM 258r	5'-TGGCCTTTAAAGCTGTCGC-3'			
RM 249f	5'-GGCGTAAAGGTTTTGCATGT-3'	5	56°C	9
RM 249r	5'-ATGATGCCATGAAGGTCAGC-3'			
RM 202f	5'-CAGATTGGAGATGAAGTCCTCC-3'	11	50°C	5
RM 202r	5'-CCAGCAAGCATGTCAATGTA-3'			

Table 3 Genetic diversity, based on SSR data

group	N	#loc.	#loc_P	PLP	Hj	S.E.(Hj)	Var(Hj)
1800	3	86	23	26.7	0.15848	0.02845	0.000809
1915	4	86	24	27.9	0.14353	0.02535	0.000643
1930	8	86	26	30.2	0.11324	0.01995	0.000398
1945	12	86	39	45.3	0.15676	0.02135	0.000456
1960	11	86	38	44.2	0.15432	0.02093	0.000438
1975	10	86	33	38.4	0.14326	0.02161	0.000467
1990	21	86	32	37.2	0.138	0.0186	0.000346
2001	54	86	46	53.5	0.15431	0.01706	0.000291

N, number of accessions within each group or subgroup
#loc P, number of loci scored
#loc P, number of polymorphic loci at the 5% level, i.e. loci with allelic frequencies lying within the range 0.05 to 0.95
PLP, proportion of polymorphic loci at the 5% level, expressed as a percentage
Hj, Genetic Diversity according to Lynch and Milligan (1994)
S.E., Standard Error of Hj
Var., variance of Hj

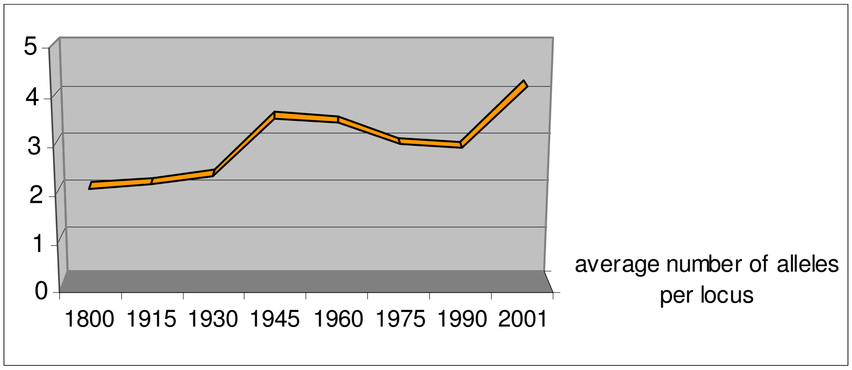

Figure 1 Average number of alleles per locus during the period 1800-2001

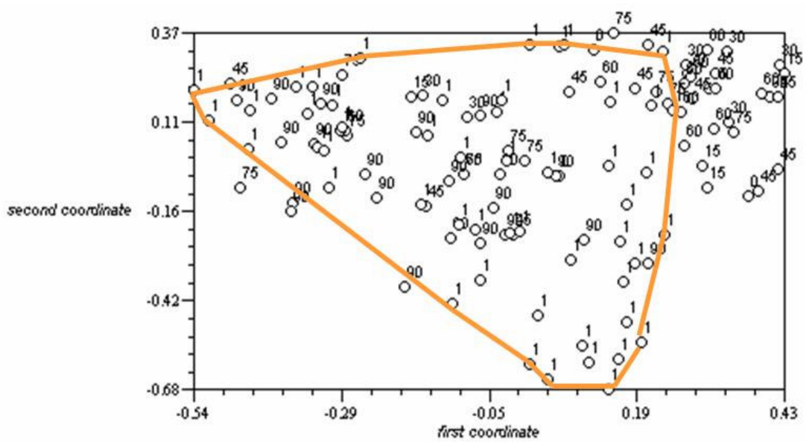

Figure 2 Principal coordinate analysis (PCO): The plot shows the distribution of diversity for the 123 varieties grown in Italy during the period 1800-2001 The lines join together the extremes of the variation for the last 15 years-varieties. Points marked 0, 15, 30, 45, 60, 75, 90, 01 belong respectively to the grops 1800, 1915, 1930, 1945, 1960, 1975, 1990, 2001. The proportion of variance accounted by the first two components is 21%.

Discussion and conclusion

The major goal of our study was to asses the temporal trends in diversity and to check if genetic erosion occurred in diversity within the Italian rice varieties.

The results show that introducing or removing cultivars at different time did not narrow the genetic pool. Thus, breeding programs have been successful in maintaining sufficient genetic potential. Moreover, the presence of new alleles combination, by the introduction of new foreign varieties, was beneficial in preserving genetic diversity. Further study will be focused on the analysis of coding sequences, with particular attention to the agronomically significant genomic region. This will help us to better understand if human driven crop evolution leads to reduction in the genetic diversity of agronomically relevant gene pools.

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PLANT CYSTATINS: A GENOMIC APPROACH IN RICE AND BARLEY

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Abstract

The plant cystatins or phytocystatins comprise a family of specific inhibitors of cysteine proteinases, involved in the regulation of different endogenous processes and in plant defence against pests and pathogens. Extensive searches in the complete rice genome and in several barley EST collections have allowed us to predict a number of twelve cystatins in rice and thirteen in barley. Structural comparisons based in alignments of all the protein sequences by the CLUSTALW program have revealed a wide conservation of the main characteristic motifs of the plant cystatins. Phylogenetic analyses have led us to establish homologies, groups of orthologous genes and protein duplications among the rice and barley cystatins. This results may be useful to assign proteinase specificities and gene function to other cystatins and use them as new transgenes against pests and pathogens.

Keywords

Barley; genomic approach; plant cystatin; rice; sequence comparison.

Introduction

Cystatins are a group of proteins specifically inhibiting cysteine-proteinases that have been identified in vertebrates, invertebrates and plants. Those from plants, referred as phytocystatins (PhyCys) comprise more than 70 members (Pfam databank; Bateman et al., 2002), which cluster in a major evolutionary tree branch of the cystatin superfamily of proteins (Margis et al., 1998). Besides the three motifs involved in the interaction proteinase-inhibitor, the reactive site motif QxVxG, a glycine (G) and a tryptophan (W) residues located near the N- and C-terminus of the protein, respectively, common to all cystatins, most of the PhyCys contain a consensus LARFAV sequence in the N-terminal half of the molecule. The phytocystatins are characterized by a molecular mass in the 12-16 kDa range and the absence of disulphide bonds. These plant inhibitors have been involved in the regulation of endogenous protein turn-over (Arai et al., 2002; Martinez et al., 2003b) and in plant defence (Lecardonnel et al., 1999; Martinez et al., 2003a).

To date, the best studied PhyCys is the oryzacystatin I (OC-I) from rice, whose three-dimensional structure consists of an α-helix and five antiparallel β-sheets (Nagata et al. 2000). Although two oryzacystatin isoforms, OC-I and OC-II, with different inhibitory properties towards cysteine proteinases have been characterized (Kondo et al., 1990), only the OC-I gene has been transgenically expressed conferring resistance to different pests (Vain et al., 1998; Lecardonnel et al., 1999; Rahbe et al., 2003). We have previously reported (Gaddour et al., 2001) the molecular characterization of a barley cDNA from developing barley endosperm encoding the cystatin Hv-CPI, which is highly related in sequence to the OC-I. The recombinant Hv-CPI protein expressed in E. coli is an efficient inhibitor of papain-like enzymes and has also antifungal properties, inhibiting the in vitro growth of phytopathogenic fungi (Martinez et al., 2003a; 2003b).

The number of cystatin members present in rice and barley has not been determined. The draft sequences of rice genome and the publicly available EST collections from barley, whose the genome sequence has still not been completed, have allowed us to achieve a comprehensive characterization of both cystatin families and to establish phylogenetic relationships among these two closely related monocots.

Materials and Methods

Searches for barley cystatins were made in publicly available barley EST libraries constructed by The Institute of Plant Genetics and Crop Plant Research (IPK, http://pgrc.ipk-gatersleben.de/b-est/login.php), Gatersleben, Germany, and The Clemson University Genomics Institute, Clemson, USA (CUGI, http://www.genome.clemson.edu/projects/barley). Nucleotide sequences were determined on both strands using vector-specific primers and the automated sequencer (ABI PRISM 377, Perkin Elmer-Applied Biosystems). Searches for rice cystatins were made at The Institute for Genomic Research (TIGR, http://www.tigr.org). Alignments of protein sequences by the CLUSTALW program were performed at the DNA Data Bank of Japan (http://www.ddbj.njg.ac.jp. Bootstrapping analysis with a PHYLIP format tree output was carried out after the neighbour-joining method and the trees were represented under the TREEVIEW (v.1.6.6) software.

Results

To assemble a complete and non-redundant set of cystatin gene family in rice and barley, an in silico approach was performed by screening the Oryza sativa ssp japonica draft genome sequence and several barley EST collections. By independent Blast searches at the TIGR database and the DNA databank of Japan, twelve non-redundant cystatin sequences in rice (OC-I-OC-XII) were identified and a total of 13 non-redundant EST sequences containing the protein PhyCys motifs (Hv-CPI-HvCPI13) were selected from the cv Morex and Barke of CUGI and IPK collections, respectively.

The deduced amino acid sequences of the 25 different cystatins from rice and barley were compared based on sequence similarity and an unrooted tree was inferred by the neighbour-joining method (Figure 1).

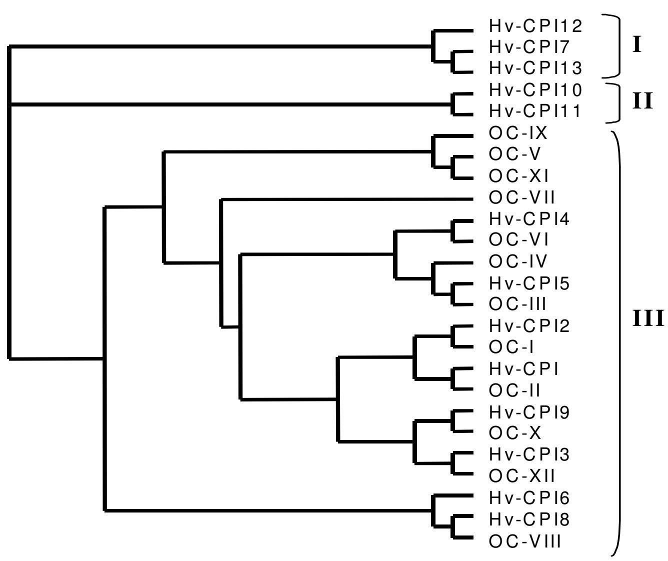
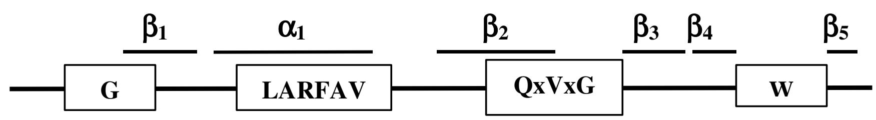


Figure 1 Phylogenetic unrooted tree of the rice and barley cystatins.

The tree topology was organized in three major homology groups (I, II and III) being the group III composed by two subgroups. Three barley proteins were grouped in branch I, Hv-CPI12, Hv-CPI7 and Hv-CPI13, and two in branch II, Hv-CPI10 and Hv-CPI11, probably originated by duplication events. Within group III, five orthologous proteins were clearly identified in barley and rice Hv-CPI14/OCVI; Hv-CPI12/OC-I; Hv-CPI/OC-II; Hv-CPI9/OC-X and Hv-CPI3/OC-XII. Besides, two putative orthologous in rice OC-IV and OC-III for the protein Hv-CPI5 of barley and other two in barley Hv-CPI6 and Hv-CPI8 for the rice OC-VIII were found. Finally, the rice OC-VII protein and the subgroup formed by OC-IX, OC-V and OC-XI had no counterpart in barley.
An alignment of the deduced amino acid sequences of the rice and barley cystatins showed that the main phytocystatin motifs, those implicated in the hydrolase-inhibitor interaction, were conserved (Figure 2).




```
Hv-CPI2    ------G---------LARFAV-----------QLVSS-------------W-----
Hv-CPI7    ------G---------LGSWAV-----------QLVSG-------------W-----
Hv-CPI13   ------G---------LGSWAV-----------QLVSG-------------W-----
Hv-CPI10   ------G---------LGNWTV-----------QIVAE-------------------
Hv-CPI11   ------G---------LGKWAV-----------QIVNG-------------------
OC-IX      ------G---------LGRWAI-----------QVVSG-------------W-----
OC-V       ------G---------LGRWAV-----------QVVAG-------------------
OC-XI      ------G---------VALWAV-----------RFEAG-------------------
OC-VII     ------G---------VGAWAV-----------QVVQG-------------------
Hv-CPI4    ------G---------LGRFAV-----------QVISG-------------W-----
OC-VI      ------G---------LGRFAV-----------QVVSG-------------W-----
OC-IV      ------G---------LGLFCV-----------QVVSG-------------W-----
Hv-CPI5    ------G---------LGRYSV-----------QVVSG-------------W-----
OC-III     ------G---------LGRFSV-----------QVVSG-------------W-----
Hv-CPI2    ------G---------LARFAV-----------QTVAG-------------W-----
OC-I       ------G---------LARFAV-----------QVVAG-------------W-----
Hv-CPI     ------G---------LARFAV-----------QVVAG-------------W-----
OC-II      ------G---------LARFAV-----------QVVGG-------------W-----
Hv-CPI9    ------G---------LARFAV-----------QTVAG-------------W-----
OC-X       ------G---------LARFAV-----------QVVAG-------------W-----
Hv-CPI3    ------G---------AARFAV-----------QVVAG-------------W-----
OC-XII     ------G---------AARFAV-----------QVVTG-------------W-----
Hv-CPI6    ------G---------LGGWAV-----------QVVSG-------------W-----
Hv-CPI8    ------G---------LGGWAV-----------QVVSG-------------W-----
OC-VIII     ------G---------LGGWAV-----------QVVSG-------------W-----
```

Figure 2 Schematic distribution of conserved motifs responsible of the inhibitor-hydrolase interaction of rice (OC) and barley (Hv-CPI) phytocystatins and predicted location of these motifs on the protein structure. Amino acids different to the consensus sequences are underlined. Accession numbers of the barley proteins: Hv-CPI (Y12068), Hv-CPI-2 to Hv-CPI-13 (AJ748337-AJ748348). Accession numbers of the rice proteins: OC-I, (PAC P0003D09); OC-II, (BAC OJ1579G03); OC-III, (PAC P0004D12); OC-IV, (PAC P0004D12); OC-V, (BAC OSJNBa0021B21); OC-VI, (cDNA AK063869); OC-VII, (BAC OSJNBb0066C12); OC-VIII, (BAC OSJNBa0083F15); OC-IX, (BAC OSJNBa0021B21); OC-X, (PAC P0667A10); OC-XI, (BAC OSJNBa0021B21); OC-XII, (BAC OSJNBb0014K18).

All the proteins maintained at least one glycine residue at the end N-terminus and most of them the tryptophan at the C-terminal part. This amino acid residue was absent in two proteins from barley (Hv-CPI10 and Hv-CPI11) and three from rice (OC-V, OC-VII and OC-XI). The reactive site QxVxG was also conserved in 10 barley sequences and 11 of rice, being the rice protein OC-XI and the proteins Hv-CPI10, Hv-CPI12 and Hv-CPI4 different in the consensus core. The common phytocystatin motif LARFAV, although mainly conserved, presented different substitutions in all its amino acid residues in rice and barley proteins (Figure 2). Finally, most of rice a barley cystatins present a predicted signal peptide sequence with the exception of the Hv-CPI protein from barley. Moreover, two barley sequences (Hv-CPI3 and Hv-CPI9) and one from rice (OC-X) have a longer C-terminal tail.

Discussion

Genomic characterization of plant proteinase inhibitors have been previously reported for two serine proteinase families, the Kunitz-type enzyme inhibitors from potato tubers and the sugarcane Bowman-Birk type proteinase inhibitors (Heibges et al., 2003, Mello et al., 2003). This is the first genomic approach to characterize cysteine-proteinase inhibitors from plants. Considering the extensive annotation work done in rice together with the analysis of barley EST collections made in this study, we assume that most or probably all of the cystatins from both species are represented in the 25 sequences documented, 12 from rice and 13 from barley. To date, only two rice cystatins, OC-I and OC-II, and one barley cystatin, Hv-CPI had been characterized, having a double role as endogenous regulators and as defence proteins against pests and pathogens (Kondo et al., 1990; Gaddour et al. 2001; Arai et al., 2002; Martinez et al., 2003a; 2003b).

Results indicate that phytocystatins are a very conserved family of proteins. Among the motifs responsible of interacting with the cysteine proteinases in the main body of several barley and rice cystatins, there is no variation in the conserved G in the N-terminal region. Surprisingly, several barley and rice putative cystatins have lacked the triptophan of the C-terminus. The importance of the triptophan residue in the second loop has been confirmed by the experiments of phage display of a soyacystatin (Koiwa et al., 2001). Finally, four proteins (3 from barley and 1 from rice) showed variations related with the QxVxG reactive site. The significance of these variants on the inhibitory effect against cysteine proteinases has to be determined. The fact that the majority of the cystatins that have significant variations in the first and the second loop are in the groups with no clear homology between rice and barley cystatins supports that these cystatins could have rapidly evolved to inhibit specific cysteine proteinases, probably to fight against pests. So, groups I and II in the unrooted tree, constituted only by barley cystatins would be an example of this specialized evolution. On the other hand, most of the cystatins of group III, which present clear homologies between rice and barley proteins, may be more ancient proteins and those proteins are probably involved in the endogenous regulation of cysteine proteinases in the plant. The existence of these groups of cystatins, may be useful to the assignment of proteinase specificities and gene function to other cystatins as new information is obtained in order to use them as new transgenes against pests and pathogens.

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FIELD STUDIES OF POLLEN-MEDIATED GENE FLOW FROM GM RICE TO CONVENTIONAL RICE AND THE RED RICE WEED

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Abstract
The objective of these studies, carried out in Spain and Italy, was to assess the frequency of pollen-mediated gene flow from a transgenic rice lines, harbouring the *pat* or the *bar* genes, to the red rice weed and to conventional rice. A circular field trial designs were set up in Spain to investigate the influence of the wind on the frequency of pollination of red rice and conventional rice recipient plants with the transgenic pollen. Frequencies of gene flow based on detection of herbicide resistant seedlings among seed progenies of recipient plants and averaged over all the wind directions were in all cases lower than 0.1%. However, for both red rice and conventional rice, a clear asymmetric distribution was observed with pollination frequency favoured in plants placed under the local dominant winds. Examination of the influence on gene flow frequency of the distance from the transgenic source to recipient plots of conventional rice planted at 1, 2, 5 and 10 m distance revealed a clear decrease with increasing distance which was less dramatic under the dominant wind direction. Results obtained in a field trial carried out in Italy suggest that it is feasible to avoid the spread of transgenes to adjacent fields by using low isolation distances and plant barriers.

Agricultural practices, since their inception, have greatly improved crops by selection and breeding. Most of the crop cultivars that are developed are compatible inter se but they are also compatible with related wild or weedy relatives, suggesting that gene flow has always taken place. With transgenic plants, the problem of gene flow has acquired a special significance. The difference between traditional plant breeding programs and those involving transgenic plants is that in the first case only genes coming from compatible species can be introduced, whereas biotechnology allows the introduction of genes from non-compatible organisms. The possibility of transgene flow from engineered crops to other varieties, to their wild relatives or to associated weeds is one of the major concerns in relation to the ecological risks of the commercial release of transgenic plants.

Given the ecological importance of the rice crop (grown on 147 million hectares world wide (FAO, 2003)), it is important to determine the extent of pollen dissemination from transgenic rice to other cultivars in various environmental conditions. Although cultivated rice (*Oryza sativa* L.) is an autogamous plant, its floral architecture being one of the main factors determining self-pollination, cross-pollination is possible and does indeed take place to some extent, the amount depending largely on climatic and variety differences.
To assess the frequency of gene flow from transgenic rice plants to non-transgenic plants of Mediterranean varieties, the first field trials were performed in Spain and Italy (Messeguer et al., 2001) by using transgenic line Thaibonnet T1506-5-6 and Ariete A2504-1-7 respectively, both containing the *pat* gene, conferring resistance to the herbicide ammonium glufosinate. Transgenic and certified non-transgenic seeds of Thaibonnet and Ariete were sown under greenhouse conditions and transplanted in the paddy field when they reached the 4-5 leaf

stage. To evaluate the gene flow from transgenic to non-transgenic plants two different designs were used: a normal side-by-side plot design in Italy to investigate the influence of distance from the margins (borders) of the non transgenic rice field on the frequency of pollination of a recipient plant by transgenic pollen, and a circular design conducted in Spain aimed at evaluating the influence of the wind on pollen dispersal and flight distance in open field conditions to nearby cultivated rice fields. In this case, transgenic plants were placed in the centre and non-transgenic plants were placed in concentric circles around transgenic plants, at 1 m or 5 m distance. All panicles of the non-transgenic plants were individually harvested and their location (in relation to the geographic orientation in the field) was recorded. Samples of the seeds of each plant were sown in a greenhouse and seedlings at the 3-4-leaf stage were treated with commercial herbicide. Integration of the *pat* gene was confirmed by PCR and Southern analysis.

In both locations analyses of phenotypic, molecular and segregation data showed that pollination of recipient plants with pollen of the transgenic source did occur at a significant frequency. A gene flow slightly lower than 0.1% was detected in a normal side-by-side plot design. Similar results were found in the circular plot when the plants were placed at 1 m distance from the transgenic central nucleus. A strong asymmetric distribution of the gene flow was detected among this circle and highest values (0.53%) were recorded following the direction of the dominant wind. A significant lowest value (0.01%) was found in the other circle (5 m from the transgenic plants) as was expected according to characteristics of rice pollen.

Recently, new forms of weedy rice -often called red rice due to the frequently occurring red coloration of the seed pericarp- have emerged in direct seeded, irrigated paddy fields of Mediterranean rice growing areas where related wild species are not found. The importance of gaining more knowledge of gene flow from cultivated rice to the red rice weed has become of acute significance with the rapid development of rice genetic engineering and the perspective of deployment of transgenic rice crops in temperate and tropical areas. Though the number of field trials of transgenic rice have been so far limited to a few traits (Oard et al. 1996, Tu et al. 2000a ,b) a very diverse range of genetically engineered genes has already been introduced into rice (recently reviewed in Tyagi and Mohanty 2000). Although no commercial release has been reported, it is expected in the next few years, and resistances to the herbicides ammonium glufosinate and glyphosate are among the first on the list. Though resistance to a total herbicide may be of interest to control red rice in paddy fields, benefits could last short if the transgene is transferred by pollination from cultivated transgenic rice to its weed derivative.

Even though it is known that red rice strains and cultivated rice can produce viable and fertile hybrids, which exhibit the dominant traits of the parental weed (Langevin 1990, Noldin et al. 1999), there were few data available to quantify the gene flow from transgenic rice to red rice weed in field conditions. So, another field trial was performed to assess
the frequency of pollen-mediated gene flow from a transgenic rice line, harbouring the *gusA* gene and the *bar* gene encoding respectively, β-glucuronidase and phosphinothricin acetyl transferase as markers, to the red rice weed and conventional rice in the Spanish *japonica* cultivar Senia (Messeguer et al, 2004). A circular field trial design was set up to investigate the influence of the wind on the frequency of pollination of red rice and conventional rice recipient plants with the transgenic pollen. Transgenic homozygous seeds, certified non transgenic seeds of Senia and red rice seeds were sown in greenhouse conditions and transplanted in the paddy field when they reached the 4-5 leaf stage. Seedlings were

transplanted to the field in concentric circles with inter-plant distance of 25 cm. The inner circle (3 m diameter) was planted with non-transgenic plants and surrounded by one circular row of red rice plants, in turn surrounded by two successive circular rows of transgenic plants, a circular row of red rice plants and then a last circular row of non-transgenic plants. The idea was to have red rice circles surrounding internally and externally a ring of transgenic plants and to protect this experimental set with Senia control plants to avoid a border effect. To evaluate the influence of the distance on gene flow frequency, 8 plots of 8 non-transgenic plants were planted at 1 m, 2 m 5 m and 10 m distances from the rings of transgenic plants, following the 8 directions of the compass card. After flowering, panicles from red rice plants were covered with an anti-hail net to avoid seed dissemination into the field. Panicles from all plants were manually and individually harvested, and their location in relation to the geographic orientation was recorded in the field.

Frequencies of gene flow based on detection of herbicide resistant, GUS positive seedlings among seed progenies of recipient plants averaged over all wind directions and considering together the inner and outer recipient plants were 0.036 ± 0.006 % and 0.086 ± 0.007 for red rice and conventional rice respectively. However, for both red rice and conventional rice, a clear asymmetric distribution was observed with pollination frequency favoured in plants placed under the local prevailing winds. Southern analyses confirmed the hemizygous status and the origin of the transgenes in progenies of surviving, GUS positive plants. Gene flow detected in conventional planted at 1, 2, 5 and 10 m distance revealed a clear decrease with increasing distance which was less dramatic under the prevailing wind direction. Results obtained in this trial together with the particular characteristics of rice cultivars such as self-pollination, pollen viability and fertilisation process, suggest that in the case of rice transgenic plants, the risk of gene flow to adjacent non-transgenic fields could be minimized by establishing a security distances and pollen containment barriers taking into account the prevailing winds that clearly improve pollen dispersal and cross-pollination.

Results obtained in this field trial suggest that within a commercial transgenic rice field, the influence of the wind appears a less determinant factor than in our circular design, because red rice plants usually will grow isolated or in patches surrounded by transgenic plants and consequently can be pollinated by all of them. On the other hand, the wind influence on cross-pollination has to be taken into account for the plants growing in the borders. This is a very essential question to consider because the real introgression of the genes will be minimized inside the field by the usual control practices tending to destroy the red rice, but the wild plants in the borders can act as reservoirs of the transgenic characters. Moreover, although the gene flow values are relatively low, the shattering and dormancy of the red rice seeds, which ensure their persistence in the field, lead into an undesirable effect of durability of the transferred genes. Therefore, whether one wants to avoid gene flow to the red rice, crop management has to be considered.

As to the peculiar case of the red rice weed, pollen-mediated gene flow between red rice and transgenic rice should be examined in both ways, since the pollination of a transgenic plant by red rice pollen may result in the conversion of its progeny into a transgenic red rice hybrid weed, because of the dominance of the wild traits (Langevin 1990, Noldin et al. 1999).

To check the spontaneous pollination from red rice to Senia transgenic plants, a preliminary assay was performed taking into account that red rice plants used in the previous field trial were much more vigorous than transgenic Senia plants. So, about 10 250 seeds collected from 41 transgenic Senia plants (250 seeds/plant/tray) were sown in greenhouse conditions. At 3-4-

leaf stage, transgenic seedlings were treated with the herbicide Finale to check their resistance. After one month in culture, the most vigorous plants (at least 2 plants per tray) were chosen and classified in two categories: 37 seedlings that clearly were much more vigorous than the rest (+) and 49 seedlings that in spite of the fact they have grown more that the rest of the plants of the tray, they were not as vigorous as the others (-). All these plants were cultured in greenhouse conditions till flowering for further analysis (AFLP pattern, colour and grain dehiscence).

The analysis of these plants shows that the 100% of analysed plants from (+) group presented grain dehiscence and red coloured grains, whereas only 14% of the other group (-) presented the same characteristics. The AFLP pattern of the analysed plants till now confirms these results.

Frequency of reverse gene flow detected in this preliminary study was of 0.45%. This value is higher than that obtained with previous studies to evaluate the gene flow from transgenic to red rice plants. This can be explained by the fact that red rice plants are taller than transgenic ones, and it's easier that pollen falls down to pollinate transgenic plants, whereas pollination in the opposite direction needs special conditions such as the influence of the wind.

The fact that the reverse flow detected is higher than the direct one, may not have a significant ecological impact on the dissemination of transgenic red rice plants because hybrid seeds remain attached to the spike and in consequence they will be collected during harvesting whereas hybrid seeds produced by pollination from transgenic plants to the red rice, may fall down to the field. In this case more studies are needed to determine the real introgression rate of transgenes into red rice in field conditions.

Another set of experiments have been performed in year 2002, in Italy, aimed at determining the proper experimental design indicated to perform GM rice field trial in a condition of complete containment. In this experiment a nucleus of GM rice plants cv.Senia, transgenic for the *bar* gene – and therefore resistant to the herbice Basta – was positioned in the middle of a 5000 m^2 room of a conventional variety taken as a barrier. The cv.Koral was chosen, being taller than the GM-Senia plants of the nucleus, also flowering a bit later than cv.Senia. Between the barrier plants and the GM nucleus, a space of 1 m was left all around, and kept free of weeds. At flowering time of the GM Senia plants, 80 contemporary flowering panicles were labelled on the edge of the containment barrier, close to the GM nucleus. This situation was aimed at recording the eventual gene flow from the GM plant to the conventional barrier plants, having no barriers between them. Moreover, an identical sampling was made on plants placed 1 m inside the barrier plant room, in order to measure the gene flow from the GM to the conventional plants with 1 m free space plus an additional 1m of barrier plants. A total number of 160 panicles were sampled, with a total number of 16500 seeds. Plantlets derived from those progeny were sprayed at the third leaf stage, in order to check for resistant seedlings: if present, in fact, they would have derived from accidental pollination of a conventional plant with transgenic pollen. The results obtained were negative: none of the seedlings tested exhibited resistance to Basta, thus confirming the absence of gene flow, either in the sampling, closer to the GM plants, either in the sampling the most distant one, in the conditions tested. It has also to be pointed out that in the tested conditions there was almost no wind detectable during the rice-growing period, thus reducing the possibility for pollen to flow and contaminate surrounding plants. The experiments also allowed to test eventual gene flow toward the weedy rice (red rice) which was spontaneously present in the room, interspersed in the barrier plants. Panicles of 35 weedy rice plants, flowering at the same time of the GM plants were sampled, for a total of 10500 seeds. After a cold treatment aimed to break dormancy, seedlings germinated at a rate of 85-90% and were sprayed with Basta. Also in this test, none of the seedling survived, thus confirming the "barrier effect" of the containment plants of the room. Our experimental design and the results obtained confirmed the possibility to perform GM rice field trials with no risk of contamination of the surrounding cultures, once defined containment strategies are adopted.

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SELECTION FOR HIGH AND LOW HARVEST INDEX IN RICE (*ORYZA SATIVA* L.) UNDER TEMPERATE CONDITION

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Abstract

The objectives of this study were to determine the response to selection for high and low harvest index, to estimate the heritability of harvest index and to compute the correlations of harvest index with the other agronomic traits in the different generations of rice (*Oryza sativa* L.) The research was conducted at Thrace Agricultural Research Institute in 1995 and 1996. Two segregating populations for each generation were used in F_3, F_4, and F_5 in this study. The results of the study indicated that the selection for harvest index was effective in establishing lines with high harvest index in early generations. The selection for harvest index would contribute as much as direct selection for grain yield; also, the selections gains for harvest index were higher than selection gains of grain yield. Harvest index had moderate heritability estimates. High harvest index selection resulted earliness in heading and shorter plant height than selection for low harvest index. However, high harvest index progenies had less biological yield than low harvest index progenies. These results revealed that high harvest index selection always did not give high biological yield. Therefore, selection based on high harvest index would be better considering high biological yield into account at the same time.

Keywords

Rice (*Oryza sativa* L.); harvest index; selection; heritability; correlation

Introduction

The productivity of a crop can be evaluated by (a) biological yield, or total biomass, and (b) economic yield or the yield of usable part, that is grain yield in cereal and legume crops. The ratio of grain yield to biological yield (excluding root weight) is defined as harvest index. Since the introduction of the term "harvest index" by Donald (1962), it has been considered an important trait for yield improvement in cereal. Donald and Hambling (1976) suggested the use of biomass and harvest index as early generation selection criterion in cereal breeding programs. Harvest index along with grain yield has been considered as a selection criterion in improving yield of cereals by several researchers. Syme (1972) studied 49 spring wheat genotypes and found that 72% of the grain yield variability in the field could be estimated on the basis of harvest index values from single plants grown in a greenhouse. Austin et al., (1980) observed the increases in grain yield due to variety improvement being associated mainly with greater harvest index in winter wheat. Sharma and Smith (1986) examined the effect of low and high harvest index selection in three winter wheat populations, selection in F_3 for high an low HI was effective in identifying F_4 lines with high and low HI, respectively. Also, selection for high HI resulted in shorter plants with earlier heading dates and lower biomass yield. Whereas, Takade and Frey (1985) reported that intensive selection for harvest index in oats resulted in high harvest index, but no grain yield improvement, because the selected lines had poor vegetative vigour.

Indirect selection for grain yield through harvest index in oats (*Avena sativa* L.) was 43% as efficient as direct selection (Rosielle and Frey, 1975). Bhatt (1977) reported that harvest index

was a useful selection criterion for improving grain yield of two wheat crosses in segregating generations.
Venkatesmarlu and Prasad (1982) reported increased maturity duration associated with higher biomass in rice but higher biomass was not related to higher HI. Selection based on high HI would be better considering the advantages, however an ideal combination would be higher HI related with higher biomass. Lin (1990) compared low and high harvest index selection in rice, and he reported that higher harvest index tended to give higher grain yield, shorter plant height and earliness of maturity. Lin (1992) reported that indirect selection for grain yield in Taiwan through harvest index was 8.5 and 35% as efficient as direct selection in the first and second crops, respectively.
Lin (1990) reported that harvest index correlated positively with grain yield and total dry weight while, its relations with days to heading and plant height were negative in rice. On the other hand, Lin (1992) found negative correlation between HI and biological yield, and positive with grain yield. Roy and Kar (1992) reported that the harvest index correlated negatively with plant height and days to flowering. Sürek at al., (1998) obtained similar correlations with days to flowering. Positive correlations observed between HI and grain yield by Roy and Kar (1992), Sunderam and Palanisamy (1994), and Sürek et al., (1998).
Rossielle and Frey (1975) reported realized heritability values for harvest index between 0.35 and 0.66 in oat lines derived from a bulk populations. Bhatt (1977) reported that realized heritability estimated for harvest index were 0.70 and 0.88 in two wheat crosses. Sharma and Smith (1986) estimated realized heritabilities for harvest index ranged from 0.44 to 0.60 in three wheat populations. Lin (1990) estimated quite high heritability for harvest index 64..3 % in rice.
The objectives of this study were to determine the response to selection for high and low harvest index, to estimate the heritability of harvest index to compute the correlations of harvest index with the other agronomic traits in the different generations of rice (*Oryza sativa* L.).

Materials and methods

Two cross populations were used for each generation in F_3, F_4, and F_5 for this study. The crosses and the number of lines utilised in the experiment were given in Table 1.
The characteristics of the parents involved in these crosses shown in Table 2. As it is seen in the table. There were large variation among the varieties for harvest index and some other characters.
Within each population, the lines shown in table-1 were randomly selected by taking one panicle from each plant in 1994, in F_2, F_3, and F_4 generations. They were grown together with parents in 2m long single plant rows spaced 25. The rice was drill-seeded on 27 May 1995. When the seedlings reached the four or five-leaf stage, the plant were thinned to an uniform density. Harvested area was 1.5 x 0.25= 0.37 m^{-2} , to avoid side effect, excluded 25 cm distance from both side in each row. After harvest, each line was studied for harvest index and grain yield. After then, 10 high and 10 low harvest index and grain yield lines were selected in each population. Also, 10 lines were randomly selected from each population to represent a random sample. These selected lines in each population grown together with their parents in a randomised complete block experiment design with three replications. Each entry was drilled in a plot which consisted of two 2m rows spaced 25 cm apart on 30 May 1996. 400 seed per m^{-2} were used in planting.

Table 1 The cross populations and the number of lines used in the experiment.

No	Cross	Generation	The number of lines
1	Trakya x Lido	F_3	51
2	Trakya x M-102	F_3	52
3	İpsala x N1-41T-1T-0T	F_4	52
4	Altınyazı x Titanio	F_4	52
5	Krasnodarsky-424 x Europa	F_5	52
6	Baldo x Balilla-28	F_5	45
	Total		304

Table 2 The characteristics of the parents used in the crossing for the populations.

Variety	Origin	Days to flowering	Plant height (cm)	Harvest index (%)	1000 grain weight (g)
Baldo	Italy	85	105.0	46	39.0
Ballila-28	France	90	87.7	42	28.0
Trakya	Turkey	85	112.3	44	36.6
M-102	ABD	91	91.0	45	29.4
Altınyazı	Turkey	86	118.0	39	36.8
Titanio	Italy	75	81.3	46	30.1
Lido	Italy	83	84.3	40	25.3
Krasnodarsky-424	Russia	77	117.7	45	30.6
Europa	Italy	90	96.0	41	29.4
İpsala	Turkey	85	116.7	45	39.7
N1-41T-1T-0T	Turkey	85	100.0	45	30.7

When the seedling reached the four or five-leaf stage, the plant were thinned to an uniform density. Harvesting area was 0.5 x 1.8 =0.9 m^2. In both years, fertilizers was applied at rate 150 kg N and 80 P ha^{-1}. All phosphorus and 1/3 part of N applied at preplanting, 1/3 at tillering and remaining at panicle initiation.

The data recorded for days to flowering, plant height, panicle length, productive panicles per square meter, the number of filled grains per panicle, 1000 grain weight, biological yield and grain yield.

Analysis of variance was carried out to study the variation among the lines for the examined traits. Phenotypic correlations were computed the procedures described by Steel and Torrie (1960), to study the associations of characters with harvest index. Heritability of harvest index and grain yield in the narrow sense was estimated from the regression of Fg_2 on F g_1 values (g_1 : initial generation and g_2 : the following generation). Realized heritability (h^2) estimated for harvest index and grain yield according to Falconer (1960) and Sharnma and Smith (1986). Using the means of high and low progenies, this formula was utilised to calculate; realized heritability = High-Low Fg_2/High-Low g_1. The paired t-test was used to compare the mean differences between lines selected for high and low harvest index and grain yield groups. The observed gain from selection for high harvest index and grain yield were computed as the difference between the mean of the high lines and the mean of random selected lines for these traits. Gains from selection for low harvest index and grain yield were computed in the same way with appropriate substitutions (Bhatt, 1977)

Results and Discussion

The result of variance analysis showed that there were significant differences among the lines for grain yield, harvest index, biological yield, days to flowering and plant height.

As it seen in Table 3 selection for high and low harvest index was effective to obtain high and low harvest index progenies in the following generations. Except one population in F_4 generation (Trakya x Lido), significant differences between high and low harvest index groups continued in F_4 , F_5 , and F_6 generations. Thus, selection for higher or lower harvest index was effective in early generations. Bhatt (1977), Sharma and Smith (1986) in wheat, Rosielle and Frey (1975) in oat, and Lin (1992 and Lin 1990) in rice reported the similar results.

Table 3 Comparison of the means for harvest index and grain yield for the low and high selection groups in different generations.

Character	Population											
	Trakya x Lido						Trakya x M-102					
	F3			F4			F3			F4		
	H	L	Dif	H	L	Dif.	H	L	Dif.	H	L	Dif
Harvest index (%)	49.6	40.8	8.8**	39.5	36.0	3.5	49.5	30.0	19.5**	29.2	19.6	9.6**
Grain yield (g/m^2)	877	583	294**	608	595	13	827	485	342**	558	503	55
	Population											
	Altınyazı x Titanio						İpsala x N1-41T-1T-0T					
	F4			F5			F4			F5		
	H	L	Dif.	H	L	Dif.	H	L	Dif.	H	L	Dif.
Harvest index (%)	46.6	28.1	15.5**	35.4	28.1	7.3**	49.3	32.9	16.4**	36.6	29.8	6.8**
Grain yield (g/m^2)	873	494	379**	611	569	42	1072	591	481**	681	603	78
	Population											
	Baldo x Ballila-28						Krasnodarsky-424					
	F5			F6			F5			F6		
	H	L	Dif.	H	L	Dif.	H	L	Dif.	H	L	Dif.
Harvest index (%)	52.2	30.6	21.6**	42.7	29.8	12.9**	48.6	28.0	20.6**	36.4	29.6	6.8**
Grain yield (g/m^2)	850	454	396**	685	542	143**	765	256	509**	549	517	32*

Table 4 The mean values of harvest index, grain yield and total biological yield for high and low harvest index selection groups in different generations.

Characters	Selection groups	Trakya x M-102 F4	Trakya x Lido F4	Altınyazı x Titanio F5	İpsala x N1-41T-1T-0T F5	Krasnodarsky-424 x Europa F6	Baldo x Balilla-28 F6
Harvest index %	High	29.2	39.5	35.4	36.6	36.4	42.7
	Low	19.6	36.0	28.1	29.8	29.6	29.8
	Difference	9.6**	3.5	7.8**	6.8**	6.8**	12.9**
Grain yield g/m^2	High	526.5	681.1	606.5	661.0	555.5	694.3
	Low	368.6	600.2	518.6	576.4	517.6	438.1
	Difference	157.9**	80.9	87.9**	84.6**	47.9	256.2**
Biological yield g/m^2	High	1849.5	1726.3	1715.1	1793.8	1580.5	1708.9
	Low	1910.3	1692.0	1930.4	1885.2	1714.7	1455.0
	Difference	-60.8	34.3	-215.3**	-91.4	-134.2**	-253.4

Table. 5 The mean values of harvest index, grain yield and total biological yield for high and low grain yield selection groups in different generations.

Characters	Selection groups	Trakya x M-102 F4	Trakya x Lido F4	Altınyazı x Titanio F5	İpsala x N1-41T-1T-0T F5	Krasnodar sky-424 x Europa F6	Baldo x Balilla-28 F6
Harvest index %	High	31.6	33.6	33.1	38.0	32.4	40.0
	Low	27.8	34.9	31.4	32.6	31.0	36.5
	Difference	3.8	-1.3	1.7	5.4**	1.4	3.5**
Grain yield g per m^{-2}	High	558.2	608.3	611.2	681.1	548.8	685.3
	Low	503.1	595.0	569.7	603.3	517.6	541.8
	Difference	55.1	13.3	41.5	77.8	31.2*	143.5**
Biological yield g per m^{-2}	High	1844.0	1809.0	1870.6	1632.5	1760.5	1734.5
	Low	1865.4	1752.0	1863.2	1845.1	1628.3	1542.5
	Difference	-21.4	57.0	7.4	-212.6**	131.7	192.0

** and ** significant at 0.05 and 0.01 level, respectively.*

Table 6 The means of days to flowering and plant height for low and high selection groups of the harvest index and grain yield in different generations.

Character	Population				
	Trakya x Lido (F4)			Trakya x M-102 (F4)	
	Selection group	Grain yield	Harvest index	Grain yield	Harvest index
Days to flowering	Low	89.2	90.0	90.8	91.8
	High	90.2	90.3	90.3	89.2
Plant height (cm)	Low	108.0	105.7	102.2	105.9
	High	105.0	106.0	102.4	103.4
	Altınyazı x Titanio (F5)			İpsala x N1-41T-1T-0T (F5)	
	Selection group	Grain yield	Harvest index	Grain yield	Harvest Index
Days to flowering	Low	90.1	91.4	90.8	91.8
	High	90.5	89.8	88.9	89.0
Plant height (cm)	Low	112.8	113.9	110.9	111.8
	High	108.2	110.9	103.2	99.9
	Krasnodarsky-424 x Europa (F6)			Baldo x Balilla-28 (F6)	
	Selection group	Grain yield	Harvest index	Grain yield	Harvest index
Days to flowering	Low	91.5	91.9	88.7	90.1
	High	92.5	91.8	87.7	85.7
Plant height (cm)	Low	104.5	98.0	111.0	104.4
	High	104.6	98.6	104.6	102.6

The high and low harvest index selection groups significantly differed for grain yield in four populations in the different generations, whereas, the differences were not significant, but positive in two populations in one F_4 and one F_6 (Table 4). The high harvest index selection groups produced lower biological yield than the low harvest index groups almost in all generations. In the different generations, the lines selected on the basis of high harvest index had higher grain yield while those selected for low harvest index had always lower grain yield. These findings are in agreement of the results obtained by Bhatt (1977), and Lin (1990). Sharma and Smith (1986) reported similar results for biological yield, however, it was contrast for grain yield.

High and low selection groups for grain yield was not effective as much as harvest index (Table 5). There were not significant difference between high and low grain yield progenies for grain yield in F_4 and F_5 generations while there were significant differences in two F_5 populations. On the other hand, high grain yield selection usually gave higher biological yield than high harvest index selection.

Selection for high harvest index resulted earliness in heading date and shorter plant height in different generations (Table 6). Shorter plants resulting from selection for high harvest index are desirable in rice breeding programs. Also, selection for high grain yield resulted shorter plant height. Sharma and Smith (1986) and Lin (1990) reported similar results for high harvest index selection.

Realized heritability (h^2) estimates for harvest index ranged from 21 to 63% and it ranged from 13 to 68% for grain yield in different F generations. Heritability in narrow sense estimated from the regression between generations ranged from 22 to 69% for harvest index and it ranged from 18 to 69 for grain yield (table-7). In general, there were similarity between realized and narrow sense heritability values in terms of magnitude. Although the magnitude of the heritability values changed generation to generation.. Similar results for harvest index obtained by Rossielle and Frey (1975) in oat, Bhatt (1977) and Sharma and Smith (1986) in wheat and Lin (1990) in rice. The gain from selection for harvest index in the crosses in the different generations resulted in an average of 6.1% to 16.8%, while it resulted in an average of 1.4% to 15.1% for grain yield (Table 7).

Table 7 Estimates of heritability and gain from selection for harvest index and grain yield.

Estimate	Population			
	Trakya x Lido (F4)		Trakya x M-102 (F4)	
	Harvest index	Grain yield	Harvest Index	Grain yield
Heritability (%) from F4/F3 regression	22	43	61	36
Realized heritability (%)	36	13	48	37
Gain from selection (expressed as % of mean) observed for;				
(a) High harvest index	10.6	0.3	2.1	2.9
(b) Low harvest index	1.6	2.5	31.4	4.6
(c) Mean gain	6.1	1.4	16.7	3.7
Estimate	Altınyazı xTitanio F5		İpsala x N1-41T-1T-0T F5	
	Harvest index	Grain yield	Harvest Index	Grain yield
Heritability (%) from F5/F4 regression	42	18	32	28
Realized heritability (%)	48	34	47	51
Gain from selection (expressed as % of mean) observed for;				
(a) High harvest index	13.0	5.3	2.8	6.9
(b) Low harvest index	10.8	1.9	16.3	5.5
(c) Mean gain	11.9	3.6	9.6	6.2
Estimate	Krasnodarsky-424 x Europa F6		Baldo x Balilla-28 F6	
	Harvest index	Grain yield	Harvest Index	Grain yield
Heritability (%) from F6/F5 regression	17	30	69	77
Realized heritability (%)	21	32	63	68
Gain from selection (expressed as % 0f mean) observed for;				
(a) High harvest index	13.7	1.8	12.3	22.8
(b) Low harvest index	7.5	4.5	21.3	7.4
(c) Mean gain	10.6	3.1	16.8	15.1

However, the gains observed for harvest index selection were higher than for grain yield in the crosses of different generations. These results for harvest index are in agreement with those reported by Bahtt (1977) in wheat.

Phenotypic correlation coefficients of harvest index with grain yield were positive and significant in all the generation studied in both years 1995 and 1996 (Table 8). Whereas, it correlated negatively significant with biological yield and days to flowering and in some population with plant height. Among, the yield components, the number of filled grains per panicle and 1000 grain weight had positive and significant correlations with harvest index in some generations in both years. Similar results reported by Sharma and Smith (1986) in wheat, Lin (1990), Roy and Kar (1992) in rice for days to flowering and plant height. On the other hand, Lin (1990); Roy and Kar (1992); Sunderam and Planisamy (1994), and Sürek et al., (1998) reported positive and significant associations between grain yield and harvest index. Also, Lin (1992) observed negative correlations between harvest index and biological yield in rice. On the contrary, Lin (1990) reported positive relation between harvest index and biological yield.

Table. 8 Correlations between harvest index and other agronomic traits in the different generations in 1995 and 1996.

Character	Population								
		Trakya x M-102	Trakya x Lido		Altınyazı x Titanio	İpsala x N1-41T-1T-0T		Krasno424 x Europa	Baldo x Balilla-28
Days to flowering	**F3**	-0.493**	–0.429**	**F4**	-0.250	-0.368**	**F5**	0.024	-0.401**
	F4	-0.641**	-0.522**	**F5**	-0.414**	-0.757**	**F6**	-0.706**	-0.885**
Plant height	**F3**	-0.334**	-0.222	**F4**	-0.286*	-0.014	**F5**	0.100	-0.275
	F4	-0.102	-0.186	**F5**	-0.292*	0.055	**F6**	-0.174	-0.438**
Panicle length	**F3**	0.142	-0.031	**F4**	0.199	0.140	**F5**	0.013	0.043
	F4	-0.383**	0.085	**F5**	-0.067	0.282	**F6**	0.118	0.056
No. of productive paniclesper m^{-2}	**F3**	0.281*	-0.001	**F4**	-0.284*	0.201	**F5**	0.039	0.053
	F4	0.176	0.062	**F5**	-0.193	-0.282	**F6**	0.118	0.0140
No. of filled grains per panicle	**F3**	0.300*	0.124	**F4**	0.592**	0.089	**F5**	0.630**	0.206
	F4	0.827**	0.446**	**F5**	0.619**	0586**	**F6**	0.575**	0.575**
1000 grain weight	**F3**	0.326**	0.352**	**F4**	0.047	0.146	**F5**	-0.063	0.415**
	F4	0.287*	0.211	**F5**	0.247	0.444**	**F6**	0.119	0.552**
Biological yield	**F3**	-0.502**	0.004	**F4**	-0.193	-0.049	**F5**	0.431**	-0.363**
	F4	-0.411**	-0.414**	**F5**	-0.476**	-0.441**	**F6**	-0.471**	-0.321**
Grain yield	**F3**	0.328**	0.412**	**F4**	0.466**	0.417**	**F5**	0.751**	0.605**
	F4	0,931**	0,749**	**F5**	0.750**	0.756**	**F6**	0.794**	0.765**

** and ** significant at 0.05 and 0.01 level, respectively.*

Conclusion

In conclusion, the results of this study indicate that selection for harvest index was effective in establishing lines with high harvest index in early generations. The selection for harvest index would contribute favourably to grain yield. It was effective as much as direct selection for grain yield : also, the selection gains of harvest index were higher than selection gains of grain yield. Harvest index had moderate heritability estimates. It indicates that harvest index may be used as a selection criteration in indirect selection in early generations. High harvest index selection resulted earliness in heading and shorter plant height than selection for low harvest index. However, high harvest index progenies had less biological yield than low harvest index progenies. This results also showed that high harvest index selection always did not give high biological yield. Higher harvest index was not related to higher biological yield. Therefore, selection based on high harvest index would be better considering high biological yield into account at the same time.

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VARIETIES AND DIRECTIONS OF RICE BREEDING IN UKRAINE

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Abstract
The main factor of increase the production of rice in Ukraine is creation and introduction in production new high-yielding varieties. Modern plant breeding work on rice in Institute is conducted with use of genetic potential samples of a world collection. Best of them, after careful study, are used in hybridization with the purpose of reception of hybrid lines combining in all necessary parameters of model of variety. For conditions of Ukraine the rice variety must have such parameters, as: high potential of efficiency, complex stability to the pests, diseases and lieing flat, cold resistance at period of reception sprouts and shaping the harvest, resistance to salinization of soil, high output of groats, short growing period. The special attention at creation of varieties is given to quality of grain, output of ready production, its marketable state. For last years the selectionists of Institute create a number of new high-yielding varieties. These Dneprovskiy, Jantarniy, UkrSRS-6570 are early ripening varieties with growing period 105-115 days and Ukraine-96, Zubets, Slavutich, Memory of Gichkin, Antey are mid-ripening varieties with growing period 116-125 days. The new rice varieties rather plastic to changes of conditions of cultivation, are characterized by high technological and economic signs, are capable to give high yields at a level 6,5-7,0 t ha^{-1} and more.

Key words
breeding; quality; yield.

Introduction
Rice is one of main agricultural cultures of our planet. In general grain balance of Ukraine it borrows an insignificant share, but as the valuable dietary product has the important meaning. In Ukraine the natural and climatic conditions, necessary for its cultivation, are available only in southern regions: at the Crimea, Kherson and Odessa regions, where in 1961-1963 the rice irrigation systems were constructed, entered on area near 60 thousand ha, mainly on salinization and demanding improvement of land-reclamation properties of soils.
Primary factor of increase of rice production in Ukraine is the creation and introduction in agricultural production new highly productive varieties.
The modern breeding work in rice in Institute is conducted with use of genetic potential samples of a world collection and local forms of cultural rice, which have a huge variety of traits and properties. Best of them, after careful study are used in hybridization with the purpose of reception of hybrid lines, combining in yourself all necessary parameters of model of a future rice variety.
For conditions of Ukraine the rice variety must have such parameters as: high potential of efficiency, complex stability to the pests, diseases and lieing flat, cold resistance at period of reception sprouts and shaping the harvest, resistance to salinization of soil, high output of groats, short growing period. The special attention at creation of varieties is given to quality of grain, output of ready production, and its marketable state.

The rice breeding process in Ukraine

The breeding work in Institute of rice carry out on the following directions:

- creation of early-maturing rice variety with productivity of 5,5-6,0 t ha^{-1}, total milled rice of 69-70%, growing period 105-115 days;
- creation middling-maturing rice variety with productivity 8,0-10,0 t ha^{-1}, total milled rice of 69-70%, growing period 115-125 days;
- creation long-grain rice variety of indian subspecies with productivity of 5,5-6,0 t ha^{-1} and high quality of a grain.

The success of breeding work is substantially defined by presence of a genetic variety of an initial material, which adapted to concrete soil and climatic conditions. Therefore, since 1992 in Institute of rice, together with the National center of plants genetic resources of Ukraine the works on introduction, formation, study and use in breeding practice of samples of a National collection are developed. In the given period the collection totals more than 500 samples from different ecological regions: Russia, Ukraine, Kazakhstan, Uzbekistan, Japan, China, France, Spain, Italy, Portugal, Bulgaria, Hungary, Romania, India and other.

The varieties are studied on duration of growing period, productivity, resistance to lodging, falling its grain and rice blast, on elements of productivity (general and productive bushing out, height of plants, length of panicle, quantity of grains on panicle, weight of grains on panicle and plant, 1000 grains weight), and also on quality of a grain (filminess, translucency, cracking, total milled rice, head rice yield).

For reception of an initial material the method hybridization is used. In hybridization only those varieties are included, which it is valid in conditions of the south of Ukraine have one or several attributes and properties, valuable in the breeding relation. Annually in hybridization 20-25 varieties of a National collection are included. For castration the pneumatic method, for pollination - twell-method are used. Average set of hybrid grains changes on years within the limits of 9,0-25,0%. The reproduction of hybrid populations will be carried out on vegetation platform and in a field.

In breeding work is applied linear-population method, which including reproduction of perspective hybrid populations, sometimes up to F_7-F_8 and parallel individual selection on a complex economic valuable attributes, since F_2 (Figure 1).

The selection of plants will be carried out in view of productive bushing out, height of plants, panicle length, setting, translucency, form and sizes of a grain, duration of vegetation period.

The early- maturing forms are selected on terms of approach of a phase shoot up of panicle.

At the first stages of breeding process percent of rejection unvaluable selection material on the average makes: selection nursery – 70,0-80,0%; check nursery – 55,0-60,0%. For the period 1963-2003 the 28 varieties of rice were transferred to the State variety test, 10 from them is brought in the Register of plants variety of Ukraine.

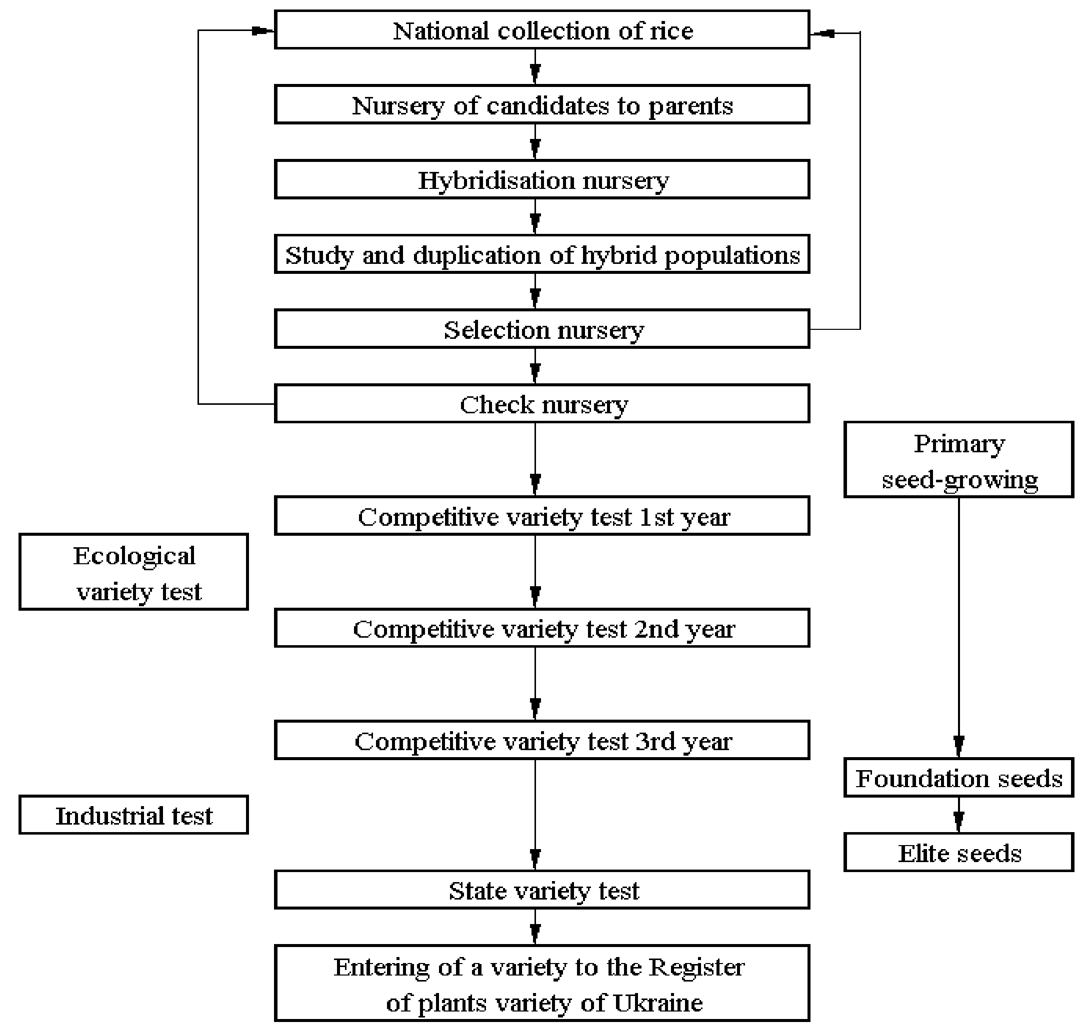

Fig. 1. The circuit of selection process.

New released varieties

In 2001-2003 four new varieties of rice: Ukraine-96 (2001), Dneprovskiy (2002), Slavutich, Zubets (2003) are brought in to the Register of plants variety of Ukraine.

Dneprovskiy is the early-maturing variety, in conditions of Ukraine ripens for 115-116 days; the top yield 8,2 t ha^{-1} is received in Institute of rice in 2000. Height of plants 75-80 cm, leaves are short, wide, green, depart under a sharp corner from a stalk. The panicle is short, dense, erect, long of 14-15 cm, carries 110-120 little-ears. 1000 grains weight is 30-32 g. Total milled rice is 68,5-69,5%, head rice yield is 90-92%.

Ukraine-96 is middling-maturing variety. The growing period is 120-125 days. Productivity of variety is 7,0-7,8 t ha^{-1}. 1000 grains weight is 30-31 g. Total milled rice is 69,0-70,0%, head rice yield is 90-91%. The variety is characterized by high ability to shooting.

Slavutich is middling-maturing variety, ripens in conditions of Ukraine for 115-118 days. 1000 grains weight is 33,0-34,0 g. The grain has lengthened form – l/b = 2,5-2,7. Total milled rice is 66,0-67,5%, head rice yield is 88,5-90,5%.

Zubets is middling-maturing, cold-resistant variety of rice, ripens for 122-126 days. Characteristic feature of a variety is intensive violet colouring of cauline units, leaf tendrils, conducting bunches, edges of leaves, little-ear scales. 1000 grains weight is 31,5-32,0 g. Productivity of variety is 7,5-8,0 t ha^{-1}.

In 2003 three new varieties of rice are transferred on State variety test:

Yantarniy is new, long-grain, early-maturing variety of rice, created on the basis of purposeful crossing subspecies indica × japonica, var. mutica. The growing period is 114-116 days. Height of plants is 90-95 cm. The panicle is long, wilting, friable, carries 110-120 little-ears. An index of a grain is 3,1-3,2. 1000 grains weight is 30,5-31,5 g. Translucency is 98,5-100,0%. Productivity of a variety is 6,0-6,5 t ha^{-1}.

Memoriy of Gichkin is middling-maturing variety, which ripening for 120-125 days. The plant is compact, erect, is resistant to lodging. 1000 grains weight is 32,5-33,5 g. Total milled rice is 68,5-69,5%, head rice yield is 91,0-92,0%. Productivity of a variety is 7,5-8,5 t ha^{-1}.

Antey is middling-maturing variety, ripens in conditions of Ukraine for 125-128 days. A variety is hy-yielding, productivity is 8,0 -8,8 t ha^{-1}. The panicle is short, dense, erect, carries 140-160 little ears. 1000 grains weight is 29,5-30,5 g. Total milled rice is 69,0-70,5%, head rice yield is 90,0-92,0%.

The new varieties answer all conditions of technology of rice cultivation, are plastic to conditions of environment, characterized by high technological and economic properties and capable to give high and stable yield.

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Річні звіти по завданню «Створити нові високопродуктивні сорти рису з високою якістю крупи і провести їх попереднє розмноження та розробити дляних основні елементи сортової агротехніки». – Скадовськ. – 2000 – 2003 р.р.

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Session 3

GENETICS AND BREEDING

Abstracts

ENHANCED RESISTANCE AGAINST THE RICE BLAST FUNGUS *MAGNAPORTHE GRISEA* IN TRANSGENIC RICE

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Rice blast, caused by the fungus *Magnaporthe grisea* (anamorph *Pyricularia grisea*) is the most devastating disease of cultivated rice (*Oryza sativa* L) due to its widespread distribution and destrutiveness. We investigated the feasibility of using antifungal genes of non plant origen, namely the *Aspergillus giganteous afp* and insect *cecropin A* genes, for engineering resistance to *M. grisea* into a commercial rice variety (*O. sativa* L. cv. Senia). These genes were choosen for genetic engineering of rice because previous studies indicated that pure AFP and cecropin A proteins possess *in vitro* antifungal activity against *M. grisea*. Questions such as the subcellular compartment to which the transgene product is targeted, i.e. extracellular space, vacuole or endoplasmic reticulum, have been addressed. We demonstrated that constitutive expression of the *afp* and *cecropin A* genes in rice plants consistently increased their resistance to the blast fungus M. grisea.

This research was supported by the European Commission (QLRT-CT99-1484, EURICE) and by the Ministerio de Ciencia y Tecnología, Spain (BIO2003-04936-C02).

OUTCROSSING STUDY BETWEEN TRANSGENIC HERBICIDE-RESISTANT RICE AND NON-TRANSGENIC RICE IN CALIFORNIA

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The introduction and rapid development of GMOs (i.e.- genetically modified organisms) has resulted in the global use of transgenic crops. Escape of engineered genes from transgenic crops through pollen flow to similar cultivars or related weedy species raises environmental concerns and can jeopardize the commercialization of conventional California rice. Our objective was to evaluate the potential for gene flow from transgenic rice plants to conventional rice plants using herbicide-resistance marker genes that had been incorporated into the most widely grown California japonica cultivar, M-202. Of particular interest was the open-field assessment of pollen flow distances and outcrossing rates under natural environmental conditions (i.e.- predominant wind flow) that occur in the water-seeded system of California.
Two types of field experiments were conducted during 2001 to 2003 by the University of California-Davis at the Rice Experiment Station (RES) in Biggs, California to assess gene flow from the transgenic rice lines 'Liberty-Link M-202' (glufosinate resistant) and 'Round-Up Ready M-202' (glyphosate resistant) to conventional 'M-202' rice. The layout of the field pollen flow consisted of a circular (4.6 m radius) central donor site of transgenic rice surrounded by an acceptor site (16.9 m radius) seeded with non-transgenic M-202. The second study evaluated the highest potential for natural outcrossing by conducting a 'forced' outcrossing study where transgenic and non-transgenic rice were grown in alternate rows of pots placed in the field subject to mechanical pollination.
The forced pollination studies revealed outcrossing rates ranging from 0.071 to 0.272% in single replication, and 0.010 to 0.216% when averaged across the four replications. In the circular pollen flow experiments, the outcrossing rates in single replication ranged from 0.010 to 0.415%. Rates averaged over four replications were from 0.007 to 0.108%. Natural outcrossing from transgenic rice to non-transgenic rice was detected no further than 1.8-meters from the transgenic source. There was no positive correlation between the effect of predominant wind flow and the extent of outcrossing from transgenic rice to non-transgenic rice.

NEW SOURCES OF CYTOPLASMIC GENIC MALESTERILITY IN RICE *ORYZA SATIVA* L.

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In rice *Oryza sativa*, wild species are important sources of cytoplasmic genic male sterility. Twenty seven accessions of seven AA genome species were crossed with two maintainers, five germplasm lines and 11 improved varieties. The resulted hybrids were evaluated for pollen / spikelet fertility. The male sterility source was identified by reciprocal difference. The three hybrids viz., *O.nivara* 105343 x CO 45, *O.barthii* 100934 x IR 50, *O.nivara* 101508 x IR 64 were found sterile and substitution backcrosses were made with their respective male parents. The hybrid *O.nivara* 105879 x CO 45 was selfed and F_2 plants were observed for sterility. One plant was identified as sterile in a population of 74 plants and backcross was also made using the respective male parent. The above four lines were in BC_5 generation of conversion. Preliminary studies on characterization through fertility restoration and biochemical studies revealed that these sources were different from existing WA source.

Keywords
Cytoplasmic genic male sterility; new sources; wild species; Rice

MARKER ASSISTED SELECTION ON RICE BLAST RESISTANCE

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More then 30 rice diseases caused by pathogenic fungi have been registered on rice. One of the most harmful of them is blast, caused by *Pyricularia grisea* Sacc. (formely *Pyricularia oryzae* Cav.).
For more than 70-years of rice cultivation in the south of Russia 10 -12 – years cycle in appearance of blast epiphytoties is fixed. One of the most effective and ecologically safe strategies of disease control is breeding of resistant rice cultivars.
On the basis of the breeding strategy, chosen by us, pyramiding of three major genes of blast resistance (Pi1, Pi2, Pi3) in Russian commercial rice cultivars with good agronomical characteristics under aegis of the program marker assisted selection is carried out. Gene Pi2 is known for wide spectrum of resistance.
Pyramiding of the specified genes is being carried out on the basis of series of backcrossing. Russian commercial cultivars: Khazar, Ametist, Snezhinka are used as recurrent parental forms. Lines C101-A-S1 (gene Pi2), C101-Lac (genes Pi1 and Pi33) are used as donors of blast resistance genes.
The balked segregant analysis is being performed in order to compare molecular data with the data of phytopathology test.
As a final result it is expected to develop series of closely isogenic lines which will be crossed among themselves in order to achieve the effect of genes Pi 1, Pi 2, Pi 33 pyramiding.
The most reasonable strategy for development of rice cultivars having durable blast resistance is combination of major genes and QTLs. For this purpose the widely known cultivar Moroberecan was included in the breeding program. Within the framework of the backcrossing program the Russian rice cultivar Belozerny (as the recurrent parental form) and Moroberecan (as the donor of durable blast resistance) are used. At the moment the QTL-analysis of durable blast resistance with use of SSR markers is being carried out.
For the realization of the QTL-analysis the polymorphism level between parental forms has been estimated as the first step while using 468 SSR markers eventually distributed on rice genome. 177 of them are appeared to be polymorphic between parental forms.
The QTLs identification at hybrid progeny will facilitate and speed up breeding cycle allowing to do more directed choice of plants for further crossings.

Keywords
Rice blast resistance; pyramiding of major genes; QTL-analysis; bulked segregant analysis

ADDRESSING ABIOTIC STRESS TOLERANCE IN RICE (*ORYZA SATIVA* L.) THROUGH A TRANSGENIC APPROACH

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Agricultural productivity is severely affected by low temperatures and soil salinity. Transcription factors have been shown to play key roles regulating tolerance to a range of abiotic stresses, including cold and salinity. In order to improve rice tolerance to these adverse environmental conditions we have used a transgenic approach. As first strategy, rice (*Oryza sativa*, cv. Taipei 309) has been transformed with heterologous transcription factor genes related to abiotic stress tolerance, two from barley and one from maize, under the control of the ubiquitin promoter. Another strategy was the isolation of rice transcription factors orthologous to those of *Arabidopsis* that have been shown to be involved in the response to cold and salinity stresses. These genes will be over-expressed in rice under the control of either ubiquitin or an inducible promoter.
Transformed and regenerated rice plants carrying and expressing transgenes for abiotic stress tolerance will be further analysed. This analysis will focus on phenotypic, biochemical (mainly oxidative stress pathway), physiological and molecular characterization of the transgenic lines to select the ones with improved stress tolerance. Several molecular markers for abiotic stress tolerance will be used.

Keywords
Abiotic stress tolerance; cold; salt; *Oryza sativa*; over-expression; transcription factors; transgenic approach.

THE MAIZE ANTIFUNGAL GENE *B32* IN RICE AS A DEFENCE GENE AGAINST FUNGAL PATHOGENS

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The maize endosperm albumin b32 is a Ribosome Inactivating Protein (RIP) normally expressed in the kernel. It has been subject of extensive studies aimed at investigating and exploiting its action as defence protein against fungi and insects. Like other RIPs present in the cereal seeds, b32 may play a double role of storage and defence protein during seed germination. Ectopic expression of b32 in plant, might result in a wider defence action. Current work developed in our laboratories aims to evaluate rice plants expressing b32 in various tissues and during the complete plant growth cycle, with the final goal to verify its potentiality as defence gene against the major rice fungal pathogens. Rice plants engineered with gene *b32* placed under two constitute promoters, p35SCaMV and pUbi-1 were obtained, and homozygous lines evaluated in phytotron as well as in field conditions for expression of the transgene and resistance against rice blast (*Magnaporthe grisea*). Results obtained confirm correct expression and stability of B32 in the plant tissues and a moderate protection against blast.

GENETIC DIVERSITY OF CURRENT AND HISTORICAL ITALIAN RICE GERMOPLASM

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Common concern is expressed that the modern intense plant breeding leads to a gradual reduction of the genetic diversity of crop (Vellvè 1993), narrowing the germoplasm-base available for future breeding. We choose Italian rice cultivars as a good model for studying temporal trends in diversity over the time. In this work 123 Italian varieties ranging from the beginning of risicolture (1800) to 2001 were investigated by means of SSR and AFLP.

Polymerase chain reaction (PCR)-based methods, such as AFLP (amplified fragment length polymorphism, Vos et al. 1995) and SSR (simple sequence repeat, Morgante and Olivieri 1993), have been successfully used to assess genetic diversity as well as temporal trends, in several crop species (Barred and Kidwell KK 1998, Donini et al 2000, Spada et al 2004)

In this study eleven microsatellite loci were analyzed, moreover 14 AFLP primer combinations.

The elaboration of both AFLP and SSR data show that genetic diversity (Lynch and Milligan 1994) of historical varieties has been maintained in the modern ones, with some changes caused by the introduction of varieties in determinate period.

The results show that the diverse breeding programs and the introduction of foreign varieties ensure a sufficient high genetic diversity to allow the steady rise in genetic potential.

CHARACTERISATION AND BREEDING OF PORTUGUESE RICE VARIETIES

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Portugal is the European country with higher rice consumption per capita, but 40% of the production still has to come from abroad, 50000 tons from EU and 30000 tons from other countries. Portuguese varieties are very sensitive to blast, they are tall and have low yield, in spite of the good grain quality. Therefore a breeding work has started to develop dwarf high yielding varieties, with blast resistance.
The donors for the hybridisation were selected from rice germplasm collections maintained at Estação Agronómica Nacional, the Rice Center and IRRI (male donors). Several crosses and backcrosses were designed and are being conducted to introgress the semidwarf gene *sd1* and blast resistance genes, accelerated by marker-assisted selection (specific PCR and microsatellites).
In parallel, other crosses are starting between seven females and nine males, in a Line x Tester matting design. The heterosis, general combining ability and specific combining ability are being analysed to select the best combining parents and hybrids, respectively. The desirable plants will be selected in the F_2 generation and forwarded to get the homozygous lines. The characterisation of rice germplasm, both at morphological and DNA levels (microsatellites) is in progress, aiming to complement data already collected within an European project.

Keywords
Breeding; marker-assisted selection; microsatellites; *Oryza sativa.*

PRELIMINARY GENOME ANALYSIS ON *PYRICULARIA GRISEA* (COOKE) SACC. BY AUTOMATED FLUORESCENT AFLP

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Blast is one of the most important rice diseases and is distributed worldwide: it is caused by the Ascomycete *Pyricularia grisea* (Cooke) Sacc. (teleomorph: *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa). Symptoms are necrotic lesions, where the pathogen can be isolated, on leaves, necks and panicles. In Italy, the most important European rice growing country, blast can causes important economic losses.
Fluorescent Amplified Fragment Length Polymorphism (fAFLP) analysis was utilized for the first time on a collection of Italian isolates of this fungus to investigate its intraspecific variability.
Infected rice plants of different Italian cultivar were collected, during four years, in a field in Northern Italy: seventy-eight strains of *P. grisea* were isolated from lesions on leaves, neck, panicle and seed. A monoconidial culture was obtained for each strains.
The dendrograms, obtained by analysing and combining the two fAFLP pattern by using Dice similarity coefficient (S_D) and clustering of fingerprints performed with the unweighted pair groups (UPGMA), does not show any apparent correlation with specifity of the fungus toward a part of the plant or a rice cultivar but emphasize the need to deepen this analysis with other pairs of primers.

BREEDING OF NEW HIGHLY PRODUCTIVE RICE FORMS IN RUSSIA

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One of the ways of further improvement of rice yield is to increase individual plant productivity at optimal plant density. World rice production proves that rice yields can reach 16-18 t/ha. This project (Super Rice) is realized in deferent rice grower countries.

Achievements of rice breeding in Russia show that under local conditions biological potential of rice plant is considerably higher compared to productivity of grown varieties. In 1983 in hybrid nursery there was selected a rice plant with large sorghum-line panicles. Five panicles contained in average 600 spikelets. Weight of grains from the main panicle was 14 g. This new form was named BZ-600 and was used in hybridization process as a donor of productivity.

As a result of step-hybridization (for many years) and replicated screening several breeding lines have been obtained, each of them had attributes which are necessary for creation of plants of new type. Samples with vertically placed erectoid leaves adapted to dense plant populations have been bred, other samples has got large panicles with high number of grains of excellent quality but their leaves have normal position and negative reaction to high density. Hybrids received from the two forms after numerous selections have produced a plant combining erectoid leaves with high grain yields. Rice form Olin-1 is characterized by plant height of 90-92 cm, maximal panicle length - 40cm (43% of total plant height), number of spikelets per plant - 702 . Grain has vitreous endosperm. These plants belong to Italica type. The other such form - Olin-2 - belongs to Zeravschanica type. This provides possibility to produce rice variety from these forms.

These rice forms of new morphological type provide new ideas about biological potential of rice plant in the South of Russia and will create a base for breeding new varieties.

Session 4

ENVIRONMENT AND ECOLOGY

Full papers

SCENARIOS AND DATA REQUIREMENTS FOR RISK ASSESSMENT EVALUATION ELABORATED BY THE MED-RICE WORKING GROUP

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Introduction

When The Uniform Principles present in the Annex VI to Council Directive 91/414/EEC were adopted, the European Council (in Document 10171/97, ADD1, Agrileg 163 dated from 22 August 1997) made the following statement (Directive 97/57/EC):

"…The Council and the Commission note that particular conditions obtain in rice cultivation. This means that certain specific criteria are inappropriate for evaluation purposes, particularly in the context of point 2.5.2.2 for the exposure of aquatic organisms in rice field waters. …"

Therefore, in 1999 the Standing Committee for Plant Health charged a small expert group with the preparation of a Guidance Document to face the risk assessment of active substances used on rice in the EU for Annex I inclusion. The task given to the expert group was both to develop a system for the risk assessment of plant protection products (PPPs) in rice, at least at the lower Steps of the risk assessment, and the preparation of a Guidance Document for data requirements and risk assessment in rice cultures to be adopted by the Standing Committee on Plant Health.

The document has been released as final on June 2003 and the SCFA took note of the rice guidance document with no votes against.

Rice cropping in Europe and scenario definitions

In Europe, year 2000, the most important countries of rice cultivation are Italy (221000 ha), Spain (111000 ha), Portugal (22000 ha), Greece (17000 ha), and France (19000 ha), with Spain and Greece recording the largest variation in the previous ten years (Figures 1 and 2)

Rice in Europe is grown under a Mediterranean climate characterised by warm, dry, clear days, and a long growing season favorable to high photosynthetic rates and high rice yields. Compared to tropical and subtropical rice-growing areas, the climate is cool, but warm summer nights during panicle development, when pollen formation takes place, helps to avoid cold-induced floret sterility. Low relative humidity throughout the growing season reduces the development, severity, and importance of rice diseases. However, cool weather and strong winds during stand establishment may cause partial stand loss and seedling drift.

Rice is grown mostly on fine-textured, poorly drained soils with impervious hardpans or clay pans. These soils principally belong to three textural classes: clays, silty clays, and silty clay loams ranging from 10 to 45 percent clay. A few of the soils are sandy, mainly in Italy and Spain.

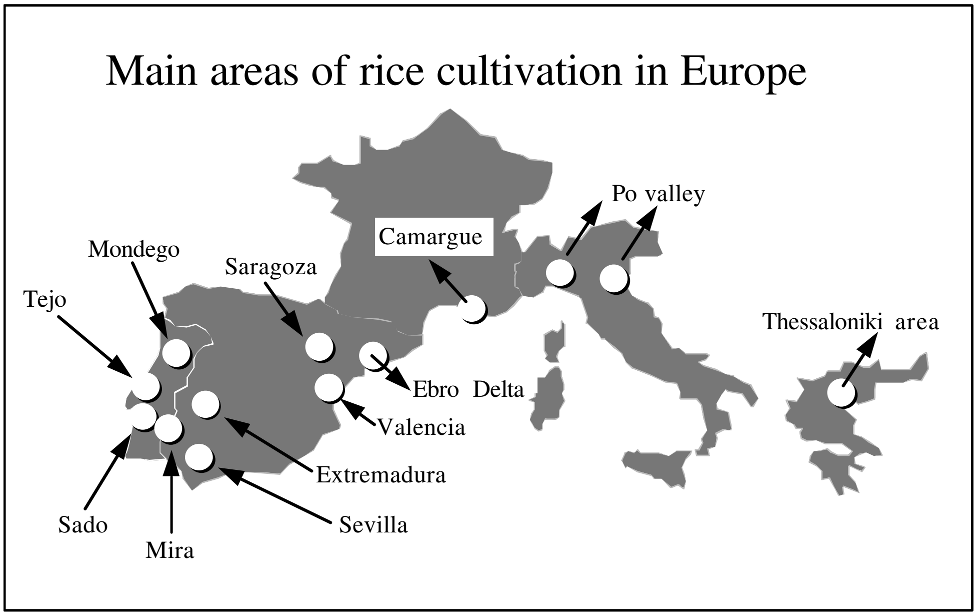

Figure 1 Areas of rice cultivation in Europe.

	Surface 2000 (ha x 1000)	1990 - 2000 variation
Italy	221	+ 3.7 %
Spain	111	+24.7 %
Portugal	22	- 6.4 %
Greece	27	+68.7 %
France	20	+ 7.8 %

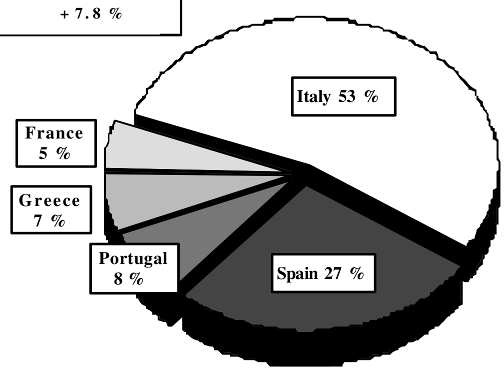

Figure 2 Rice surface in European countries in 2000.

These soils are well suited to rice production, since their low water permeability enhances water use efficiency. Most of the irrigation water for European rice comes from rivers (Po river in Italy, Ebro river in Spain, Rhone river in France, the Tejo, Sado and Mondego rivers in Portugal, etc.) and lakes. It has been estimated that less than 5 percent of rice irrigation water is pumped from wells in areas where surface water is not available, or as a supplement to surface supplies.

In all European countries, rice is commonly cultivated with a permanent flood with short periods during which the soil is dried up to favor rice rooting (in the early stages) or weed control treatments.

The workgroup made an inventory of the rice agricultural practices in the 5 South-European member states, France, Greece, Italy, Portugal and Spain, considering the main similarities and differences. Table 1 gives information concerning the rice cropping strategies in the South European countries. The main characteristics of the cropping are indicated to illustrate the similarities and differences in these countries.

Table 1 Overview of rice cropping strategies.

Characteristic	France	Greece	Italy	Portugal	Spain
Soils:					
* texture	**Silt loam/ Silty clay loam**	**Silty loam**	**Clay/Sandy**	**Sandy Clay/ Clay loam/ Clay**	**Clay/Loam/ Sandy loam**
* % o.m.	**1 – 4**	**1.8 – 2.0**	**0.8 – 10**	**2 – 3**	**0.5 – 3**
* pH	**8.0**	**7.4 – 8.0**	**4 – 8**	**5 – 7**	**5.5 – 8.5**
* % clay	**10 – 40**	**20**	**Sandy: 2 – 6 Clay: 20 – 30**	**30 – 40**	**10 – 40**
Drainage system	**Yes**	**Yes (75%)**	**Yes**	**Yes, 2 systems**	**Yes**
Water level	**Max. 20 cm, average 10 cm**	**2 – 10 cm**	**10 cm**	**2 – 10 cm**	**< 20 cm, average 10 cm**
Water velocity:					
*drainage (outflow field)	**0.4 l/s/ha**	**0.5 l/s/ha**	**0.15 l/s/ha**	**2 – 2.5 l/s/ha**	**0.15 l/s/ha**
* field (inflow field)	**2 – 3 l/s/ha**	**4 l/s/ha**	**1.01 l/s/ha**	**2 – 4 l/s/ha**	**0.5 – 1.5 l/s/ha**
Flooding conditions	**May – Aug**	**May – Sept**	**May – Sept**	**April – Sept**	**April – Aug**
Time of closure of field	**7 days**	**2 – 5 days**	**5 days**	**2 – 5 days**	**2 – 5 days**
Depth of drainage channels	**1.5 – 2.5 m**	**1.5 – 2.0 m**	**1 – 2 m**	**1 – 2 m**	**2 m**
Crop rotation	**Yes**	**Yes (80%)**	**No**	**No**	**In some areas**
Infiltration (leakage) rate	**Max < 8 mm/d, Mean 4 mm/d**	**5 – 10 mm/d**	**6 – 11 mm/d**	**1 – 10 mm/d**	**1 – 10 mm/d**
Usage of outflow water	**No**	**No**	**No**	**Occasionally for irrigation**	**No**
Aerial application	**Yes**	**No**	**No**	**Yes**	**Generally no**
Irrigation system	**No**	**Yes (75%)**	**Yes, 3 systems**	**Yes**	**Yes, flooding**
Temperature (ºC)	**> 14**	**> 12**	**> 14**	**> 16 – 22**	**> 14 – 20**
Aerobic/anaerobic conditions at soil/water interface	**Aerobic**	**Aerobic**	**Aerobic**	**Aerobic**	**Aerobic**

From this comparison two European standard scenarios were abstracted, corresponding to two different and representative situations, in particular with respect to contamination of surface waters and leaching of substances applied to the paddy field. In fact, the analysis of the rice practices in the Southern European Member States showed that in general two different environmental situations might occur in the regions under consideration. Two different

scenarios were identified covering the impermeable soils with high clay content on the one hand and more permeable, sandy and low organic matter soils on the other. The first scenario can represent more vulnerable conditions with regard to surface water exposure, whereas the second scenario represents conditions vulnerable to leaching and groundwater contamination. These two scenarios are characterised by different infiltration rates: 1 mm d^{-1} for the clayey scenario and 10 mm d^{-1} for the sandy scenario. Both scenarios are intended to represent the two extremes of actual situations (realistic worst case), and most real situations in the Member States will be in between these two.
Table2 shows the basic parameters of the two scenarios defined by the working group.

Table 2 Scenario definition

Characteristic	Scenario 1	Scenario 2
Soils:		
* texture	**Clayey**	**Sandy**
* % clay	**30**	**5**
* % o.m. (% o.c.)	**3 (1.8)**	**1.5 (0.9)**
* pH	**8**	**6**
Water level	**10 cm**	**10 cm**
Water velocity:		
* outflow	**0.5 l/s/ha**	**0.5 l/s/ha**
* field	**1.8 l/s/ha**	**2.8 l/s/ha**
Flooding conditions	**May – August**	**May – August**
Time of closure of field	**5 days**	**5 days**
Depth of drainage channel	**1 m**	**1 m**
Crop rotation	**No**	**No**
Infiltration (leakage) rate	**1 mm/d**	**10 mm/d**
Evapotranspiration rate	**10 mm/d**	**10 mm/d**
Usage of outflow water	**No**	**No**
Temperature (ºC)	**20**	**20**
Conditions in soil	**Aerobic**	**Aerobic**

Additional data requirements for rice cropping
A revision of data requirements as defined in Annex II and III of the Directive 91/414/EEC was undertaken in order to assess their appropriateness to rice culture.
The workgroup concluded that as regards the requirements for Fate and Behavior in the environment, some adaptations were necessary considering the agricultural peculiarities of this crop. The main changes are related to the evaluation of the route and rate of degradation. It was concluded that a flooded soil degradation study would better address the degradation of active substances under paddy field conditions. The suitable protocol developed by the Society for Environmental Toxicology and Chemistry (SETAC) and the Organisation for Economic Co-operation and Development (OECD) 307 for aerobic and anaerobic transformation in soil is therefore recommended. Following this protocol a typical soil study representative of rice growing should be used. Additionally, a small-scale or full-scale outdoor dissipation study with radiolabelled material may give useful information for certain compounds (e.g. where photolysis may be important).
For the ecotoxicology data requirements, since the application of PPPs in rice culture may coincide with the breeding season of birds, it is possible that birds or nesting sites could be exposed to these products during application. Furthermore, rice paddies are often located in or

in the vicinity of Natural Reserves and are of great importance as habitats for waterfowl and migratory bird species. Nevertheless it was concluded that current guidance was considered sufficient for Annex I inclusion and that additional testing was not required.

Taking into consideration the scenario definition (Table 2), the Step 1 for PEC calculation methods for surface water, groundwater and soil were developed for plant protection products applied in rice crops, following as much as possible the current approaches used at the EU level. It should be noted, however, that the approach followed to calculate PEC in groundwater at Step 1 of the risk assessment differs from the procedure adopted for plant protection products used in other field crops.

Proposal for a standard risk assessment

The working group developed a tiered approach in three steps, starting from a relatively simple calculation of the PECs up to a more sophisticated approach using complex modeling and monitoring at the highest level. The group focused on three environmental compartments, surface water, including sediment, groundwater and soil. For these three compartments a method was developed to estimate the actual predicted environmental concentration (PEC) values and the Time Weighted Average concentrations (TWA) over relevant time periods. These PECs are then used in the risk assessment for relevant non-target organisms.

The working group limited itself to developing a standard Step 1 assessment, as advanced mathematical modeling tools are not yet sufficiently validated to be used in a regulatory context. The generalised tiered approach is shown in Figure 3.

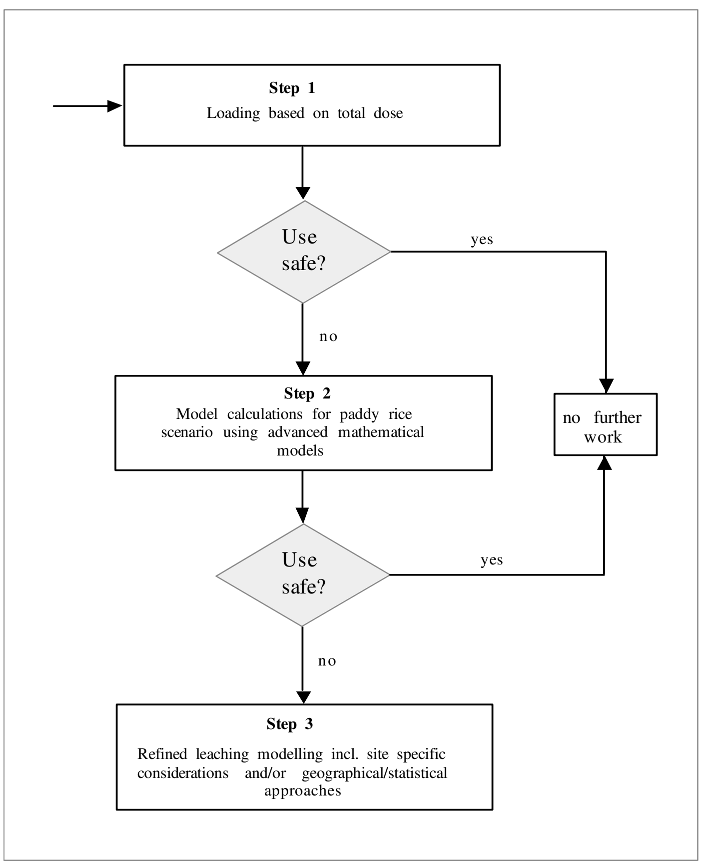

Figure 3 Generalised Tiered Approach

Estimation of PECs and TWAs in rice paddy fields

The working group developed a method to estimate the PEC in different environmental compartments. These compartments are surface water, including sediment, groundwater and soil. A distinction is made between the actual PEC estimates and the TWA for different time points or periods.

Surface water, including sediment (both degradation and sorption considered):

water phase (outflow):

$$PEC_{sw}(t_{close}) = (PEC_{sw,drift}(t_{close}) \cdot fact_{dilution} + PEC_{pw}(t_{close}))/(1 + fact_{dilution})$$

sediment phase:

$$PEC_{sed}(t_{close}) = PEC_{sed,drift}(t_{close}) + \frac{PEC_{pw}(t_{close}) \cdot depth_{water} \cdot F_{sorbed}}{fact_{dilution} \cdot depth_{sed} \cdot BD_{sed}}$$

For the time weighted average concentrations the following equations are used:

water phase:

$$TWA_{sw}(T) = \frac{PEC_{sw,initial} \cdot (1 - e^{-T \cdot \ln(2)/DT50_{sw}})}{T \cdot \ln(2)/DT50_{sw}}$$

sediment phase:

$$TWA_{sed}(T) = \frac{PEC_{sed,initial}(T) \cdot e^{-T \cdot \ln 2/DT50_{sed}}}{T \cdot \ln(2)/DT50_{sed}}.$$

Groundwater (both degradation and sorption considered):

For the estimation of the concentration in groundwater the following equations have been derived for the concentration in groundwater:

$$PEC_{pgw}(t = 365) = \frac{M_{leak(>1000)} \cdot 100}{365 \cdot leakage}$$

Soil (both degradation and sorption considered):

For soil the following equations are proposed for the calculation of the PEC in soil as concentration and time weighted average:

$$PEC_{soil}(t) = PEC_{soil,initial} \cdot e^{-t \cdot \ln 2/DT50_{soil}}$$

$$TWA_{soil}(t) = \frac{PEC_{soil,initial} \cdot (1 - e^{-t \cdot \ln(2)/DT50_{soil}})}{t \cdot \ln(2)/DT50_{soil}}$$

A simple mechanistic new model RIPACEM (now called SWAGW), with a pre-computation performed by the model CODEWS, has been developed by Cervelli et al., but till now it has not yet been sufficiently checked and validated.

Conclusions

- The proposed methodology gives notifiers and authorities the information on the data requirements needed to be considered for fate and behavior and ecotoxicology as well as a standard tool to calculate the appropriate PECs for the purpose of review for inclusion in Annex I of Council Directive 91/414/EEC.
- The group has identified the following main areas, which need consideration within the scope of the guidance document: soil, groundwater, surface water and sediment in the paddy and in the drainage canals.
- The review of the cropping conditions in the five Southern EU countries involved in cultivation of this crop has revealed many similarities. The two different standard scenarios proposed represent dominant situations occurring in the MS of concern and offers limited but relevant differences, one based on vulnerable conditions for leaching and the other being more suited to estimate risks in surface waters.
- Limited changes are proposed in Annex II of Directive 91/414/EEC with regard to the evaluation of the fate and behavior of plant protection products in the environment. These affect mainly the test system to be used for investigation of route and rate of degradation in soil. It was concluded that an aerobic flooded soil study would be more appropriate and should replace the normal aerobic soil degradation study. In addition, a decision scheme is proposed with regard to the possible necessity of higher tier (e.g. small-scale or full-scale outdoor dissipation) studies. For registration at the national level, relevant regulatory authority judgment would be required.
- For ecotoxicological data requirements the workgroup has concluded that current guidance on how to perform the risk assessment for non-target species is acceptable. Aquatic organisms in the rice paddy itself do not require the same level of protection as those in the non-target water bodies adjacent to the fields. However, other species that may use the treated rice paddies as a feeding ground (e.g. birds and mammals) do require the normal level of protection. In addition, the ecological function of aquatic organisms within the paddy field should be considered at Member State level if appropriate. If specific concerns are identified at a national level higher tier studies should be considered on a case by case basis.
- The major contribution concerns Annex III. Regarding PEC calculations for soil, ground waters and surface waters, currently adopted methods for PEC calculations for surface water, soil, and groundwater were found to be not fully appropriate for paddy field conditions. Therefore, a stepwise approach has been developed for the estimation of PEC in these compartments after application of plant protection products in rice. Simple calculation methods for the step 1 assessment were developed. Based on current practice separate steps are defined for taking into account degradation and sorption of the active substance under consideration. The specific case of outflow canals has been taken into particular account.
- Relevant simulation models for paddy rice conditions have been reviewed. Among the readily available simulation models, RICEWQ was considered to be appropriate for the assessment of exposure in surface waters. Further research would be needed to fully evaluate the applicability of RICEWQ or other models. The model RICEWQ is proposed to be used in connection with the RIVWQ model to estimate the PECs in surface waters. The model RICEMOD uses a fugacity approach, and may be an appropriate model if more information on it becomes available.

A STEP 2 APPROACH TO COMPUTE WATER, SOIL AND SEDIMENT PECS AND TWAS FOR THE INCLUSION OF RICE PESTICIDES IN ANNEX I OF THE EU COUNCIL DIRECTIVE 91/414/EEC

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Abstract

When Annex VI to Council Directive 91/414/EEC was adopted, the Council and the Commission recognised that due to particular conditions associated to rice cultivation the specific criteria and principles referred to in Annex VI were inappropriate. Therefore, the Commission committed to identify any specific data requirements and develop criteria for environmental risk assessment and decision-making which specifically addressed the use of plant protection products (PPPs) in rice cultivation.

Currently, regulatory models to predict both surface water and groundwater contaminations are limited in their ability to simulate the flooded conditions of a paddy rice field, and no model is recommended for such simulations. The Standing Committee for Plant Health charged in 1999 a small group (MED-Rice Working Group) to prepare both a document for the European Commission in the framework of Council Directive, and a system for the risk assessment of PPPs in rice, at least at the lower Steps, for the inclusion of a substance in Annex I of the Directive. The Working Group developed a simple procedure (Step 1) to estimate PECs in different environmental compartments.

Here we present a more complex model (Step 2) to estimate PECs and TWAs in surface water and groundwater, sediments and soil.

Introduction

In rice cultivating EU member states, the rice crop is characterized by a general current cropping practice consisting of two time periods of water submersion. While during the first period, usually 5 days, the field is submerged by a static body of water (closed rice environment), during the second one, usually about 3 months, the water flows freely to and from the field, leaving a constant water level (open rice environment). According to this practice, charged in 1999 by the Standing Committee for Plant Health, the MED-Rice Working Group developed a low level model (step 1) for screening purposes. Here we present a more complex mechanistic model (step 2) to estimate PECs and TWAs in surface water, including sediment, groundwater and soil.

The Model

As a first improvement of the assumptions for paddy and surface waters of step 1, we propose a more realistic adsorption to be modeled in step 2. The amount of substance adsorbed on the solid phase (soil and sediment) is in constant equilibrium with the amount in the water phase (paddy field and canals), while in step 1 sorption takes place instantaneously.

For groundwater, step 2 assumes a miscible displacement behavior of PPP, a constant moisture content corresponding to saturation, and a constant addition of the pesticide corresponding to its TWA in paddy water, both during the closing and the opening time.

Surface Water, Sediment and Soil

Closed rice system

Figure 1 shows the chemical-physical model of the rice system during closing time. Following the scheme and symbols of Figure 1, for the conservation of the mass in the paddy environment we have:

$$A_{Total} - A_{LL} = A_L + A_S + B_L \tag{1}$$

where A_{Total}, A_L, A_{LL}, A_S and B_L are the total amount of PPP added, the amount of PPP leached through soil, the amount of PPP in water and adsorbed on soil, and the amount of residue formed, respectively. By differentiating with respect to time, we have:

$$\frac{dA_L}{dt} + \frac{dA_{LL}}{dt} + \frac{dA_S}{dt} + \frac{dB_L}{dt} = 0 \tag{2}$$

Due to the elementary equations:

$$\frac{dA_{LL}}{dt} = k_{inf} A_L \quad , \quad \frac{dB_L}{dt} = k_{2w} A_L \quad , \quad \frac{dA_S}{dt} = K_2 \frac{dA_L}{dt} \tag{3}$$

we have from (2):

$$\frac{dA_L}{dt} = -\frac{k_{2w} + k_{inf}}{1 + K_2} A_L \tag{4}$$

where k_{inf} is a pseudo first order degradation constant to take into account PPP leaching, having the dimension of t^{-1}, and:

$$K_2 = K_{2d}\, \delta_2 \frac{L_{2S}}{L_{2w}} \tag{5}$$

where K_{2d} is the soil adsorption constant, δ_2 the soil bulk density, L_{2S} the depth of the soil, and L_{2W} the level of the water pounding on the soil, respectively.

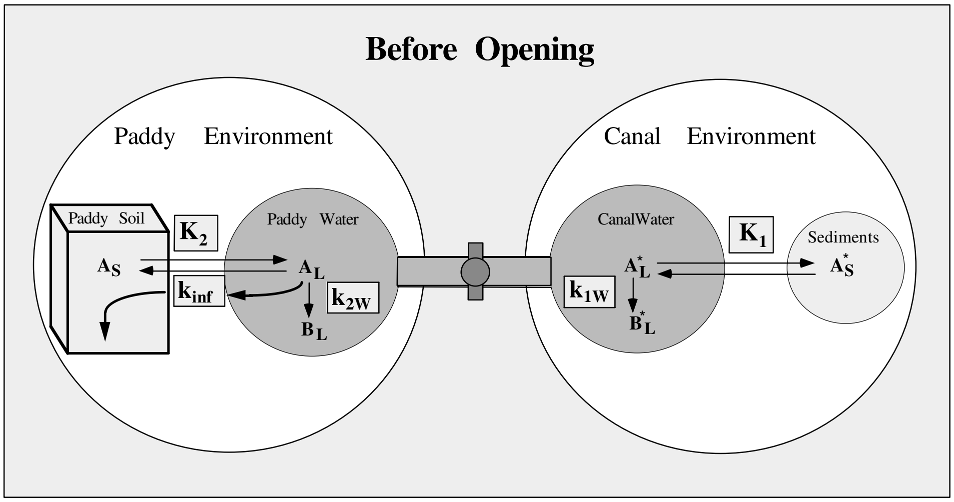

Figure 1 Chemical-physical model of the rice-paddy and canal environments before opening. A_L and A_S are the PPP amount in the water and soil compartment, respectively. B_L is the residue amount in water. A_L*, A_S* and B_L* are the same amounts in the water/sediment system. K_1 and K_2 are the adsorption ratios, k_{inf} is a pseudo first order leaching constant, and k_{1W} and k_{2W} are the first order rate constants in the two environments.

After integration of (4) with the initial condition:

$$t = 0 \quad \longrightarrow \quad A_L = A_0 \tag{6}$$

where A_0 is the initial amount of PPP in the water phase following the adsorption equilibrium, we have:

$$A_L = A_0\, e^{-\frac{k_{2w}+k_{inf}}{1+K_2} t} \tag{7}$$

The TWA in the time interval $0 \le t < T_1$ is:

$$TWA_{A_L} = \frac{A_0\,(1+K_2)}{t\,(k_{2w}+k_{inf})}\left(1 - e^{-\frac{k_{2w}+k_{inf}}{1+K_2} t}\right) \tag{8}$$

where T_1 is the end of the closing time.

For the canals, following the scheme and symbols of Figure 1 (the superscript "*" indicates any compound present in the canal system), analogously to (2) without infiltration we have:

$$A_L^* = A_0^*\, e^{-\frac{k_{1w}}{1+K_1} t} \qquad \text{where} \qquad K_1 = K_{1d}\, \delta_1 \frac{L_{1S}}{L_{1w}} \tag{9}$$

with k_{1d} the sediment adsorption constant, δ_1 the sediment bulk density, L_{1S} the depth of the sediments, and L_{1W} the level of the water in the canals, respectively.

We have also:

$$A_S^* = K_1\, A_L^* \tag{10}$$

In the interval $0 \le t < T_1$ we have for canals:

$$TWA_{A_L^*} = \frac{A_0^* (1 + K_1)}{t\, k_{1w}} \left(1 - e^{-\frac{k_{1w}}{1+K_1} t} \right) \tag{11}$$

where T_1 is the end of the closing time.

Open rice system

Figure 2 shows the chemical-physical model after opening the rice field. Following the scheme and symbols of Figure 2, the relationship for the conservation of the mass in the paddy environment is:

$$A_{Total} - A_{LL} - \overline{\overline{A_L}} = A_L + A_S + B_L \tag{12}$$

where A_{LL} is the amount of A_L lost by leaching through soil and $\overline{\overline{A_L}}$ is the amount of A_L flowing from paddy to canals. After differentiation with respect to time we have:

$$\frac{d A_L}{d t} + \frac{d A_{LL}}{d t} + \frac{d \overline{\overline{A_L}}}{d t} + \frac{d A_S}{d t} + \frac{d B_L}{d t} = 0 \tag{13}$$

and after some substitutions:

$$\frac{d A_L}{d t} = -\frac{k_{2w} + k_{inf} + k_{out}}{1 + K_2} A_L \tag{14}$$

due to the elementary equations:

$$\frac{d A_{LL}}{d t} = k_{inf} A_L \quad , \quad \frac{d \overline{\overline{A_L}}}{d t} = k_{out} A_L \quad , \quad \frac{d A_S}{d t} = K_2 \frac{d A_L}{d t} \quad , \quad \frac{d B_L}{d t} = k_{2w} A_L \tag{15}$$

where k_{out} is equal to $k_{outflow}/V_p$ and takes into account the water outflow, having the dimension of t^{-1} (the volume of water in the paddy field is assumed to be constant), $k_{outflow}$ is the volume of water leaving the paddy environment during time (L day^{-1}) and V_p is the volume of the paddy water. The constant K_2 has been previously defined in (5).

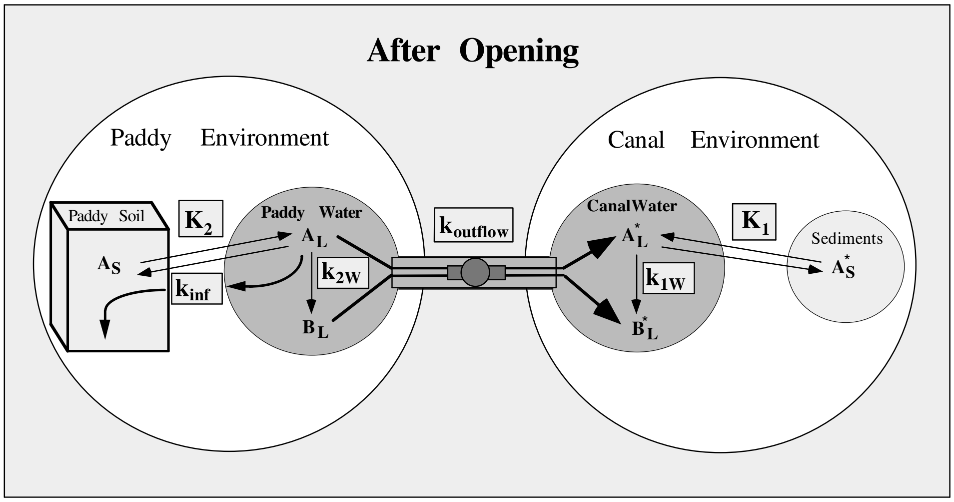

Figure 2 Chemical-physical model of the rice-paddy and canal systems after opening. A_L, A_S, B_L, A_L*, A_S*, B_L*, K_1, K_2, k_{inf}, k_{1W} and k_{2W} are defined as in Figure 1. k_{inf}, and $k_{outflow}$ are pseudo first order constants.

The solution of (14), with the initial condition:

$$t = T_1 \qquad \longrightarrow \qquad A_L = A_0^{\circ} \tag{16}$$

is:

$$A_L = A_0^{\circ}\, e^{-\frac{k_{2w} + k_{inf} + k_{out}}{1+K_2}(t - T_1)} \tag{17}$$

where A_0° is the amount of PPP at the end of the closing time T_1.
The equation taking into account the adsorption of A_L is:

$$A_S = K_2\, A_L \tag{18}$$

TWA is calculated from (17) in the interval $T_1 \le t \le T_2$:

$$TWA_{A_L} = \frac{A_0^{o}\,(1+K_2)}{(t-T_1)\,(k_{2w}+k_{inf}+k_{out})}\left(1 - e^{-\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}(t-T)_1}\right) \tag{19}$$

where T_2 is the harvest time. Following the opening of the paddy field, water and active substance outflow to the canals, and following the scheme of Figure 2 the relationship for the mass conservation in this environment is:

$$A^*_{Total} + \overline{\overline{A_L}} = A^*_L + A^*_S + B^*_L \tag{20}$$

where A^*_{Total} and $\overline{\overline{A_L}}$ are the amount of PPP in the canals due to drift and flowing from paddy to canals, respectively. During the flow, we consider a dilution factor DF of 10. After differentiation with respect to time we have:

$$\frac{dA_L^*}{dt} - \frac{d\overline{\overline{A_L}}}{dt} + \frac{dA_S^*}{dt} + \frac{dB_L^*}{dt} = 0 \qquad (21)$$

and after some substitutions:

$$\left(1+K_1\right)\frac{dA_L^*}{dt} = -k_{1w}A_L^* + \frac{k_{out}}{DF}\,A_0^{\circ}\,e^{-\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}\left(t-T_1\right)} \qquad (22)$$

due to the elementary equations:

$$\frac{dB_L^*}{dt} = k_{1w}A_L^* \quad , \quad \frac{dA_S^*}{dt} = K_1\frac{dA_L^*}{dt} \quad , \quad \frac{d\overline{\overline{A_L}}}{dt} = \frac{k_{out}}{DF}\,A_L \qquad (23)$$

The constant K_1 has been previously defined in (9). The solution of (22) with the initial conditions:

$$t = T_1 \longrightarrow A_L = A_0^{\circ} \quad , \quad t = T_1 \longrightarrow A_L^* = A_0^{*\circ} \qquad (24)$$

is:

$$A_L^* = \frac{e^{-\frac{k_{1w}}{1+K_1}\left(t-T_1\right)-\frac{k_{2w}+k_{inf}+k_{out}}{\left(1+K_2\right)}\left(t+T_1\right)}}{DF\left[-k_{inf}\left(1+K_1\right)+k_{1w}\left(1+K_2\right)-\left(1+K_1\right)\left(k_{2w}+k_{inf}+k_{out}\right)\right]}$$
$$\left\{-A_0^{\circ}\left(e^{\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}\left(t+T_1\right)} - e^{\frac{k_{1w}}{1+K_1}\left(t-T_1\right)+\frac{2\left(k_{2w}+k_{inf}+k_{out}\right)}{1+K_2}T_1}\right)\right.$$
$$\left(1+K_2\right)k_{out} - A_0^{*\circ}\,DF\,e^{+\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}\left(t+T_1\right)}$$
$$\left.\left[k_{inf}\left(1+K_1\right)-k_{1w}\left(1+K_2\right)+\left(1+K_1\right)\left(k_{2w}+k_{inf}+k_{out}\right)\right]\right\} \qquad (25)$$

TWA in the interval $T_1 \le t \le T_2$, where T_1 is the end of the closing time and T_2 the harvest time, is:

$$
TWA_{A_L^*} = \frac{e^{-\left(\frac{k_{1w}}{1+K1}+\frac{(k_{2w}+k_{inf}+k_{out})}{1+K_2}\right)(t+2T_1)}}{(t-T_1)\ (k_{2w}+k_{inf}+k_{out})\ \left[-k_{inf}(1+K_1)+k_{1w}(1+K_2)-(1+K_1)(k_{2w}+k_{out})\right]}
$$

$$
\frac{1}{DF\ k_{1w}}\left\{ A_o^{*0}\ DF \left(e^{+\frac{(k_{2w}+k_{inf}+k_{out})(t+T_1)+\frac{k_{1w}(1+K_2)+(1+K_1)(k_{2w}+k_{inf}+k_{out})}{1+K_1}T_1}{1+K_2}} - \right.\right.
$$

$$
\left. e^{+\frac{k_{1w}}{1+K1}t+\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}(t+2T_1)} \right) \left(k_{inf}(1+K_1)-k_{1w}(1+K_2)+(1+K_1)\,(k_{2w}+k_{out})\right) \tag{26}
$$

$$
(1+K_1)\,(k_{2w}+k_{inf}+k_{out}) + A_o^0\ \ k_{out}(1+K_2)\left(-e^{+\frac{k_{1w}}{1+K1}t+\frac{3\,(k_{2w}+k_{inf}+k_{out})}{1+K_2}T_1}\ k_{1w}\,(1+K_2)\ + \right.
$$

$$
e^{+\frac{(k_{2w}+k_{inf}+k_{out})(t+T_1)+\frac{k_{1w}(1+K_2)+(1+K_1)(k_{2w}+k_{inf}+k_{out})}{1+K_1}T_1}{1+K_2}}\ (1+K_1)\,(k_{2w}+k_{inf}+k_{out})+
$$

$$
\left.\left. e^{+\frac{k_{1w}}{1+K1}t+\frac{k_{2w}+k_{inf}+k_{out}}{1+K_2}(t+2T_1)}\ \left(-k_{inf}(1+K_1)+k_{1w}(1+K_2)-(1+K_1)\,(k_{2w}+k_{out})\right)\right)\right\}
$$

We have also:

$$
A_S^* = K_1\ A_L^* \tag{27}
$$

The concentrations in water of paddy field and canals are calculated by dividing the amounts of PPP in paddy/canal water by the volume of paddy/canal water, 10^6 and 10^7 liters/ha in paddy and canals, respectively. The amount of PPP adsorbed *per* weight units of soil/sediments is calculated by dividing the amounts of PPP present in the soil/sediment by the weight of the soil/sediment, both 7.5x 10^5 kg/ha.

Groundwater

The complete chemical-physical model assumed in developing the step 2 approach for leaching is reported in *Figure 3*.

The equation used to calculate the PECs at different times and depths are derived from the general equation for the conservation of the mass (Jury, Gardner and Gardner, 1991):

$$
\frac{\partial C_T}{\partial t} = -\frac{\partial J_S}{\partial x} \pm \Phi \tag{28}
$$

where C_T is the total concentration and J_S the flow of PPP, t the time, x the depth and Φ a source–sink term. We have also (Hutson and Wagenet, 1992):

$$
C_T = \rho\,K_{ads}\,c + \theta\,c\,,\ J_S = -\theta D_i(\theta,q)\frac{\partial c}{\partial x} + q\,c\,,\ D_i(\theta,q) = D_W\,a e^{b\theta} + \lambda\left|\frac{q}{\theta}\right|, \Phi = -\,\theta\,k_{react}\,c \tag{29}
$$

where K_{ads} is the adsorption constant, D_W is the diffusion coefficient in pure water, θ the volumetric water content, ρ the soil bulk density, c the PPP concentration, q the water flux, and λ the dispersivity, having the value: $0.2\ cm < \lambda < 8\ cm$ (Hutson and Wagenet, 1992), and where k_{react} is the degradation first order kinetic constant.

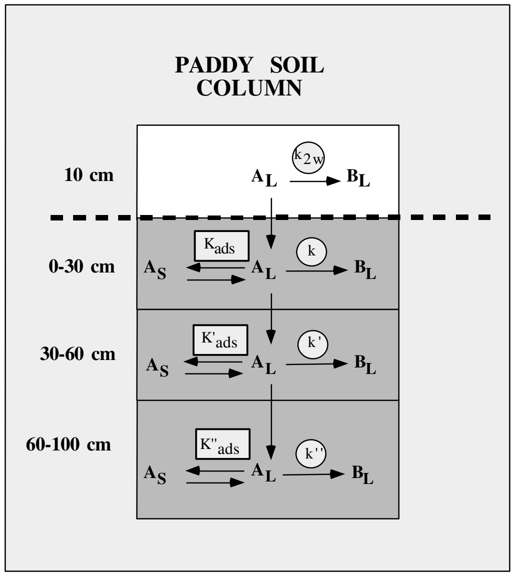

Figure 3 Chemical-physical model of the rice-paddy leaching system. A_L, A_S and B_L are defined as in Figure 2. K_{ads}, K'_{ads} and K''_{ads} are the soil adsorption constants, and k, k' and k'' are the first order rate constants at different depth intervals, taking into account the respective biofactors.

After substitution of (29) in (28) we obtain the following equation:

$$\frac{\partial c}{\partial t} = Disp\ \frac{\partial^2 c}{\partial x^2} - v\ \frac{\partial c}{\partial x} - k\,c \qquad (30)$$

where:

$$R = \theta + \rho K_{ads} \quad , \quad Disp = \frac{\theta}{R}\left(D_W\, a e^{b\theta} + \lambda \left| \frac{q}{\theta} \right| \right), \quad v = \frac{q}{R} \quad , \ k = \frac{\theta}{R} k_{react} \qquad (31)$$

If *Disp* and *v* are constant and PPPs are added at a constant concentration for a time T_1, according to the following initial and boundary conditions:

$$c = C_0 \qquad x = 0 \qquad 0 < t < T_1 \qquad (32)$$
$$c = 0 \qquad x = 0 \qquad t > T_1 \qquad (33)$$
$$c = 0 \qquad x \geq 0 \qquad t = 0_1 \qquad (34)$$
$$c = 0 \qquad x \to \infty \qquad t \geq 0 \qquad (35)$$

equation (30) can be solved analytically (Misra et al., 1974). The solution is:

$$c(x,t) = P(x,t) \longrightarrow 0 < t < T_1 \tag{36}$$

$$c(x,t) = P(x,t) - P\left[x,(t - T_1)\right] \longrightarrow t > T_1 \tag{37}$$

with:

$$P(x,t) = \frac{1}{2} C_o \left\{ exp\left[\frac{x}{2\,Disp}\left(v - \sqrt{v^2 + 4\,Disp\;k}\right)\right] erfc\left[\frac{x - t\sqrt{v^2 + 4\,Disp\;k}}{\sqrt{4\,Disp\;t}}\right] + exp\left[\frac{x}{2\,Disp}\left(v + \sqrt{v^2 + 4\,Disp\;k}\right)\right] erfc\left[\frac{x + t\sqrt{v^2 + 4\,Disp\;k}}{\sqrt{4\,Disp\;t}}\right]\right\} \tag{38}$$

where *exp* is the natural exponential and *erfc* is the error function complementary.
During closing time, the constant concentration of the added PPP is assumed to be the TWA_{A_L} given by equation (8) in paddy water at $0 \le t < T_1$, while during the open time the same concentration is assumed to be the TWA_{A_L} given by equation (19) at $T_1 = t \le T_2$.
According to the assumptions introduced by the FOCUS Ground Water Scenario Workgroup (EC Document Sanco/321/2000), in the second horizon the organic carbon percentage is 50% of the value of the first horizon (biofactor = 0.5) and in the third horizon it is 30% of the value of the first horizon (biofactor = 0.3). Therefore both adsorption and degradation can be decreased by these values in the second and third horizons.
Since it is not possible to use equations (36) and (37) for k and K_{ads} changing along the soil profile, we assume that the reaction kinetic and adsorption constants to be used in groundwater calculations can be expressed as weighted averages along the soil depth according to the following equations:

$$k_{react} = \frac{1}{\sum_i x_i} \sum_i bio_i\, k_{react,i}\, x_i \quad , \quad K_{ads} = \frac{1}{\sum_i x_i} \sum_i bio_i\, K_{ads,i} x_i \tag{39}$$

Finally, TWAs are computed according to the following equation:

$$TWA = \frac{1}{\sum_i \Delta t_i} \sum_i f_i(t) \Delta t_i \tag{40}$$

where Δt_i is the elementary time interval (usually 1 day) and $f_i(t)$ are the functions (36) and (37).

Dummy Variable Examples
Table 1 reports TWA data computed for three dummy variables having different degradation and adsorption properties in different scenarios (sandy and clayey soils).

Table 1 Different Scenarios and TWAs of various PPPs (Rate 100 g ha^{-1}, Diff 0.3 cm^2 day^{-1}, [TWA] =μg L^{-1})

DT_{50}	K_{OC}	TWA-sw* Paddy (5 d)	TWA-sw Paddy (120 d)	TWA-sw Canal (5 d)	TWA-sw Canal (120 d)	TWA-sw Paddy (5 d)	TWA-sw Paddy (120 d)	TWA-sw Canal (5 d)	TWA-sw Canal (120 d)
		Scenario clay				Scenario sand			
3	10	54.1	1.1	1.6×10^{-1}	5.3×10^{-3}	47.6	4.9×10^{-1}	1.6×10^{-1}	4.2×10^{-3}
30	100	40.1	10.4	2.4×10^{-1}	2.4×10^{-1}	49.9	3.6	2.3×10^{-1}	1.4×10^{-1}
100	1000	6.6	5.3	1.2×10^{-1}	1.6×10^{-1}	12.5	4.8	1.2×10^{-1}	1.7×10^{-1}
		TWA- gw** (120 d)				TWA-gw (120 d)			
3	10	1.9×10^{-25}				1.7×10^{-2}			
30	100	4.3×10^{-90}				8.6×10^{-1}			
100	1000	0.0				7.8×10^{-54}			

** sw = surface water*
*** gw = groundwater*

Sensitivity Analysis

Sensitivity analyses of the SWAGW software, developed from the step 2 model, were carried out for the most vulnerable sandy soil scenario. Sensitivity of the model was investigated using a "one-at-a-time" analysis with a dummy pesticide, the physical-chemical properties of which were preliminarily found to be the most critical for groundwater pollution (DT_{50} in paddy and canal waters 10 days, and K_{OC} for soil and sediments 100 ml/g).
The magnitude of the sensitivity of the models was found to depend more on the dispersivity and the water flow rate than on the diffusion coefficient in water and the constant *a*. Table 2 reports the data of simulations following change of dispersivity.

Table 2 Uncertainty of TWA computations due to dispersivity λ, measured as percentage variation from a reference value (λ=1 cm).

λ (cm)	TWA Variation (%)
0.2	-21.6
0.5	-12.0
1.0	0.0
2.0	20.7
4.1	52.0
6.0	74.7
8.0	108.4

In terms of uncertainty of prediction by the step 2 model, the dispersivity λ and the water flow *q*, which can vary in different soils and the value of which is not well known in all the Mediterranean countries cropped to rice, have the greatest influence on TWA computations. However, as far as the water flow is concerned, according to the work of Greppi *et al.* (1998) and Greppi (1999) on Italian paddy soils, a constant value of one cm day^{-1} as realistic worst case for sandy soils has been chosen in the simulations. In conclusion, according to this analysis, the only source of uncertainty that can severely affect TWA computation is dispersivity.

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PESTICIDE VOLATILIZATION FROM PADDY FIELD

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Abstract

Volatilization may represent an important process influencing the environmental fate of pesticides. In this research study the volatilization rate of five chemicals with different chemical-physical characteristics has been assessed in an artificial paddy field using Theoretical Profile Shape (TPS); a versatile micrometeorological method based on Trajectory Simulation Model, and Integrate Horizontal Flux (IHF), a method based on a time-averaged mass balance technique..

The mixture of chemicals was applied by a spray bar directly on the paddy field which was submerged with 10 cm of water. This water level was maintained constant for the whole study period.

Residues in the air were continuously assessed for a period of one week following pesticide application using polyurethane foam dynamic samplers. For a mass balance, pesticide residual has been measured: (i) in the water immediately before treatment and at the end of the study period; (ii) in the sediment at the end of the study.

Traces of all selected chemicals in air have been detected during the study: higher fluxes have been assessed especially for the chemicals with higher Henry constant during the first three days after the application, in correlation with the water temperature.

Introduction

Many studies have been conducted to determine the processes of pesticide dissipation in rice paddies. Capri et al. (1999) reviewed most of the published data on the pesticide monitoring programme carried out in the paddy areas of Italy. Results showed a diffuse pollution of both surface and groundwater bodies with pesticides at concentrations varying from 0.1 to 30 mg/L. Amongst the pesticides found, the authors reported specific pesticides used in rice crops (propanil, molinate, bethiocarb, bensulfuron-methyl) as well as pesticides used in other rotational crops like maize and soya (alachlor, atrazine, metolachlor and bentazone). In some provinces of Italy, water contamination is so persistent that the use of pesticides has been limited. For example, the use of molinate and bentazone has been prohibited since 1987 (OM, 1987a; OM, 1987b). On the other hand, there are no studies showing how the rice paddies has the potential to contaminate the air phase with pesticide residue. The potential for contamination of air during rice cultivation is of continuing concern to residential population, legislators, farmers and the agrochemical industry.

Materials and methods

The experiment was carried out at the University experimental field (Università Cattolica del Sacro Cuore) in Piacenza, Italy. The field was situated on a flat alluvial plain in the Po valley. The soil (Vertisuol Cambisol) was a silty loam with a 1.5 % organic carbon content and pH 7.8. An automated climatic station installed inside the field site (model AD2, Silidata spa, Modena - Italy) for recording air and soil temperature (10 cm depth), wind speed and wind direction, precipitation, air humidity, and solar radiation on 10-minute intervals.

Evapotranspiration was estimated / calculated from pan evaporation daily data using RadEst 3.0 software.
Five test substances with different chemical properties were selected: insecticides chlorpyriphos, chlorpyriphos methyl and ethoprophos, and fungicides procymidone and metalaxyl. These test substances were applied for the purpose of this study in October 2002 by a calibrated pneumatic spray bar. This non-label application was justified because this was a scientific study to evaluate processes, not a regulatory study to assess risk. The pesticide application was performed with a hand-carried sprayer with a boom of 5-m width in a 16 x 17.5 m water surface submerged with 10 cm of water. This water level was maintained throughout the study period by regular additions of water when needed. Since considerable volatilization normally takes place immediately after application the sample collection started 10 minutes after application. During the course of the experiment, no spray treatments were carried out within 5 km of the experimental field.

The air-sampling system adopted for the field study was developed by evaluating a number of different procedures. The field measurements of volatilization were performed with four air-sampling stations, each consisting of a glass tube sampler of 20-mm diameter containing a plug of polyurethane foam (PUF). These were connected by a Tygon® pipe to an air-sampling pump (model 224-PCEX4, SKC ltd, - USA), which was operated with an airflow of 2 L min-1. The PUF sampling plugs were positioned at the centre of the plot at Z_{inst} height (Z_{inst} is described below) and at the other three heights defined by application of the IHF method . After sampling, the plugs were returned to their sample jars and stored at -22°C until analysis.

The extraction procedure of PUFs was carried out using a triple extraction with acetone in ultrasonic bath, followed by concentration and GLC-MSD analysis. An Agilent Model 6890 Series gas liquid chromatograph (GLC) equipped with an Agilent model 5973 mass detector (MSD) and a fused-silica capillary column DB-17 by J. & W. Scientific (30 m × 0.25 mm I.D. and 0.25 μm film thickness) was used for pesticides quantification. The detection limit was in the order of 0.01 mg/kg and good linearity was achieved (R2 > 0.98) for all pesticides. Active ingredients were identified by: (i) comparison between sample and standard retention times, (ii) comparison between sample and standard mass spectra, (iii) comparison between sample and mass spectra present in library; concentrations were determined by linear regression technique recovery tests. Air concentrations were obtained by considering the volume of sampled air (Capri et al., 1999). Static recovery, retention efficiency, and sampling efficiency were also assessed for each test substance.

The volatilization of pesticides from the soil surface was calculated using the theoretical profile shape (TPS) method described by Wilson et al. (1982) and the integrated horizontal flux (IHF) (Denmed et al., 1977).

Volatilization calculation by TPS
TPS is used to determine the gaseous mass transfer from field experiments. The TPS method, recently applied by Ferrari et al. (2003), offers a series of advantages compared to the aerodynamic method (Yates et al., 1996): (i) the large fetch requirement is not necessary, (ii) measurement of air concentration and wind speed are needed at only one height, and (iii) the sensor is placed at a height that is relatively insensitive to the atmospheric stability so temperature, wind gradients, and stability correction are not required. This approach is based on the Trajectory Simulation Model (TSIM) (Wilson et al., 1982; Wilson et al., 1981) and was used to simulate movement of pesticides away from a treated field (Yates, 1993).

Volatilization V is calculated according to:

$$V = \left. \frac{[u(t)c(t)]}{\Omega} \right|_{Zinst} \quad [\text{mg m}^{-2}\ \text{s}^{-1}]$$

where $u(t)$ is the wind speed during sampling time (m s^{-1}) and $c(t)$ is the pesticide concentration in the air during the sampling time (mg m^{-3}) at sampling height Z_{inst} (cm). The ratio of the horizontal to vertical flux Ω is obtained using the 'trajectory simulation method'. Ω and Z_{inst} (calculated by TSIM) depend on the surface roughness Z_0, and the upwind fetch distance (i.e. the radius of the treated surface).

The roughness length, Z_0, was estimated by measurements of the wind speed at three heights continuously for a week prior to pesticide treatment. The roughness length was then obtained from the logarithmic wind profile where Z_0 is the intercept with the height-axis. A constant value of 9.3 cm was obtained; Z_{inst} were calculated at 127 cm from the water surface.

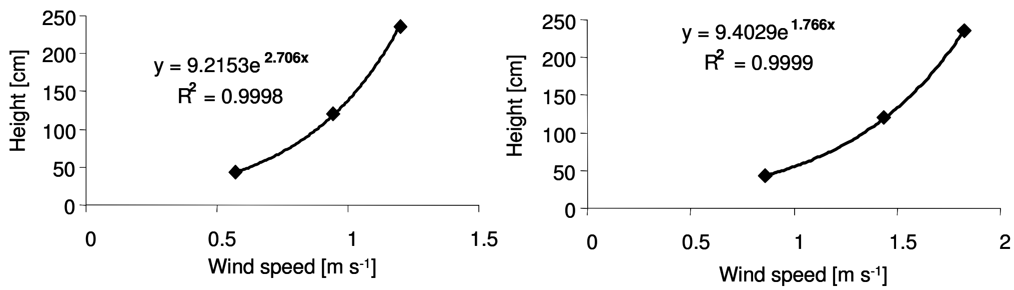

Figure 1. Wind profile measured in two different days, at 12 p.m. and 12 a.m.; wind speed has been measured at three different heights from the water surface: 40, 125,, and 240 cm. Dots represent measured points (one hour average) while the line represents a regression line. The constant (9.2 ÷ 9.4) in the exponential function of the regression line corresponds to the roughness length Z_0 (expressed in cm).

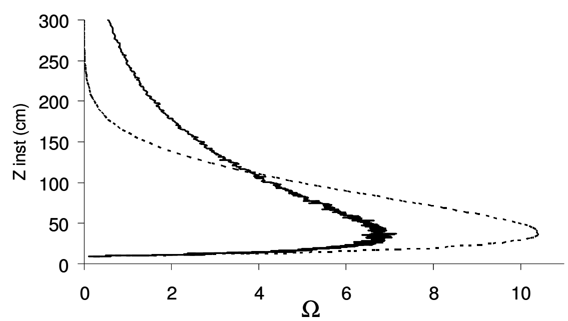

Figure 2. Normalized flux of air (ratio of the horizontal to vertical flux) obtained using a Monin-Obukhov length of 500 and –500 cm for stable and unstable atmospheric conditions, respectively, for different heights calculated with the Trajectory Simulation Model. The full line represents unstable atmospheric conditions and the dotted line stable one. The crossing point in common with the two lines indicate Z_{inst} and Ω.

Volatilization calculation by IHF

This method is a time-averaged mass balance technique where the flux is determined according to:

$$V = \frac{1}{X}\int_{0}^{\infty} \overline{c}\,\overline{u}\ \mathrm{d}z$$

where X is the upwind distance on the leading edge of the source, and *u* and *c* are the averaged wind speed and air concentration at height *z* (Denmed et al., 1977; Majewski et al., 1990). This method requires a uniformly surface source, and an uniform source strength, but not require atmospheric stability corrections and the long upwind fetch distance. During the study four heights *z* from the water surface (33, 84, 127 and 153 cm) were chosen and air samples were collected continuously for four days after the treatment.

Results and discussion

The field experiment was performed in absence of rainfall, with an air temperature ranging from 5.4 to 22.8°C and a water surface temperature ranging from 10 to 21.5°C. During the study period the inflow water volume in the studied paddy was 277 m^3. In contrast outflow water volume from the paddy was considered to occur due to evaporation, leaching or lateral drainage.

The mixture of the five pesticides applied on the experimental plot was analyzed by GC-MS before treatment and application rate for each pesticide (Table 1) was calculated considering the concentration of the active ingredients and the distributed volume in the tank mix.

Pesticides in surface water and sediment

Surface water samples were collected before the treatment and four times after: traces of pesticides were found only after application. The highest concentrations of pesticides were recorded 30 minutes after application and ranged from 514 $\mu g\ L^{-1}$ for metalaxyl to 103 $\mu g\ L^{-1}$ for chlorpyriphos. This discrepancy between expected and measured concentrations of pesticides observed immediately after application not be explained by the application rate. However, it could be related to the mixing speed of each individual pesticide in the paddy water after application. As a result, the higher pesticide concentrations in water were measured for metalaxyl and ethoprophos which have the higher water solubility (8400 and 700 mg L^{-1}, respectively), compared with procymidone, chlorpyriphos methyl and chlorpyriphos which had low water solybilities (4.5, 4 and 1.4 mg L^{-1}, respectively) (The Pesticide Manual). A daily gradual decrease of pesticide concentration in the paddy water was observed during the study and the concentration of all pesticides were below 1 $\mu g\ L^{-1}$ a week after treatment. The highest persistence of procymidone, chlorpyriphos and chlorpyriphos methyl at the end of the study could be related to their high k_{ow} (1380, 50000, 17000 respectively) explaining the concentrations of these pesticides detected in the water phase at the end of the study. This is also in accordance with the pesticide concentration found in the sediment (0-5 cm depth) where chlorpyriphos, the chemical with the lower solubility and the higher k_{ow} was found at level of 433 $\mu g\ kg^{-1}$ (Table 1).

Table 1 Application rates and pesticides concentration in surface water and sediment

Sample	Ethoprophos	Procymidone	Metalaxyl	Chlorpyriphos	Chlorpyriphos methyl
	---------------------------------------mg m^{-2}---				
Appl. Rate	105	73.0	105	117	84.2
	---------------------------------------µg L^{-1}---				
Water 30min	488	279	514	103	121
Water 2 days	1.52	2.77	3.08	6.92	1.39
Water 4 days	0.43	0.82	0.51	1.57	0.38
Water end	n.d.	0.38	n.d.	0.96	0.15
	-------------------------------------µg kg^{-1}--				
Sediment end	14.7	49.9	9.7	433	18.7

Volatilization calculated by Theoretical Profile Shape method

Volatilization rate was assessed by TPS show a typical daily trend (Figure 3) where the higher fluxes were measured during morning and afternoon in correlation with the higher water temperature. Volatilization of ethoprophos was observed for the first five days after treatment. Chlorpyriphos and chlorpyriphos methyl followed a similar trend but they were still volatilizing at the end of the experiment. In contrast, for procymidone a volatilisation flux was observed three times and always in the afternoon, while metalaxyl residues were present in the air only for the first 24 hours after application.

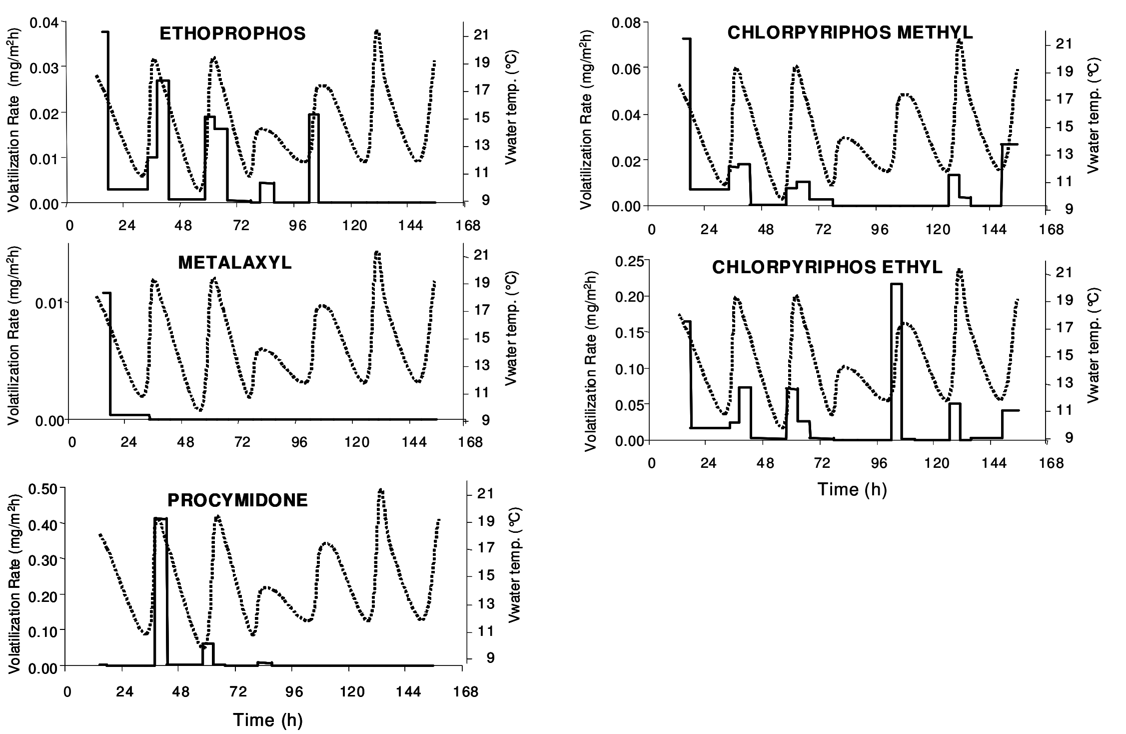

Figure 3 Volatilization fluxes measured for each pesticide by TPS (full lines) in relation to the water surface temperature (dotted lines). Hour zero indicate the midnight before the treatment.

The cumulated volatilization of the selected chemicals measured within a week after treatment represents only a minor amount of the total pesticide mass initially applied, ranging from the 0.03 to 3.27 % of the application rate. The total pesticide mass lost through volatilisation during the study period were 0.62, 2.39, 0.04, 3.11, 0.86, mg m^{-2} for ethoprophos, procymidone, metalaxyl, chlorpyriphos and chlorpyriphos methyl, respectively. These values are in good agreement with the respective Henry constants (1.6 10^{-2}, 1.14, 2.49 10^{-5}, 6.75 10^{-1}, 4.51 10^{-1}) of the studied pesticides.

Volatilization calculated by Integrated Horizontal Flux method

Volatilization of selected chemicals has been determine for the first four days after the treatment also by IHF method and gradient concentration (Figure 4) in air were measured continuously by collecting four samples of air from the middle of the plot at four different heights from the water level.

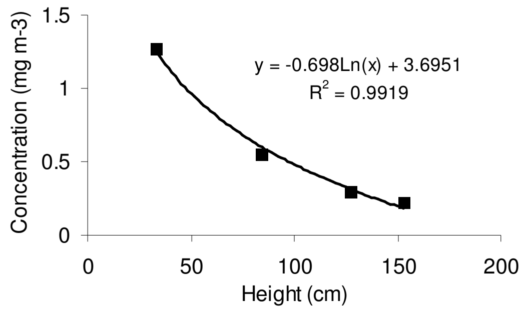

Figure 4 An example of gradient of chemicals in air: concentration of chlorpyriphos in air at different heights in the first samples collected after the treatment.

Applying the IHF method by coupling wind gradient to each concentration profile, we were able to recalculate cumulated volatilization of the five pesticide and compare the results with those results obtained by the TPS method (Figure 5). An acceptable correlation was evident for four of the five chemicals tested -ethoprophos, chlorpyriphos methyl, chlorpyriphos and metalaxyl. For these pesticide, a discrepancy in the cumulated volatilisation calculated by the two methodes varied from 10 to 25 %. The higher uncertainty was observed for the descriptions of the volatilization of procymidone given by the two micrometeorological methods and a discrepancy of four times in the cumulated volatilizations indicates a failure of one of the two methods. This discrepancy could be attributed to secondary processes affecting volatilization of chemicals (e.g. co-distillation, non-uniform mixing of the solute in the water, condensation from air etc.) which are not considered by these methods, but could significantly influence volatilization of procymidone.

This simple analysis of data suggest that micrometeorological methods used usually to asses fluxes of chemicals from the soil could be applied also on water surface. However, the accuracy of those methods should be evaluated every time in order to avoid mistakes in the interpretation of the results; mistakes which could be due to particular application conditions where secondary processes may became relevant.

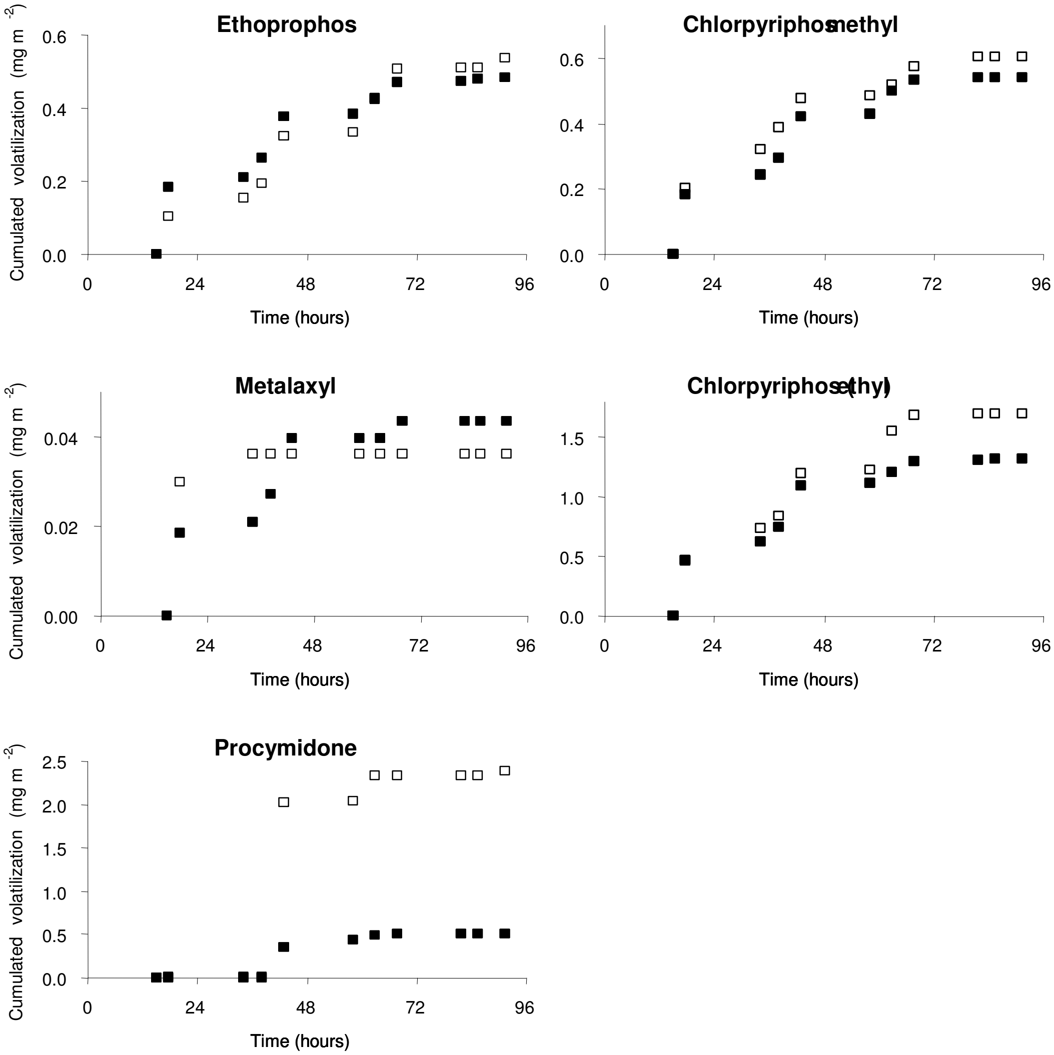

Figure 5 Comparison of cumulated volatilization measured for each pesticide by TPS (empty dots) with cumulated volatilization measured by IHF (full dots). Hour zero indicate the midnight before the treatment.

Conclusion

Volatilization of chemicals from surface waters is a process affecting the environmental fate of xenobiotics. Results reported in this paper show that under realistic field conditions, volatilisation of pesticides with relatively high vapour pressure ($5x10^{-2}$ - $7x10^{-4}$ Pa) could account for more than 3% of the initially applied pesticide mass, in autumn which is a period usually characterized by mild air and relatively low water temperature (< 23°C). This implies that higher pesticide losses due to volatilization might be expected during late spring and summer when application of pesticides on water surfaces becomes more intensive and the climatic conditions (high water and air temperature) favour volatilisation of pesticides from water surfaces. This study was a first attempt to measure volatilisation losses from water surfaces. However, further work is required in order to more accurately assess the contribution of volatilisation from water surfaces on pesticide environmental fate and risk assessment procedures.

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UNCONFINED AQUIFER IN PADDY FIELD AND WATER QUALITY

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Abstract

In paddy irrigation the water infiltrated from the soil surface: at first saturates the soil stratum over the unconfined aquifer at field saturation then raises the groundwater level. The soil structure and the soil texture characterize the infiltration process. This hydrologic process in first approximation is one-dimensional; the flow in the horizontal plane is of one order lower than that in the vertical direction, mainly but not only because of the distance to travel. In this way the phreatic level rising is closely correlated to the vertical infiltration. The resultant of the gravitational force and the capillary forces gives the energy to the water molecules to push the air entrapped out of the pores, reaching a full saturation of the soil and raising then up the phreatic level. There is a complex interaction between the agent forces on the water and the air resistant forces in the soil micro-pores. In paddy fields irrigation a groundwater level stable condition is reached when there is equilibrium between the capillary forces. A stable soil stratum with pressured air is always present in the soil over the phreatic level and under the soil surface submerged. From the collected data it seams that the soil stratum thickness from the soil surface to the groundwater table is proportional to the modulus of the capillary force. In a stable surface ponding depth, during the groundwater table rising, the level growing velocity may be assumed proportional to a medium hydraulic conductibility of the soil stratum. When the phreatic level is approaching the saturated soil surface, the rising rate gradually changes until it gets to a point of equilibrium where the groundwater table is stationary at stable surface conditions. The local rise rate of the groundwater-table is used to define local medium soil conductivity in the vertical direction and then local pollution risk. It is well known that water is a primary vector for the transport of substances in the environment. The irrigation waters and the rain infiltrated into the ground seep through the soil agricultural chemicals into the aquifer.

Keywords

Groundwater level; Soil conductivity; Monitoring Equations; Aquifer contamination.

Introduction

In a paddy field the irrigation causes a water layer ponding over the soil surface. The experience shows the close correlation between the surface water ponding and the phreatic level raising. Bradley, 1996, hyphened that groundwater-table rising depends for a great part on the infiltration of the water present on the surface. The soil texture of the ground stratum over the unconfined aquifer and the soil structure are relevant to characterise this phenomena. In 1967 Allavena showed that the groundwater table level in a paddy field raises during the irrigation season till a certain depth that in that case was about 1 m under the soil surface (Figure 1and 2).

The process of water infiltration into the soil is usually described by the one dimensional Richards equation, because water flow is predominantly vertical (Romano et al.,1998) . The solution of this equation with a quasi permanent water layer on the soil surface gives a continuum profile of the water pressure head through the soil to the surface, i.e. the groundwater level reaches the soil surface (van Dam and Feddes, 2000). But in our experience with paddy field piezometers it was always found a soil stratum at field saturation with air in the micro-pores between the groundwater level and the soil surface. In a surface ponding condition the forces exerted by the air trapped into the soil cannot be left aside in the study of the infiltration in an unsaturated soil. Wang et al. (1998) showed the importance of the air pressure in the infiltration process, and before them Corrants et al. (1988) evaluated a reduction of about 20% of the hydraulic conductivity at saturation for the presence of the air in the porous media.

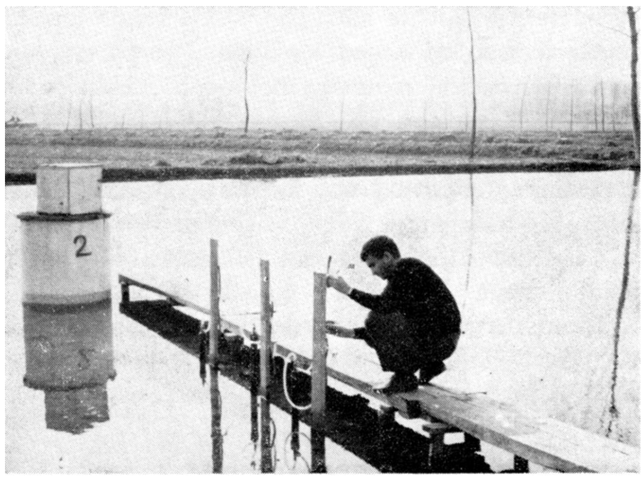

Figure 1 Piezometric wells in a paddy field (1967).

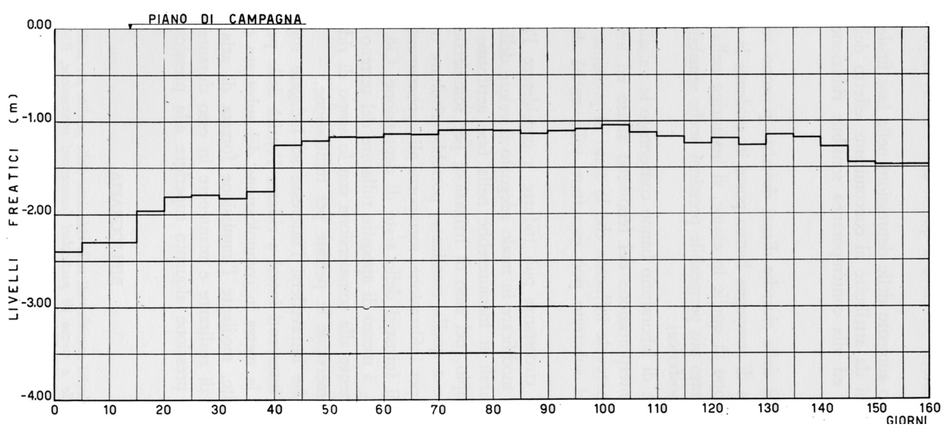

Figure 2 Groundwater level during irrigation season.

The surface soil structure is very important to define an initial infiltration velocity. Into the soil the infiltrated water first saturates the soil stratum over the groundwater table at field saturation, and then contributes to raise the groundwater level. It is a matter of fact that, after a first period of consistent raising and high infiltration, the groundwater-table tends to stabilize around a fixed depth from the soil surface, which is a soil parameters function. The equilibrium is reached between the capillary forces, the gravitational force and the air pressure in the micro-pores.

The rising of the aquifer takes a different amount of time depending on the structure and the texture of the surface soil. In an area with a surface coarse-textured soil the transfer time is

shorter, the volumes transported are greater and the levels rise very rapidly. The groundwater table stabilises right below the soil surface. On the contrary, in an area with surface fine-textured soil the rise is slower and the groundwater table stabilises at a deeper depth from the soil surface.
In the pictures of Figure 3 are plotted the groundwater level trend of the year 2000 in two piezometric stations, as example of two different regions. The piezometric well in the area denominated Bianzé has a surface soil structure with higher clay content. The other one in the area denominated Stroppiana has a surface soil structure quite different like a sandy soil. For the same piezometric stations the level excursion and the rain occurrences during the year 2000 are daily compared in Figure 3.

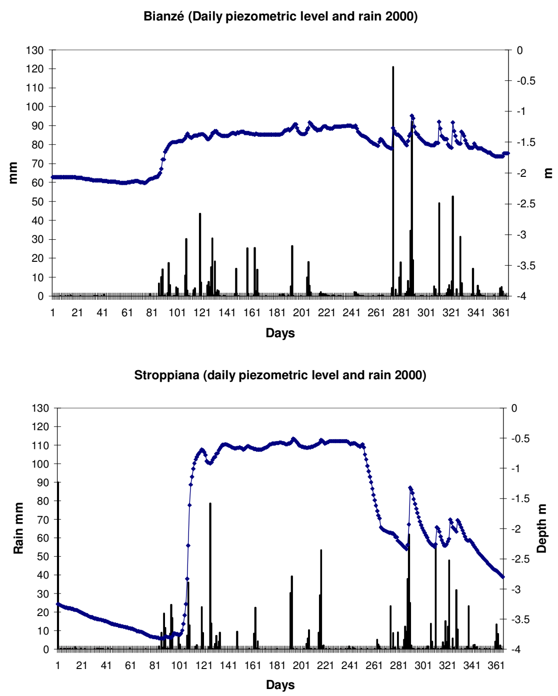

Figure 3 Groundwater level excursion and rain occurrences in two different surface soil texture

From these pictures it is evident that the groundwater table rising starts after the irrigation of paddy fields or after an intensive rain. Before irrigation or during dry spells the phreatic level decreases slowly. The excursion of the groundwater-table is lower in Bianzé than it is in Stroppiana and, before irrigation period, the phreatic level is closer to the surface in a clayey surface soil than it is in a sandy soil. At the same time, in Bianzé, where there is a higher capillary fringe, during the irrigation period the phreatic level gets to a point of equilibrium at a higher depth than in Stroppiana. As is known, there is much less infiltration for a fine structured surface grounds; the transfer flow is in fact very slow when the soil is not fractured, the rise of the groundwater-table as a consequence more gradual.
These piezometric wells are part of the network of fourteen piezometers which were installed in North West Italy paddy fields on a wide area of about 70.000 hectares in the second half of the 1960's to measure the groundwater level trend on a daily basis. The map of this region is plotted in Figure 4. This is an interesting example of a continuum groundwater table monitoring.

The groundwater level dynamic

In the fourteen piezometers the groundwater level trend shows a quite different behaviour. Nevertheless it is possible to identify two sub-regions where the trend of the levels is similar. In the North West area, showing a trend similar to the Bianzé piezometer, the groundwater table is not too deep under the soil surface in the dry season and its raise during the irrigation period is slower than in the other piezometers. While in the South East part of the region, the groundwater level comes down to a higher depth from the surface during the dry season and, after the irrigation beginning, the raise is quicker with a trend similar to that of Stroppiana piezometer. In this area the stationary level under the soil surface reached is much more near to the surface than the first ones.

To better understand the water flow in the unconfined aquifer and the diffusion process of transported substances, a series of surface wells (6 piezometers) located on the board of a 1.5 hectare paddy field were installed in a 2 m side square (see "*Sito sperimentale*" in Figure 4). The wells are made up of steel tubes approximately 6 cm in diameter and they were implanted into the soil. The length varies between 2 and 19 meters. Each tube was perforated along the last 30 cm, in order to capture the water only at the desired depth. The well tops were capped with a threaded steel stopper to prevent contamination.

Recently a piezometric well has been equipped with an STS Data Logger and a rain gauge station was installed in the site *"Sito sperimentale"*. The groundwater level is monitored every three hours to better appreciate its dynamic, starting February 4th 2003. In the Figure 5 is plotted the trend of one year from February 4th 2003 to February 3th 2004. From this picture it is interesting to remark that during paddy field flooding the rainfall does not influence significantly the level of the aquifer; on the other hand in the non irrigated period heavy rainfall causes a rapid rising of the level. When the infiltration of the water stops the level descends quite rapidly, but with a rate lower than during the raise.

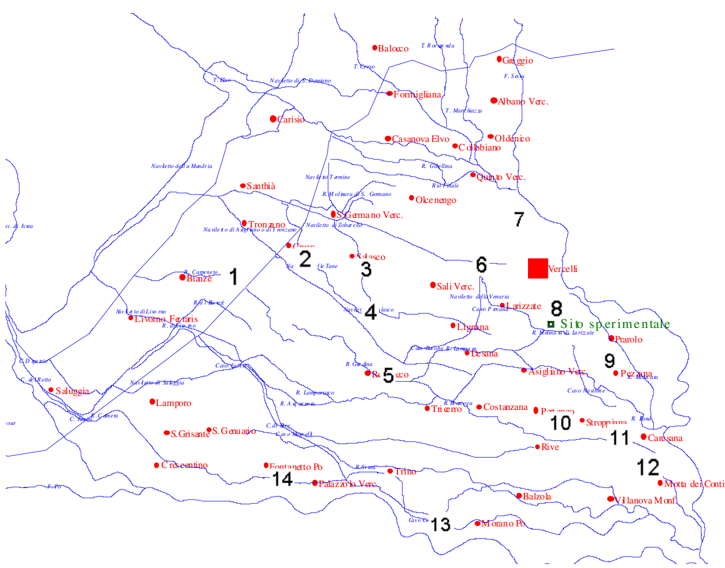

Figure 4 Map of the piezometric wells in the paddy region.

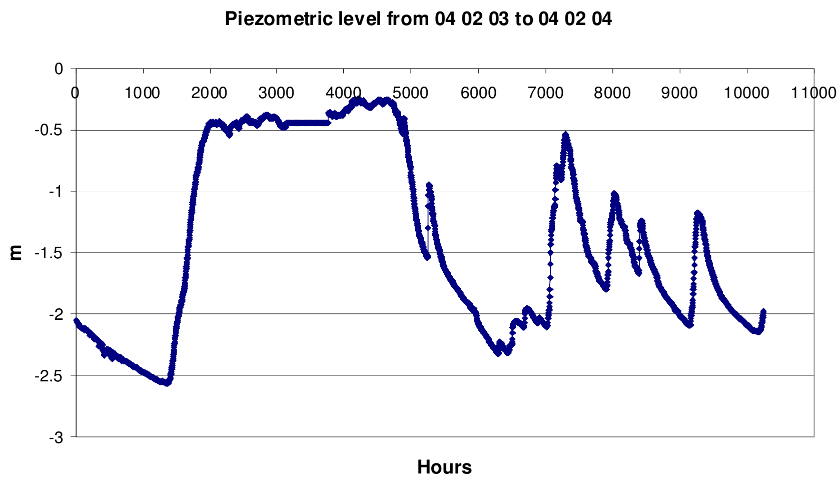

Figure 5 Groundwater level trend, monitored every 3 hours.

In Figure 6 rain events and groundwater level corresponding to the period 7010 - 7490 hours of the previous Figure 5 are plotted. The rain events are very intense so that the precipitation infiltrated raises the groundwater level of a 1.2 m step and it maintains this level near the soil surface for some days after.

The groundwater level trend is a function of the rainfall and of their intensity. The soil structure characterizes the level path in the raising speed, in the curvature before reaching the equilibrium at a top level of a flooded field and in the downing curvature.

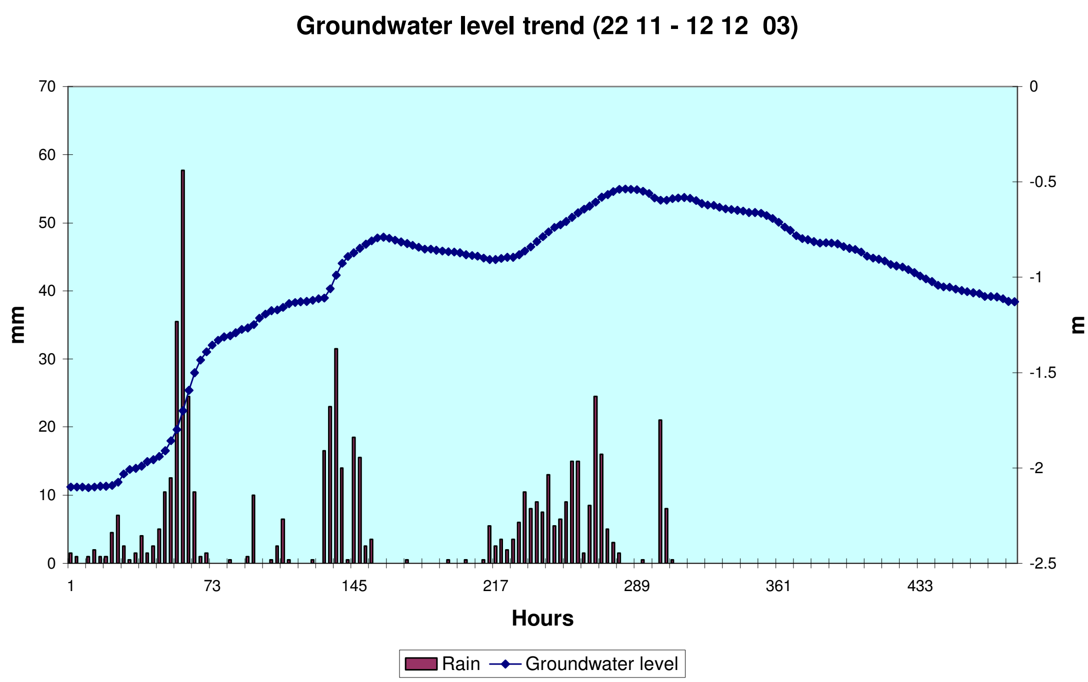

Figure 6 Correlation between rain occurrences and groundwater level in twenty days

The equations to describe the process

During the ponding, the infiltration process can be divided schematically into three phases:

1) In the first phase through the unsaturated soil water infiltration acts under the push of the gravitational force and the capillary forces. The infiltration flow can be described by the one dimensional equation:

$$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial z}\left[D(\theta)\frac{\partial \theta}{\partial z} - K(\theta) \right] \tag{1}$$

where the soil water diffusivity is defined by $D(\theta) = K(\theta)d\psi/d\theta$, with ψ the matric potential head and with $K(\theta)$ the hydraulic conductivity.

The water retention capacity of the soil, which depends on its structure, is usually defined as the measure of the energetic state of the water present in the soil called matric potential head ψ_p. It depends by the force of adhesion of the water to the solid particles of the soil, which in the tiny canals present in the soil gives the capillary effect, so some authors call capillary head (Corradini et all, 2000, Smith et all, 1999).

The hydraulic conductivity $K(\theta,,z)$ is a function of the position and the state of saturation of the soil, for this reason usually the field saturated hydraulic conductivity K_s is used.

2) This process of soil saturation at a field condition lasts until the soil stratum over the groundwater table is all saturated. After this stratum has reached the condition of field saturation, the surface ponding persisting, a second phase starts with a constant and prevalent flow q_z from the surface that causes the phreatic aquifer level raising. The term "field saturated" is used (Mertens et al. 2002) because, under field condition, a certain amount of air is usually entrapped in the soil during the infiltration process (Reynolds et al. 1983, Elrick et al. 1989)

The flow rate along the vertical direction, when the groundwater table is deep from soil surface (>> of capillary fringe) could be studied, using the Darcy equation written with the relationship:

$$q_z(t) = \overline{K}_s \frac{H + l(t)}{l(t)} \tag{2}$$

where the conductivity $\overline{K}_{zs}$ is a medium value in the vertical (z direction), H is the irrigation water depth and $l(t)$ is the soil stratum thickness crossed by water filtering, which change with groundwater level rising and therefore is a function of time t. The depth H is small (0.1 m) compared with l usually over 1 m, then the previous relation with a first approximation becomes:

$$q_z(t) \cong \overline{K}_s \tag{3}$$

In this description the air pressure effect into the pores is neglected. From the experimental fields, as is seen in Figure 1, it is known that the groundwater table never raises as far as ground surface. When t goes to infinity, in ponding condition, $l(t)$ tends to a value which is not zero but a function of the soil stratum characteristics.

The relation between the mass of water per unit of surface that enter in the groundwater and the water-table variation in the hypothesis of neglecting the horizontal flow with respect to the vertical flow and the air pressure into the soil pores becomes:

$$\rho q_z \Delta x \Delta y = \frac{\partial}{\partial t}\left(\rho n_e \Delta V\right) + n_e \frac{\partial h}{\partial t} \rho \Delta x \Delta y \tag{4}$$

where the left hand term represents the flow rate entering in the aquifer, ρ is the water density; whilst h is the groundwater–table level depth from the ground surface. The two right hand terms represent respectively: 1) the changes in time of the liquid mass contained in soil volume ΔV, due to the difference in the effective porosity n_e and in the density ρ, and 2) the quantity due to the increase or decrease of the specific water content. The hypothesis to neglect the groundwater horizontal flow against vertical flow contribution, during surface aquifer level rising (Bradley 1996), is particularly justified in a flat plane with paddy fields like Po Valley.

Leaving aside the first of the two terms on the right with respect to the second in the previous relation, given its reduced numerical dimension (Bras 1990), and substituting the expression for q_z, the following relationship is obtained:

$$\overline{K}_s = C\frac{\partial h}{\partial t} \tag{5}$$

where C is a constant in a quasi homogeneous soil.

The groundwater table level h rises due to the full saturation of the pores in the ground under it. The process is governed by the combination of the two forces acting on the water infiltrated into the ground: the gravity force and the capillary force. The resultant has its greatest value in the vertical direction, like gravity. This force is able to introduce the water inside the micro-pores getting out the air present.

When the groundwater table is at a greater distance from the soil surface than the capillary fringe, the pressure exerted by gravity and the capillary force on the air in the micro-pores is greater than the air resistance, causing the infiltration water to go into the micro-pores and push out the air. Therefore the groundwater table rises. Furthermore the soil surface submerged is a condition of no limit for the infiltration water in a water balance of the aquifer. Following Eq: 1 the groundwater table has to raise to the soil surface, because the flow in the vertical direction is greater than that in the horizontal plane. But the experience shows that this is not true. There is a reduction of flow in the vertical direction and the groundwater level reaches a stationary condition at a fixed depth under the soil surface.

3) A third phase starts when the groundwater table approaches a distance from the soil surface approximately equal to the capillary rise; the capillary force from the surface water stratum and that from the groundwater table annihilate, and gravity is not enough to push all the air out of the pores. The groundwater table level reaches then a stationary distance from the soil surface that is roughly the capillary fringe. Besides when the level approaches the soil surface, the infiltration reduces significantly its speed.

In this phase the relation written above is not valid, the conductivity becomes a function of the air pressure into the pores. Therefore it is proposed to describe the phreatic level depth dynamic, using the conductivity at saturation multiplied by a damping function, as in the following equation:

$$\frac{\partial h}{\partial t} = \frac{\overline{K}_s}{C}\left(1 - e^{-\alpha(h-h_c)}\right) \tag{6}$$

where α is a factor that depends on the soil texture and h_c is the capillary fringe depth. This equation for the phreatic level h greater than h_c tends rapidly to Eq. 5.

In the *"Sito sperimentale"*, using the 2003 data recorded with the STS probe, the rising velocity is V_h=0.126 m/d and the groundwater table depth is approximately h_c=0.44 m. The description of the phreatic level before reaching the stationary level fits with the value 0.13 for α in Eq. 6.

Table 1 Surface aquifer rising velocity.

Piezometric well	V_h (m/d)
1 – Bianzé	0.033
2 - Crova	0.036
3 - Salasco	0.044
4 - Veneria	0.037
5 – Ronsecco	0.051
6 – Boraso Farm	0.070
7 – Caresanablot	0.161
8 – Boschine Farm	0.078
9 – Pezzana	0.187
10 – Pertengo	0.095
11 – Stroppiana	0.316
12 – Motta dei Conti	0.261
13 – Pobietto	0.040
14 – Fontaneto Po	0.154

From this analysis it can be seen that the phase of the unconfined aquifer rising allows the evaluation of a rise velocity that can be considered at first approximation constant and proportional to the average hydraulic conductivity K_s of the soil stratum involved.
It can also be observed from Figs. 2, 3, 5 and 6, that the rising velocity is more rapid than the going down velocity. This coincides with the literature known on the greater capacity of the soil to absorb water in the wetting process than to release water during drying.
In the fourteen piezometers of Figure 4 the rising velocity V_h (m/day) of the aquifer have been calculated as the average value of the thirty years of observation; their values are then reported in table 1.
The local velocity of the groundwater rise, as seen, is directly proportional to the flow rate infiltrated and hence to the quantity of water that has fed the aquifer in the time considered. But, given that the water is the principal conductor of the substances present in the surface, a greater velocity means also a higher possibility that a substance from the surface should be transported in the aquifer. At last, the transfer of the chemical substances, used in agricultural practice, results much higher where the aquifer rising velocity is higher.

Conclusions

In this alluvial region the texture is generally finer near the surface and becomes coarser at a greater depth. The hydraulic conductivity of the surface soil stratum is directly correlated with transport capacity of chemical substances from soil surface into the aquifer. Water is the primary vector of transport of pesticides and herbicides used in agriculture, and the process of infiltration of water is the method of transport of the substances into the surface aquifer. The movement of the water and the conditions in which this happens can contribute to accelerate their degradation.

A mean value of the surface soil conductivity could be estimated using the piezometric level rising during initial irrigation period, as it is shown ahead. On the other hand it is simpler to monitor the velocity of the groundwater level rising in a piezometric well than to measure the hydraulic conductivity of the undisturbed soil. In this way using the rising velocity it is possible to estimate a local soil stratum medium hydraulic conductivity at saturation and to evaluate the interchange of water in the rice paddies through the unconfined aquifer. Thus it is also possible to study the environmental fate of the main herbicides used in rice production. Nevertheless evaluation of the environmental persistence of herbicides is very important in understanding their potential to enter into and remain in the unconfined aquifer. In general, the longer herbicides persist in the environment the greater the possibility of contamination of the aquifer is.

For most herbicides, the impact on surface water is closely related to the application period and the quantity used. The impact of herbicides on surface aquifer is over all a function of the infiltration process then of the quantity of herbicides used and of the characteristics of the various herbicides.

For this reason the velocity of the rise of the first aquifer has been studied where piezometric wells are located, having demonstrated that a linear relationship exists between the groundwater level raise and a medium value of the hydraulic conductivity of the soil layer involved. On these bases where are found similar values for the soil saturation hydraulic conductivity $\overline{K}_{zs}$, as previously defined, could be identified in a regional mapping land zones with the same contamination possibility.

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PREDICTING THE ENVIRONMENTAL FATE OF PESTICIDES IN RICE PADDIES IN EUROPE – CATCHMENT SCALE RISK ASSESSMENT

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Abstract

Rice is a crop cultivated in large river basins mainly in southern Europe and the extended use of herbicides in rice paddies may entail a risk for such an aquatic system. Therefore, it would be more relevant to assess the risk from pesticide use at a catchment rather than at a field scale. Accordingly, two catchment-scale scenarios were developed for the specific crop, water and pesticide practices used in rice cultivation in Greece. The scenarios developed were utilized for the parameterization of the RICEWQ 1.6.2v model and the surface water model RIVWQ 2.02 which operating in a linked mode could provide Predicted Environmental Concentrations (PECs) of pesticides in groundwater (GW) and surface water (SW) systems within the rice cultivated catchment. The application of these scenarios using RICEWQ and RIVWQ model enabled us to perform risk assessment exercise for most of the herbicides registered in Greece for use in rice paddies. Such an approach could be further improved in order to be used a regulatory tool in pesticide registration in rice.

Introduction

In recent years mathematical modeling has been introduced as a necessary tool for pesticide registration in Europe. Although, a uniform approach for proper model use has been now established in Europe this could not be used in rice cultivation due to its unique flooding conditions which are used in all European countries. Therefore, there was a need for the development of a uniform approach for performing pesticide risk assessment in rice cultivation in Europe. The MED-RICE group which was established under the aspicies of EU produced general guidelines for how risk assessment should be performed in rice paddies and developed a simple Tier 1 spreadsheet which could be used for calculating PECs in groundwater and adjacent SW bodies. However, the Tier 1 spreadsheet produced by the MED-RICE group could not be used when refined modeling approach is needed and the use of more sophisticated mathematical model was suggested. RICEWQ 1.6.2v is a model which was produced after the integration of a surface run off model like RICEWQ and a vadose zone transport model like VADOFT. RICEWQ 1.6.2v is the only current model which could predict with good accuracy pesticide losses due to runoff/overflow and also provide GW PECs beneath rice paddies (Miao et al 2003; Karpouzas et al 2004a; Karpouzas et al 2004b).
A review of the crop, water and pesticide management practices applied in rice cultivation in Europe revealed significant differences among the various rice-producing countries. The existence of such differences suggested that it would be more appropriate to develop several well-defined national rice scenarios instead of one or few pan-European scenarios. Therefore, in a similar way to the FOCUS SW scenarios developed for other crops (FOCUS, 2001), the tiered approach for rice may consist of step 3 including representative scenarios developed at Member States level for estimating PECs at paddy field scale, and step 4 including basin level scenarios where mitigation processes could be also included (Karpouzas & Capri, 2004). Only recently, a novel FOCUS group was established in order to produce guidelines and scenarios for Step 4, pesticide landscape risk assessment. A similar pesticide exposure assessment at

basin scale level would be also appropriate for rice which is cultivated in Europe at large river basins such the Axios river basin in Greece. Therefore, this study aimed to develop standard catchment-scale scenarios for rice cultivation in Greece which could be used for pesticide risk assessment at basin scale using the two model RICEWQ 1.6.2v and RIVWQ in a linked mode.

Materials and methods

Description of models

RICEWQ 1.6.2v, using daily time steps, simultaneously tracks mass balance of chemical in rice foliage, water column and sediment. Subsequently the amount of pesticide and the volume of water which are predicted to seep below the sediment layer of the paddy system are considered as inputs for vadose zone transport sub-model VADOFT which subsequently calculates, also at daily time steps, pesticide concentrations at the selected groundwater level. The top 5 cm of the soil profile is represented by the active sediment layer in RICEWQ and the remainder of the soil profile is represented as multiple compartments in VADOFT. The bottom of the active sediment layer is the interface between the two sub-systems represented by the two models. The model also provides the amount of pesticide and water lost by the paddy water system due to overflow. This output of the RICEWQ 1.6.2v model was used an a water and pesticide mass input for the chemical transport model for riverive environments RIVWQ 2.02.

RIVWQ simulates the transport and fate of organic chemicals in riverine systems based on the theory of constituent mass balance. System geometry is represented using a link-mode approach in which the water body is divided into a number of discrete junctions connected by flow channels. Dynamic constituent transport occurs between junctions via links and is a balance between river-driven flows and dispersion processes. Chemical transformation occurs within each node including dilution, volatilization, partitioning between water and sediment, decay in water and sediment and resuspension from bed sediments. A detailed description of the models is given in the related users manuals for RICEWQ 1.6.2v (Williams et al 1999) and RIVWQ 2.02 (Williams et al 2004).

Development of scenarios

Two catchment-scale scenarios were developed based on the agricultural, water management and pesticide practices used in the Axios river basin which constitutes more than 70% of the total rice cultivated area in Greece. For the development of the scenarios a total area rice-cultivated area of 24 h was considered including 12 paddies of 2 ha each. Scenario A is a conservative scenario where pesticide mass and water lost from paddy by oveflow and controlled drainage enter directly into a large river system (Figure 1). In contrast, scenario B is a more realistic situation, where pesticide mass and water lost from each 2 ha paddy by overflow and controlled drainage is initially discharged into a series 400 m drainage canals which subsequently discharge their water into a large river system (Figure 2). In Scenario B, four paddy areas consisting of three 2-ha paddies each, were considered. Each of the paddy areas was linked to a drainage canal which receives pesticide mass and water lost by each paddy due to overflow or controlled drainage as loading. The drainage canals included in Scenario B had a rectangular shape, length of 400 m, width and depth of 4 and 2 m and a flow velocity of 0.1 cm/s. Subsequently, each of the four drainage canals is considered as pesticide and water inputs for a larger river system. A rectangular shaped river system was simulated, with a length of 9000 m, width and depth of 70 and 3 m respectively, and a flow velocity of 0.5 cm/s. Pesticide and water input from the drainage canals to the river system occurs at 1000, 2000, 3000 and 4000 m from the beginning of the river.

The simulation period for the scenario was the whole rice cultivation season in Greece; from 1 May until 10 October. The dates of rice crop seeding, emergence, maturation and harvest were set to be 1 May, 7 May, 15 September and 10 October, respectively. Rice is maintained under permanent flood until physiological maturity of rice grains except when drainage is necessary for herbicide application. During flooding the water level in paddy fields is kept to *ca* 10 cm.
Meteorological data for the years 1992-94 (daily precipitation and evapotranspiration) were collected from a station adjacent to the Axios River basin and used for simulation purposes. In accordance to the lower tier MED-RICE scenarios, two hydrological situations for rice paddies were considered in the RICEWQ model: i) a clay soil horizon with an infiltration rate of 0.1 mm/day representing a high risk for SW contamination and ii) a sandy loam soil horizon with an infiltration rate of 1 mm/day representing a high risk for GW contamination. A uniform soil horizon of 1 m depth was considered in accordance with FOCUS GW models and annual concentrations of the pesticide at 1 m were produced.
For RIVWQ parameterization, a clay loam sediment was considered for both drainage canal and the riverine system. Other RIVWQ parameters like the dispersion coefficient E_L (m^2/s), coefficients c and d which are related the depth D and flow Q of each surface water system (drainage canal and river) were calculated according to Williams et al (2004). The muscingum routing option of the model was only used for the river system and the values were calculated according to Williams et al (2004).

Risk assessment example
A risk assessment exercise was performed to assess the possibility of using such an approach for regulatory purposes in Greece. The pesticides included in this study are all herbicides registered in Greece for use in rice paddies (Table 1).The maximum recommended dose for each pesticide was used for model parameterization. Pesticide properties such water solubility, adsorption coefficient (K_d), and pesticide decay rates in paddy water and sediment which are essential parameters for RICEWQ were derived from literature databases (Tomlin 2000; Wauchope et al, 1992; Augustin et al, 1994).

Table 1 Herbicides used in rice cultivation in Greece

Herbicides	Application Rate (kg/ha)	Mode of application	Application Time (DAS)
Azimsulfuron	0.025	Drained	25
Bentazone	1.200	Drained	40
Pretilachlor	1.000	Flooded	10
Molinate	3.750	Flooded	10
Propanil	5.950	Drained	35
Cyhalofop butyl	0.300	Drained	25
Quinclorac	0.750	Drained	25

The annual average concentration leaching below the 1 m soil zone for each of the three years were used as outputs for assessing the potential risk for the studied pesticides to contaminate groundwater resources. The concentration of pesticides predicted by the model in the drainage canal downstream of the rice paddy inputs and also in specific points in the river system downstream of the input junctions (5000, 7000 and 8000 m) were used as exposure concentrations. These concentrations were used for the calculation of the acute toxicity exposure ratios (TERs) using the following equation:

$$\text{TERs acute} = \frac{\text{LC50 or EC50}}{\text{PECs}}$$

The toxicological endpoints used for TERs calculation were derived from literature databases (PAN Pesticide Ecotoxicity).

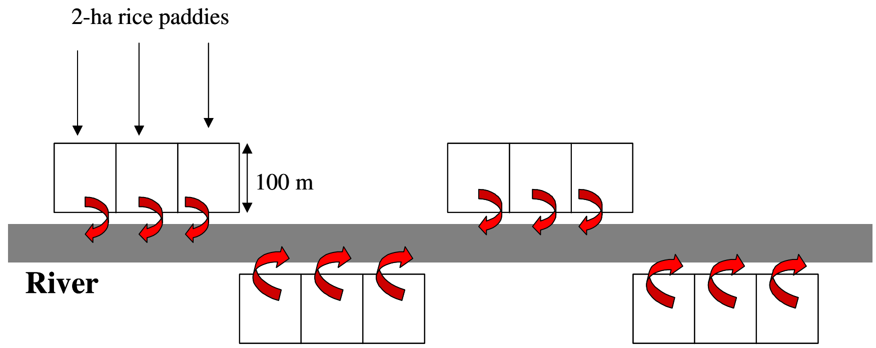

Figure 1 Scenario A (conservative) for risk assessment of rice applied pesticides at basin scale.

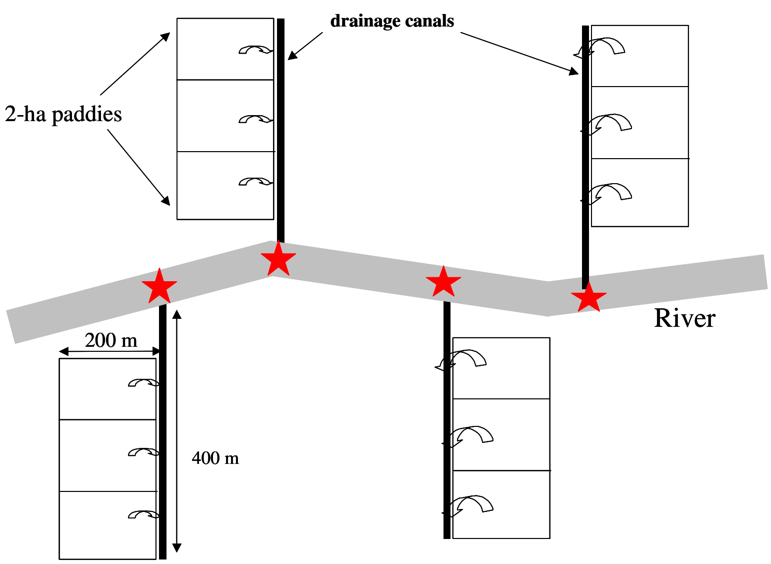

Figure 2 Scenario B (realistic) for risk assessment of rice applied pesticides at basin scale level.

Results and Discussion

The two catchment-scale scenarios were used for performing a risk assessment exercise for several herbicides used in rice paddies in Greece. The PECs in GW and SW derived by the two models used in the scenarios were utilized for calculation of appropriate acute and long term TERs, mainly for aquatic organisms. The risk for groundwater contamination in paddy systems by the use of the specific herbicides as predicted by the model are shown in Table 2. The results suggest no risk for GW contamination for all herbicides when a clay loam horizon with low infiltration rate was simulated. On the contrary, bentazone, quinclorac and molinate appear to pose a risk for GW contamination when a sandy loam horizon with a high infiltration rate was simulated. These results were expected since bentazone and quinclorac are relatively mobile chemicals as suggested by its low K_{oc} and its high water solubility. In addition, quinclorac is rather persistent chemical which increases the possibility for groundwater contamination. Molinate is also a relatively mobile chemical but its high volatility increases its dissipation rate in the field and therefore diminishes the amount of pesticide in soil which is available for leaching. The annual concentrations of molinate predicted by the model to leach to 1 m depth in a sandy loam soil horizon are marginally higher than the 0.1 μg/L limit which has been set by EC.

Table 2 Groundwater PECs (μg/L) for rice herbicides used in Greece

Pesticides	Clay (0.1 mm/d)	Sandy loam (1 mm/d)
Azimsulfuron	-	0.03
Bentazone	-	2.36
Propanil	-	-
Molinate	-	0.13
Quinclorac	-	5.10
Pretilachlor	-	-
Cyhalofop butyl	-	-
Cyhalofop acid	-	-
Cyhalofop amide	-	-
Cyhalofop diacid	-	0.01

The aquatic risk assessment exercise suggested that pretilachlor, molinate, azimsulfuron and propanil appear to pose a risk for several aquatic organisms (Table 3). Pretilachlor appears to pose an aquatic risk for algae and *Procambarus* in both scenarios. It should be stressed that pretilachlor is the only herbicide which failed the risk assessment exercise in the river body of Scenario B (realistic situation).

Table 3 Aquatic risk assessment (TERs acute) for most of the herbicides used in rice paddies in Greece when a clay loam soil horizon beneath rice paddies was simulated.

Pesticides	Scenario A	Scenario B Canals	Scenario B River
Azimsulfuron	AP[a]	AP	+
Bentazone	+[b]	+	+
Propanil	+	AL	+
Molinate	F, D	F, AL, Ga	+
Quinclorac	+	+	+
Pretilachlor	AL, Pr	AL, F, Pr	AL, Pr
Cyhalofop butyl	+	+	+
Cyhalofop acid	+	+	+
Cyhalofop amide	+	+	+
Cyhalofop diacid	+	+	+

[a] *Risk for AP: Aquatic Plants; AL: Algae; Pr:* Procambarus *sp.; F: Fish; Ga:* Gamarus *sp.*
[b] *(+) no risk for aquatics.*

The use of molinate appears to pose a risk for certain aquatics including fishes and invertebrates like *Daphnia* and *Gamarus* sp. and also algae. This was expected since molinate is a high input herbicide and the duration of the closure of paddy after its application has been found to be a significant parameter controlling its appearance in adjacent surface water bodies (Crepeau & Kuivila, 2000). In our exercise a 5-day paddy closure after application of molinate was used which is a normal agricultural practice in Greece. Azimsulfuron, appears to pose a risk for aquatic plants like *Lemna* sp. This could be attributed mainly to its high toxicity for aquatic plants (EC50 0.93 μg/L) and not its high concentrations in the surface water bodies.

The scenarios developed in this study could be a first step towards the development of a standard catchment-scale risk assessment approach for the registration of pesticides used in rice paddies. However, further improvements should be incorporated in these scenarios in order to be further implemented in the risk assessment process. Acquisition of 10 to 20 years meteorological data series would offer the option for a probabilistic risk assessment and further calculation of the 80^{th} percentile annual average concentration at 1 m depth in accordance to the FOCUS groundwater scenarios (FOCUS, 2000).

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RECYCLING OF RICE STRAW TO IMPROVE CROP YIELD AND SOIL FERTILITY AND REDUCE ATMOSPHERIC POLLUTION

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Abstract
Burning of rice straw is a common practice in northwest India where rice-wheat cropping system is extensively followed. This practice results in loss of nutrients, atmospheric pollution and global warming due to emission of greenhouse gases such as carbon dioxide, methane and nitrous oxide. A field experiment was conducted at Indian Agricultural Research Institute, New Delhi in 2002-03 to evaluate the efficacy of different modes of rice straw recycling in improving yield and soil fertility and reducing atmospheric pollution. Removal of rice straw from the field and application of recommended level of inorganic N, P and K gave the highest yield of wheat in the rice-wheat rotation. Recycling of rice straw with additional amount of inorganic N or inoculation of microbial culture to enhance decomposition, however, gave similar yield to that of straw removal. Lowest yield was recorded in plots where rice straw was incorporated in soil without additional N. Rice straw incorporation, however, increased organic C and Olsen P and available K status of soil. Emission of nitrous oxide was more when additional N was added. The study showed that burning rice straw could be avoided without affecting yield of subsequent wheat crop by recycling it with additional doses of inorganic N and/or inoculation of microbial culture.

Keywords
Farmyard manure; microbial culture; nitrous oxide emission; rice straw; soil organic carbon; wheat.

Introduction

Rice is the primary staple food for more than 40% of the world's population. Globally about 155 million ha of rice is harvested annually with a production of about 596 million tons (IRRI, 2001). More than 90% of this is produced and consumed in Asia. India, with 42 million ha of land under rice, produces about 110 million tonnes of rice and 250 million tones of rice straw every year (FAI, 2000). The disposal of rice straw is a major concern, particularly in the northwest India, where rice-wheat cropping system is extensively followed. Rice straw now-a-days is neither used as animal feed due to low digestibility, low protein, high lignin and silica contents nor recycled in soil due to limited time left before sowing of wheat. Farmers in the northwest India, therefore, dispose a large part (about 90%) of it by burning *in situ*.

The burning of rice straw is environmentally unacceptable as it leads to 1) emission of greenhouse gases such as carbon dioxide, methane and nitrous oxide (N_2O) causing global warming, 2) release of soot particles causing human health problems such as asthma or other respiratory problems and 3) loss of plant nutrients such as N, P, K and S. Therefore, burning should be avoided and alternate measures of disposal of straw should be developed. One potential solution to the problem of rice straw burning would be its recycling in the succeeding crop. However, incorporation of rice straw with high C:N could accelerate immobilization of N causing N deficiency and increase N_2O emission by adding organic carbon required for microbial growth (Pathak *et al.*, 2002). To increase the mineralization of N, additional application of inorganic, organic or microbial N source may be made. The objectives of the present study were to 1) evaluate the effect of various modes of rice-straw recycling on wheat yield, 2) assess the impact of straw recycling on soil fertility and 3) measure N_2O emission from soil due to rice straw recycling.

Materials and methods

A field experiment was conducted at Indian Agricultural Research Institute farm during the rabi (November to April) season of 2002-03. The alluvial soil of experimental site was Typic Ustochrept with pH 8.0, loam in texture, bulk density 1.38 g cm^{-3}, electrical conductivity 0.43 dS m^{-1}, CEC 7.3 C mol (p^+) kg^{-1}, organic carbon 0.42%, and available N, P and K 232, 17 and 335 kg ha^{-1}, respectively. The experiment had six treatments with three replications in plots of 6 m long and 5 m wide in a randomised block design. The details of the treatments are given in Table 1.

Table 1 Treatments

Treatment	Details
1. NPK	N, P and K 120, 26 and 40 kg ha^{-1}, respectively; No rice straw incorporation.
2. NPK + rice straw	N, P and K 120, 26 and 40 kg ha^{-1}, respectively; rice straw (5 t ha^{-1}) incorporated after chopping into 5-6 cm pieces.
3. NPK + rice straw + extra N	N, P and K 180, 26 and 40 kg ha^{-1}, respectively (extra 60 kg ha^{-1} inorganic N was applied to bring the C:N of rice straw to 20); rice straw (5 t ha^{-1}) incorporated after chopping into 5-6 cm pieces.
4. NPK + rice straw + FYM	N, P and K 120, 26 and 40 kg ha^{-1}, respectively; rice straw (5 t ha^{-1}) incorporated after chopping into 5-6 cm pieces; FYM at 5 Mg ha^{-1}.
5. NPK + rice straw + Microbes	N, P and K 120, 26 and 40 kg ha^{-1}, respectively; rice straw (5 t ha^{-1}) incorporated after chopping into 5-6 cm pieces; microbial culture.
6. NPK + rice straw burnt	N, P and K 120, 26 and 40 kg ha^{-1}, respectively; rice straw (5 t ha^{-1}) burnt in the plot.

Farmyard manure (FYM) consisting of well-rotten cattle dung and cattle-shed wastes was incorporated into the soil 2 weeks before sowing of wheat. Nitrogen, through urea, was applied in 3 equal splits. Phosphorus and K was incorporated into the soil at the time of sowing using single super phosphate (SSP) and muriate of potash (KCl), respectively. For microbial culture 50 g mycellial mat of *Aspergillus awamori*, 50 g mycellial mat of *Trichoderma viridii* and 1 L of *Bacillus polymyxa* (10^8 viable cells) per 100 kg of straw as applied. Wheat (variety HD 2329, 100 kg seed ha^{-1}) was sown in rows 22.5 cm apart. The plots were irrigated as per requirement. Collection of gas samples was carried out through out the cropping period by closed chamber technique and the samples were analysed for N_2O using gas chromatography with ECD detector (Pathak *et al.,* 2002).

Wheat grain and straw yields were recorded from the total plot area by harvesting all the plants excluding plants bordering the plot. Grain weights were expressed at 120 g kg^{-1} water content whereas straw weights were expressed on an oven-dry basis (65^{o}C).

Soil samples from the 0-15-cm soil layer in 3 locations in each plot were collected using a core sampler. Representative sub-samples were drawn to determine various physico-chemical properties of soils using standard procedures.

Results and Discussion

Wheat yield

Yields of wheat ranged from 4.17 Mg ha^{-1} in NPK + rice straw treatment to 4.72 Mg ha^{-1} in NPK treatment where straw was removed from field and recommended levels of NPK were applied (Table 2). Lower yield in NPK + rice straw amended plot was due to immobilization of N because of addition of rice straw with high C:N (Kumar and Goh, 2000). In the NPK + Straw + extra N treatment, additional amount of N overcame the problem of N immobilization resulting in higher yields (Table 2). Lower yields in NPK + rice straw + FYM treatment was due to slow release of N from FYM and immobilization of N. Production of some organic acids in the process of decomposition of rice straw could also cause lowering of yields in rice straw amended plots (Kumar and Goh, 2000). The addition of microbial culture assisted in the faster decomposition of rice straw and more N being available leading to higher yields in the NPK+ rice straw + microbes treatment, which were similar to extra inorganic N treatment.

Table 2 Effect of rice straw amendment in soil on yield of wheat, soil fertility and N_2O emission.

Treatments	Grain (Mg ha^{-1})	Straw (Mg ha^{-1})	Soil organic C (%)	$KMnO_4$ N (kg ha^{-1})	Olsen P (kg ha^{-1})	Ammo. acetate K (kg ha^{-1})	N_2O emission (g ha^{-1})
NPK	4.72a†	6.36a	0.47c	233a	18.8a	342c	588c
NPK + rice straw	4.17c	6.67a	0.56b	243a	18.7a	385a	646b
NPK + rice straw + extra inorganic N	4.56a	6.40a	0.59b	265a	18.5a	377a,b	749a
NPK + rice straw + FYM	4.22b,c	6.78a	0.68a	250a	18.8a	422a	651b
NPK + rice straw + Microbes	4.61a	6.22a	0.57b	251a	17.7b	377a,b	623b,c
NPK + rice straw burnt	4.50a,b	6.67a	0.50c	240a	17.6b	362b	601c

†In a column, values followed by the same letter are not significantly different at P*<0.05 by Duncan's multiple range test.*

Straw yield ranged from 6.22 Mg ha^{-1} in NPK + rice straw + microbes treatment to 6.78 Mg ha^{-1} in NPK + rice straw + FYM treatment (Table 2). However, different treatments did not show any significant effect on straw yield of wheat. Total biomass of wheat ranged from 10.83 Mg ha^{-1} in NPK+ rice straw and NPK + rice straw + microbes treated plot to 11.17 Mg ha^{-1} in NPK + straw burnt plot. Like straw yield different treatments were statistically similar in terms of total biomass yield.

Soil fertility

Organic carbon content of soil at wheat harvest ranged from 0.47% (NPK treatment) to 0.68% (NPK + straw + FYM treatment) (Table 2). The treatments NPK + straw, NPK + rice straw + extra N and NPK + rice straw + microbes were on par in terms of soil organic carbon. Thus application of rice straw or/and FYM significantly improved the organic carbon status of soil. Organic carbon content was lowest in case of NPK and NPK + straw burnt treatment. KMnO4 extractable N (a measure of available N) ranged from 233 kg ha^{-1} (NPK treatment) to 265 kg

ha^{-1} (NPK + rice straw + extra N treatment). But the difference in available N among different treatments was not significant.
Olsen P content in the soil from different plot ranged from 17.6 kg ha^{-1} (NPK + rice straw burnt) to 18.8 kg ha^{-1} (NPK treatment). Difference of available P between NPK, NPK + rice straw, NPK + rice straw + Urea and NPK + rice straw + FYM plots was not significant (Table 2).
Ammonium acetate extractable K in different treatments ranged from 342 kg ha^{-1} (NPK plot) to 422 kg ha^{-1} (NPK + rice straw + FYM plot) (Table 2). The highest content of ammonium acetate extractable K in NPK + rice straw + FYM plot was due to addition of K through FYM. Higher value of available K in NPK + rice straw burnt plot than that of NPK plot was because of the ash left in the field after burning of straw contains a good amount of K. Verma and Bhagat (1992) observed increased available N, P, and K contents in soil with incorporation of crop residues in the rice-wheat rotation.

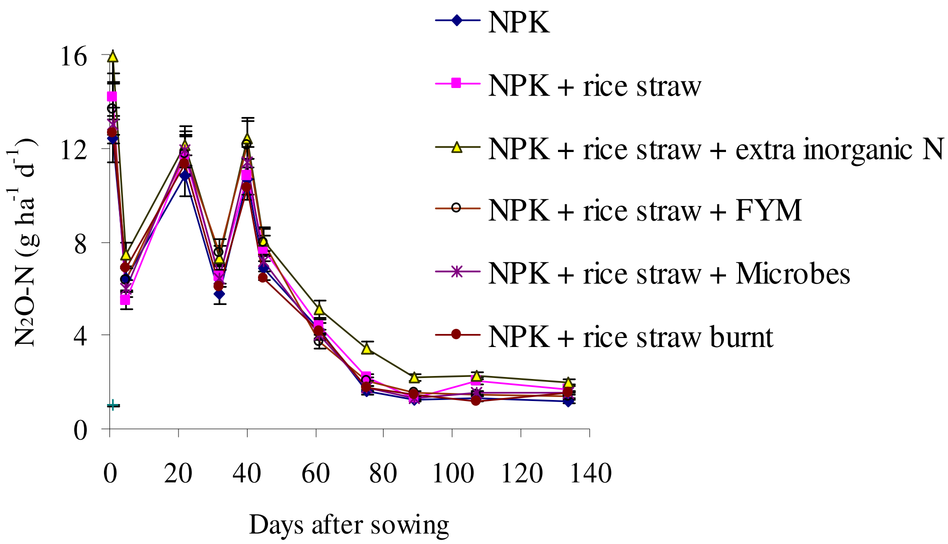

Figure 1 Emission of nitrous oxide (N_2O) from soil under wheat with various straw management practices

Nitrous oxide emission

Emission of N_2O on day 1 after sowing of wheat ranged from 12 to 16 g ha^{-1} d^{-1}, which reduced thereafter till the next dose of N was applied (Fig. 1). High emission of N_2O on day 1 in all the treatments was due to formation of N_2O during nitrification of ammonium N already present in soil as well as ammonium N produced by the hydrolysis of urea. A peak was observed in all the treatments following the addition of N through urea followed by a decline. Total emission of N_2O during the wheat season (120 days) ranged from 588 g ha^{-1} with the NPK treatment to 749 g ha^{-1} with NPK + rice straw + extra inorganic N treatment (Table 2). In terms of the total emission of nitrous oxide the treatments followed the sequence of NPK + rice straw + extra inorganic N > NPK + rice straw = NPK + rice straw + FYM > NPK + rice straw + microbes > NPK + rice straw burnt = NPK. Application of additional amount of N through urea was responsible for higher N_2O emission in the NPK + rice straw + extra inorganic N treatment (Pathak *et al*., 2002). In the straw amended plots, there were higher availability of organic carbon and greater microbial activity, which enhanced nitrification and denitrification (in microsites) resulting in higher emission of N_2O.

Conclusion

The study showed that adding additional inorganic N or microbial culture could avoid the problems of N immobilization and yield reduction of the subsequent crop due to rice straw amendment. Addition of FYM with rice straw is another option as it improves organic C and available nutrient status of soil offering advantages in the long run. It was observed that the plots treated with additional inorganic N emit maximum amount of N_2O. It may be noted out that there may not be significant difference of N_2O emission between NPK and NPK + straw burnt plot as the later treatments emits N_2O during burning (Lee and Atkins, 1994). Moreover, increased emission of N_2O due to straw incorporation could be countered by carbon sequestration in soil as observed in the study.

The study suggested that recycling of rice straw in soil offers good promise in reducing environmental pollution and improving yield and soil fertility. However, the hindrance of such a practice is difficulty in chopping and spreading rice straw in the field and no much economic benefit in terms of grain yield. Suitable machinery, therefore, has to be developed to overcome this problem and farmers have to be made aware of the long-term benefit in terms of improved soil fertility.

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VALIDATION OF A NEW MODEL (SWAGW) TO ESTIMATE PECS IN PADDY FIELDS

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Abstract
In the context of Directive 91/414/EEC, the assessment of risk to the environment, due to application of Plant Protection Products (PPPs), follows a tiered approach, which implies the use of more and more realistic (and complex) models to refine the exposition. Methods currently used to estimate exposition (Predicted Environmental Concentrations - PECs) to PPPs in different environmental compartments are not suitable to paddy fields. Concerning rice, the European group MED-Rice established just a first step. The use of a new mathematical model, SWAGW, to calculate PECs for a Step 2 of the tiered approach is discussed. The model is conceived and proposed for European and national authorizations in Italy and can provide results for groundwater and surface water both in paddy field and in the surrounding canals. Two PPPs, applied in flooded rice growing areas in different ways and amounts, were considered to study the behaviour of SWAGW. Data derived from field studies, kindly provided by Companies, have been used to perform a sort of first validation of this model.

Keywords
Rice; PEC calculation; risk assessment; models

Introduction
A tiered approach is widely applied to assess the risk to the environment following application of Plant Protection Products (PPPs), as outlined in Directive 91/414/EEC. The use of models of growing complexity is required to refine exposition, when threshold values are not met.
Tools currently used to estimate exposition (Predicted Environmental Concentrations - PECs) to PPPs in different environmental compartments are inappropriate to paddy fields, which need specific evaluation. According to the peculiarity of the practice used for this crop, in 1999 an expert group, the MED-Rice Working Group, was designated to develop a common system for the risk assessment of PPPs in rice, at least at the lower Steps of the risk assessment. This was especially intended for the inclusion of active ingredients (a.i.) in Annex I of the Directive. The Group developed a standard Step 1 assessment to determine environmental concentrations in surface water and groundwater, as at that time advanced mathematical modelling tools were not enough validated to be used in a regulatory context.
A new and more complex mechanistic model (SWAGW), which can model adsorption in a more realistic way than in Step 1, is available to calculate PECs in paddy environment for a Step 2 of the tiered approach. The SWAGW model, developed by Cervelli et al. (2004), can be used to estimate PECs and TWAs in paddy field, including sediment, canals, groundwater, and soil. However, evaluation of its behaviour and validation are still needed.

Materials and methods

Six PPPs, applied in flooded rice growing areas, in different ways and amounts, were considered to study the behaviour of the SWAGW model.

Laboratory and field studies data, both generated in the evaluation process for registration of agrochemicals, were kindly provided by different Companies (DuPont, Bayer, Dow AgroSciences), with claim of data protection. Available data on the compounds were evaluated for their suitability to the aim of this testing, and two PPPs were selected.

SWAGW requires data on degradation in paddy field, adsorption constant in soil and sediments, and degradation in canals. These data are in part available from the dossiers prepared by Companies for registration purposes. Degradation in paddy field can be represented by DT_{50} in flooded soil; adsorption constant K_{oc} for soil is a data requirement for a.i. registration; degradation in canals can be identified with water DT_{50} from the water/sediment study. Adsorption constants in sediment are not directly available from data packages. A different model, CODEWS (Cervelli et al., 2003), was used to derive proper values for K_{oc} in sediments, starting from the water/sediment study row data. When this study is not available, K_{oc} sediment can be reasonably assumed equal to K_{oc} soil.

In this work, the simulation capacity of the model was tested by comparing PECs calculated by SWAGW with concentrations measured in paddy, as derived by field studies provided by Companies.

Results

SWAGW modelled the behaviour of two selected PPPs on the basis of their dossier "endpoints". Data from the two PPPs allowed the evaluation of the model behaviour in predicting concentrations both in paddy water and groundwater. In particular, data from one PPP allowed comparisons to be made with paddy water data (data on canals were not available). In this case, the photo degradation DT_{50} value was used in surface water PEC estimations. Data from the other PPP were suitable to make comparisons for groundwater only.

PECs obtained with the model were of the same order of magnitude of the concentrations measured in field studies, as shown in Table 1.

Table 1 Comparison between measured concentrations of compound XA and PEC estimations calculated by SWAGW in paddy water, assuming 7 days closing time. Values are given in $\mu g L^{-1}$.

Paddy SW sand			Paddy SW clay		
Day after treatment	Company	SWAGW	Day after treatment	Company	SWAGW
day 0	33.1	17.50	day 0	24.9	12.98
day 1	21.35	15.73	day 1	17.75	12.55
day 2	12.25	14.14	day 2	16.1	12.14
day 6	8.8	9.23	day 5	8.85	10.97
day 7	6.8	8.30	day 7	5.25	10.26
day 8	6.3	7.24	day 8	3.6	9.70
day 14	0.25	3.20	day 15	0.25	6.58

Data from compound XB allowed only to compare concentrations in soil at day 1 after treatment. Predicted values for sand and clay were respectively 2.1 $\mu g\ kg^{-1}$ and 2.0 $\mu g\ kg^{-1}$, while field study reported 19.1 $\mu g\ kg^{-1}$.

Discussion

Rice cropping in Europe is characterized by two periods of water submersion. In the first period, lasting 5 days, the field is covered by a static body of water (closed rice environment); in the second one, approximately 3 months, water flows through the field, although maintaining a stable water level (open rice environment). SWAGW makes a distinction between a closed and an open system for surface water, and the amount of substance adsorbed to the solid phase (soil and sediment) is assumed to be in constant equilibrium with the amount in the water phase (paddy field and canals). In case of groundwater, the model assumes a miscible displacement behaviour of PPPs, a constant moisture content corresponding to saturation, and a constant addition of the pesticide corresponding to its TWA in paddy water, both during the closing and the opening time.

SWAGW, initially developed with the purpose to be a Step 2 in EU registration process for PPPs to be used on rice, was submitted to the MED-Rice Group, and then was also proposed for national authorizations in Italy. The model had not been validated yet.

As performing both laboratory and field studies is very expensive and time-consuming, the idea was to exploit suitable data previously generated by Companies in the frame of Directive 91/414/EEC, and try to get a first rough validation of SWAGW in a quick way. To this purpose, results from field studies were compared with PEC estimations from the model.

An agreement between field studies results and SWAGW outputs was found for the two selected compounds XA and XB, in paddy water and groundwater respectively. In fact, concentrations measured in field studies and PEC estimations obtained by the model were in the same order of magnitude, especially in regard to paddy water.

For paddy water, differences between predicted and measured values are less than expected, if the high degree of uncertainty introduced with the choice of input values is taken into account. This means that the model can be able to perform good simulations in paddy water environment. Moreover, results show that, when photo degradation is the most relevant degradation pathway, the use of DT50 from photolytic degradation as input parameter allows a realistic estimation of concentrations.

For groundwater, even if SWAGW is flexible enough to allow a certain degree of variations from the standard scenarios parameters, the main point is that PEC values obtained from the model can be reliable only if taken in the end of the simulation period, whatever it is. This brings to choices in the simulation period, which are not in complete agreement with the model.

The limitation of applying SWAGW to the experimental data provided by Companies is that field data were obtained in special situations, since conditions of rice cropping, as described in the available studies, do not comply completely with those described in MED-Rice scenarios. Preliminary results are encouraging, but further work is necessary before reaching a complete evaluation of the model. More PPPs have to be taken into account and "ad hoc" field studies should be designed and performed.

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ECOLOGICAL RISK ASSESSMENT IN RICE PADDIES: INNOVATIVE CONCEPTUAL MODELS AND HIGHER TIER APPROACHES

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Abstract

Rice paddies constitute a very special agrobiosystem, which for some circumstances could be considered as a human driven wetland. The complexity of such dynamic systems is a key element for conducting ecological risk assessments, in addition, the proximity of rice paddies to areas of high ecological value must be considered. This paper presents a new conceptual model for conducting ecological risk assessments of pesticides and other agrochemicals in rice, based on a combination of three main elements, the source of the stressor, the exposed environmental compartments and the relevance of the ecological receptors. A tiered testing strategy, and specific higher tier studies for risk refinement are also presented.

Keywords

Ecological risk assessment; rice pesticides; agrochemicals.

Introduction

Ecological or environmental risk assessments are currently an essential part of the evaluation/authorization process for pesticides, other agrochemicals and chemicals in generals. In the EU several guidelines and scenarios have been developed for assessing the environmental risk of different groups of chemicals, including industrial chemicals, pesticides, feed additives, veterinary pharmaceuticals, etc. A revision of the scientific bases of these protocols was conducted by the EU Scientific Steering Committee (SSC, 2003). Obviously, these protocols include agricultural scenarios, covering the direct use of pesticides, or the indirect exposure due to the use of sludge from wastewater treatment plants or animal manure as agricultural fertilizer. However, the agricultural scenarios cover mostly generic situations such as arable land and permanent crops (e.g. orchards and vineyards) and are developed as independent assessments for terrestrial (basically “in crop”) system and for aquatic bodies located in the vicinity of the agricultural area and potentially exposed due to drift, drainage or run-off.

Due to obvious reasons these scenarios cannot be applied to rice paddies, which required a common assessment of aquatic and terrestrial communities. Some efforts, such as the MED-Rice (2003) report, have been conducted for establishing harmonized scenarios. However, there is a real need for setting innovative and specific assessment models. Following conceptual models initially developed for addressing complex terrestrial ecosystems (Tarazona et al., 2002; Tarazona and Vega, 2002) this paper presents the development an implementation of a conceptual model created for assessing the ecological risk of chemicals in rice paddies and the associated communities, combined with a tiered testing strategy.

The Conceptual Model

The proposed conceptual model has been adapted from a previous model developed for covering the complexity of terrestrial ecosystems (Tarazona et al., 2002; Tarazona and Vega, 2002) based on the principles presented by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2001). The model covers three main points, the source or origin of the stressor, their distribution among environmental compartments, and the exposed ecological receptors. The first aspect is represented by the assessed emission or use patterns, establishing the conditions and time scale of the expected inputs, e.g. direct application for pesticides according to the recommended use patterns. The distribution of the stressor within environmental compartments is regulated, in the case of chemicals, by their fate and behaviour properties. This phase must consider the distribution among both biotic and abiotic compartments, using, if available, toxicokinetic information. The ecological receptors establish different categories for the same taxonomic group depending on the exposure route, and opposite to simplistic models must consider simultaneous or consecutive exposures of the same group from several environmental compartments.

The conceptual model has been modified for covering the characteristics of rice paddies. Basically, the main difference is that for the in-crop assessment, the systems are flooded part of the year, and, similarly to wetlands, the same surface supports consecutively modified aquatic and terrestrial systems. Therefore, the exposure routes from soil and sediment can be combined, and a paddy invertebrate community covering both aquatic and non-aquatic organisms has been considered. The off-crop aquatic assessment is also modified for covering the particularities of European rice paddies, which in several cases are located in the vicinity of wetlands of high ecologically relevance in terms of both flora and fauna. Particular exposure conditions, such as releases of contaminated water from the paddy to adjacent water bodies or the use of the paddy as a relevant food source for wetland animals have been included. The graphic representation of the proposed models appears in Figure 1.

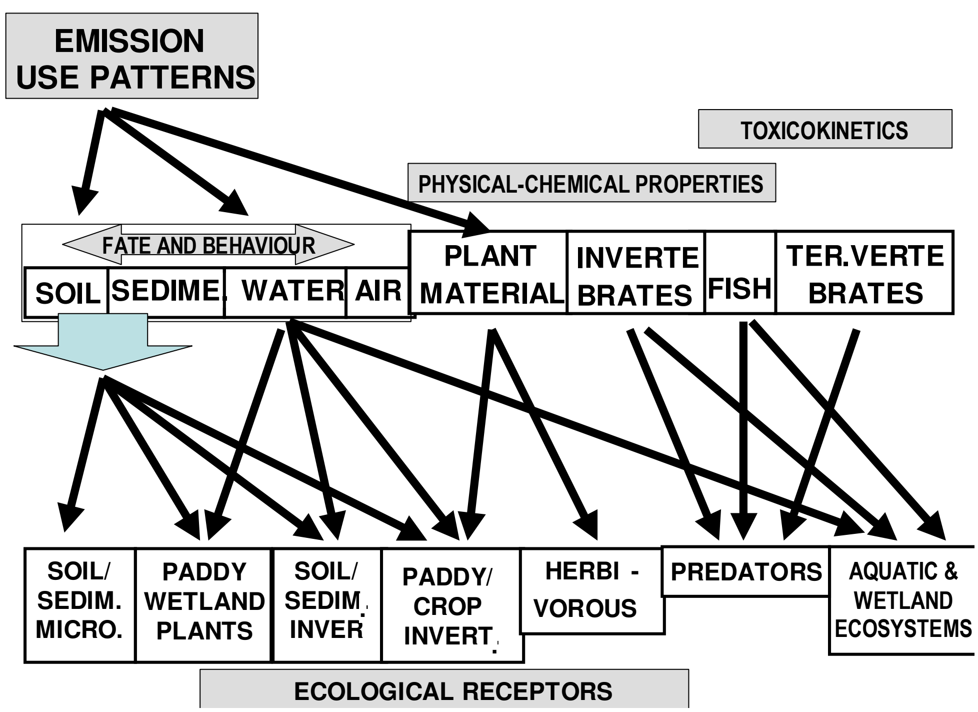

Figure 1. Conceptual model proposed for the ecological risk assessment of chemicals in rice paddies.

The implementation of this conceptual model follows the selection of the key combinations of environmental compartment → exposure route → ecological receptors that must be assessed; on the base of the available information (see Tarazona and Vega, 2002, for a general description). Worst-case assessments can be initially considered, and if required, refinement processes for specific compartment/route/receptor combinations can be conducted. In addition, the specificity of the paddy and wetland communities can require specific testing methods.

The Testing Strategy

The proposed testing strategy has been initially developed for rice pesticides, it can be applied to other agrochemicals with minor modifications. The strategy covers an initial level, or Tier 0, and three options for refinement, Tiers 1 to 3, summarized in Table 1 and described below.

Table 1 Testing strategy for pesticides in rice paddies

Level	Description	Advantages
Tier 0	Screening level based on the “standard” registration dataset	No additional assays are required
Tier 1	Direct toxicity assessments (water, soil) during a semi-field dissipation assay	Parent and metabolites covered under realistic conditions at a low cost
Tier 2	Rice-paddy mesocosms	Ecological realism and replicable conditions
Tier 3	Full field study in a paddy area and associated wetland including effect monitoring	Higher tier realism

The initial assessment or Tier 0 can be done on the base of the “typically” available information, e.g. the data set required for the registration of a pesticide in the EU, which include physical-chemical properties, fate studies in the laboratory on soils and water-sediment systems, and a collection of acute and chronic toxicity tests on aquatic and terrestrial organisms (see guidelines developed under Directive 91/414/EC and SSC, 2003 for details on EU data requirements; similar requirements are also implemented in North America and other parts of the world). If no information is available, e.g. desorption from soil to water after flooding; worst-case assumptions, e.g. 100% desorption for assessing exposures from water column and 0% desorption for assessing exposures from the sediment, should be employed at this initial level.

The first and second refinement possibilities, Tier 1 and 2, are innovative proposals that can be run in parallel to a semi-field dissipation study. In Tier 1, samples of water and/or sediment are collected at regular time intervals, transferred to the lab and used for direct toxicity assessments through modified OECD standardized guidelines. In Tier 2, the evolution of the paddy community is directly monitored before and after the pesticide application, using the proper negative and positive controls. A combination of positive controls, selected according to the application patterns, is recommended. The suitability of both approaches has been already checked and results employed for the EU assessments conducted by INIA.

The final refinement possibility is a full field study where the effects on exposed communities are directly monitored.

Discussion

Rice paddies in Europe are exclusive of the Mediterranean region. In Spain, two main situations can be observed: the traditional paddies associated to wetland areas of very high ecological value such as Doñana National Park, the Albufera or the Ebro river delta, and the "new" paddies associated to irrigation channels such as those located in Extremadura or Aragón. The ecological relevance of the vicinity of the "new" paddies is lower than for the traditional ones, but cannot be disregarded. In fact, some reports suggest a very high increase in bird abundance and biodiversity following the transformation of arable land in rice paddies. The alternative proposed in this paper covers the complexity of this situation moving from the traditional risk assessment conceptual models based on compartments (soil, water, secondary poisoning), to a conceptual model based on combinations of ecological receptors potentially exposed from several routes. This terrestrial model has been already modified, with the incorporation of the aquatic compartment, for assessing veterinary medicines (Montforts et al., 2003), but the modifications required for assessing paddies are obviously of much more magnitude. As already mentioned a key situation is the biodiversity with a combination of aquatic and terrestrial communities, and the application of ecological features developed for wetland risk assessments (e.g. Lemly, 1997) can be particularly useful. The use of direct toxicity assessment for both water and sediments is receiving and increasing attention (e.g. Latif and Licek, 2004; Bejarano, 2004), and these tools can be easily incorporated to semi-field dissipation studies. Initially, the adaptation of the standardized tests is proposed, as the interpretation of test results can follows the triggers already agreed for Tier 0 (e.g. the Directive 91/414/ EC triggers in the EU). However, additional possibilities, such as the water/sediment multispecies invertebrate test (Sanchez and Tarazona, 2002) can be applied.

Tiers 2 and 3 offer the alternative to typical mesocosm studies, which are conducted under conditions far away from those expected for paddies and the associated wetlands. However, if mesocosm studies are available, the possibility for extrapolating the observed responses to the paddy and wetland communities should be assessed before starting new higher tier assays.

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NEW COUPLED MODEL OF PESTICIDE FATE AND TRANSPORT IN PADDY FIELD

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Abstract
A new coupled model associating PCPF-1 and SWMS-2D had been developed. This new coupled model simulates fate and behavior of pesticides in paddy water and paddy soil along the full agronomic crop season (from transplanting, after puddling, to harvest including mid term drainage).
PCPF-1 is a lumped model, based on water and pesticide mass balance (Watanabe and Takagi, 2000a, b). It simulates the fate and behavior of pesticide in surface compartment (including paddy water and the first centimeter of paddy soil), by taking into account paddy water management. SWMS-2D is a widely used numerical finite element code which is used in HYDRUS-2D, software package for simulating water flow and solute transport in variably saturated porous media (Simunek et al, 1998). It solves the Richard's and advection-dispersion equations, including adsorption and degradation phenomena in the soil compartment. Longitudinal dispersivity, halflife and adsorption parameters were used to simulate pesticide transfer in soil.
The coupling involved some improvements in exchanges of water and pollutant at the interface between both compartments.
The monitoring data collected form experimental plots in Tsukuba (NIAES) in 1998 and 1999 were used to calibrate hydraulic properties and functioning of paddy soil. Hydraulic functioning had been complementary evaluated with tracer (KCl) experiment and thus with pesticide data (Pretilachlor). The hydraulic functioning study confirmed that the hard pan layer controls percolation rate and pollutant dissipation. Matric potential and tracer monitoring highlighted the evolution of hard pan layer properties such that the saturated hydraulic conductivity was slightly decreased during days after surface soil puddling by clay clogging and significantly increased after mid term drainage by cracks formed during drying process. The tracer and pesticide travel times in paddy soil were about 30 and 90 days after application at 15 cm and 45 cm depths, respectively. The model calculates residential time in every soil layers and assesses leaching below hard pan layer. It provides a good estimation of pesticide persistence after one full crop season.

Keywords
pesticide; paddysoil; model; persistence; SWMS; PCPF.

Introduction
For paddy field rice production, pesticide concentration in surface drainage reveals a high disparity and depends mainly on water management practices. However pesticide losses in soil profile are not often taken into consideration. In terms of non point source pollution and protection of ground water resources, prediction of pesticide concentration and residential

time in soil profile is crucial. A review of literature shows that models are separated into two categories: water management models including surface and soil compartments (e.g. SAWAH, FEMWATER…) and pollutant models (mainly surface models such as PADDY, RICEWQ, PCPF-1, and soil model for nutrient). A single attempt of coupling models including soil and surface compartments and describing pesticide transfer exists: RICEWQ and VADOFT (Miao, et al 2003). Both models are linked by a common boundary limit: top soil surface.

This paper presents a new coupled model between a surface compartment model PCPF-1 and a soil compartment model HYDRUS (Simunek et al 1999) to simulate the pesticide fate and transport in paddy fields. The herbicide active ingredient is the Pretilachlor (2-chloro-2',6'-diethyl-N-(2-propoxy-ethyl)acetanuilide) used for the selective control of some annual weeds in lowland rice including barnyardgrass. This paper is organized into two sections: the "materials and methods" section presents a site description, monitoring data and modeling principles. The second section presents the hydraulic functioning, tracer and pesticide results.

Materials and Methods

Site Description

Field experiments were conducted at experimental rice paddy field of National Institute for Agro-Environmental Sciences (NIAES) in Tsukuba, Japan (36°104N, 139°564E). The local average annual precipitation and temperature are 1406 mm and 14°C, respectively. The experiments were carried out in 1998 and 1999 in two 9×9 m² plots surrounded by concrete bunds and plastic borders.

The paddy soil preparation usually comprised one or two passes of rotary tiller about 0.15 to 0.2 m deep before the first irrigation or pre-saturation usually in April or May. After this pre-saturation, the soil is mechanically puddled by one or two passes of rotary tiller, and a final leveling under water-saturated field conditions within a few centimeters of water ponding condition. After a few days, 17-day-old rice seedlings (*Orysa sativa* L. cv. Nihonbare) were transplanted with 16×30cm² spacing on May 8, 1998, on May 7, 1999. Plots were irrigated and ponded with about 4cm water depth until mid-term drainage. Mid-term drainage, which de-saturates soil surface to improve the root environment, was carried out for 17 days from July 17, 1998 and for 10 days from July 16, 1999, respectively, that corresponded to a period from the maximum tillering stage to the panicle formation stage. During the reproductive stage after mid-summer drainage, intermittent irrigation that repeated ponding every few days was performed until one week before harvesting (September 7, 1998, and September 27, 1999).

Six soil horizons were identified in the experimental area. Three of them are located in the upper part (< 23cm) that is considered as agricultural layer. This was a sandy clay loam (SCL), characterized by a high clay content (between 30 and 40%). This was composed by muddy or puddled layer, and hard pan layer. The last three horizons formed the lower part that is considered as the original non puddled soil. Those were composed by a transitional subsoil clayed layer (23-50 cm), by a first volcanic ash mixture layer (clay-Kuroboku mixture, Loam CL, 50-65 cm), and from 65 cm, by the original second volcanic ash layer (Kuroboku soil, SiCL). Hydraulic properties such as saturated water content, saturated hydraulic conductivity, Van Genuchten parameters for water retention curves, bulk density, cone penetrometer data were measured in laboratory from soil core samplers.

A commercial preparation of granule herbicides, Hayate® containing 1.5% of pretilachlor, and other active ingredients was applied at a rate of 4968 mg/82m² on each plot on May 12, 1998 and May 14, 1999, respectively. Those dates used in this paper are referenced time as days after herbicide application (DAHA=0).The tracer inputs (KCl) were 1317 g and 2633 g respectively for 1998 and 1999 for an area of 82.5 m^2. Those data provided an input chloride tracer concentration of 256 and 486 mg/l in 1998 and 1999 respectively. All the dates for important events were summarized in table 1. A permanent monitoring (DAHA=0 to 63) was set up and described in Figure 1.

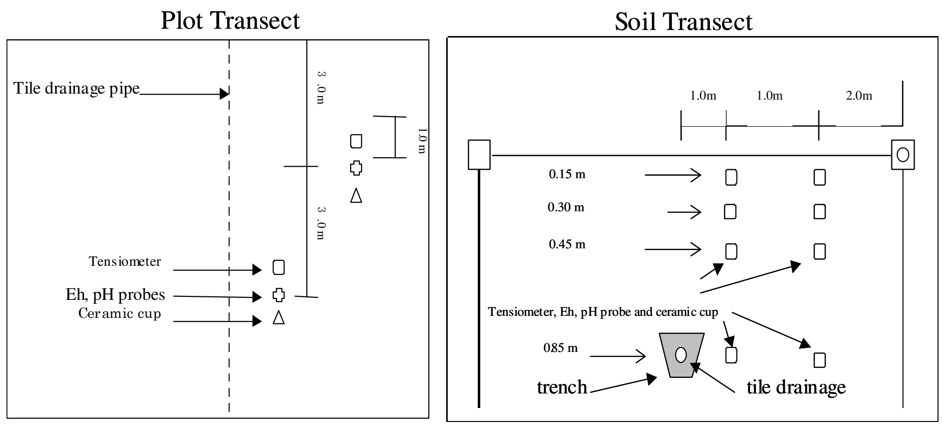


Figure 1 Schema (plot and soil transect) of in situ monitoring equipments in paddy soil (Tsukuba, Japan).

Models

PCPF-1

PCPF-1 is a lumped model which simulates the pesticide fate and transport in two compartments, the paddy water compartments and the paddy surface sediment layer compartment which is defined as a 1.0 cm thick conceptual surface paddy soil layer (Watanabe and Takagi, 200a). These two compartments, are assumed to be a completely mixed reactor having variable water depths. Both compartments are assumed to be homogeneous, and having uniform and unsteady chemical concentrations. The water balance of a puddled rice field is determined by the following components such as irrigation supply, rainfall, surface drainage, evapotranspiration, lateral seepage and vertical percolation. Pesticide concentration is governed by dissipation (dissolution, volatilization, biochemical and photochemical degradation, sorption) and transport (surface run-off, percolation) processes under oxidative flooded conditions. Detailed description of the model can be found in Watanabe and Takagi (2000a and 2000b).

SWMS

SWMS_2D is the open source FORTRAN code in HYDRUS-2D, a Windows-based modeling environment for analysis of water flow and solute transport in variably saturated porous media (Simunek et al 1999). The program solves the Richards' equation for saturated-unsaturated water flow and a Fickian-based advection-dispersion equation for solute transport including provisions for linear equilibrium adsorption, zero-order production, and first-order degradation. The governing equations are solved using a Galerkin type linear finite element scheme. Specifically for pesticide, the degradation processes are modeled by a first-order kinetic with half life coefficient and the sorption processes by water/soil partitioning coefficient (Kd). A single study dealing with simulation of pesticide with HYDRUS (Pang et al., 2000) could be found in the literature.

Coupling model PCPF-1 / SWMS

The coupling of models is carried out by linking the percolation flux, induced by ponded water depth, and the predicted concentration at soil surface, between paddywater compartment and paddysoil compartment using PCPF-1 and SWMS-2D. Interaction between these two model compartments for water movement and solute exchange could be summarized as follows: PCPF-1 provides paddy water depth as top boundary condition in SWMS (pressure prescribed data) and SWMS-2D determines the vertical percolation which is an input data in the PCPF water balance equation. For the solute transport, PCPF-1 provides the top boundary solute concentration which, associated with the percolation rate, determines the input solute flux for the SWMS-2D simulation.

Practically, we first incorporated a water balance equation in the main program of SWMS-2D and then added a new subroutine, called PCPF, which is the Fortran version of PCPF-1 code. The calculation is carried out in a way that, daily ponded water depth imposed prescribed pressure conditions and then Watflow subroutine calculated percolation rate, which was used in water balance for the next simulated day. For the solute transport, the boundary concentration (cBnd) value is replaced by the predicted value by PCPF subroutine. Note that the PCPF-1 model was modified and simplified for the tracer case. The new coupled model PCPF-1/SWMS is applied to simulate, first, the flow of water and the transport of chloride and to assess transport parameters, and then, to simulate pesticide fate and transport through the soil profile.

PCPF requires daily precipitation (cm), runoff (cm), evapotranspiration (cm) during the simulation period. The pesticide properties were determined from experimental soil samples in laboratory studies by Takagi et al., (1998) and Fajardo et Takagi (2000).

Performed simulations and boundary conditions

In agreement with the different agricultural water management, the full crop season was divided into three periods (table 1): continuous irrigation, during which PCPF-1 and SWMS are coupled; mid term drainage, during which atmospheric boundary conditions are applied on top soil surface; intermittent irrigation, during which a prescribed pressure head condition is applied on soil surface (we assume that no pesticides remain in ponded water compartment) until final drainage before harvest.

Table 1 Crop periods and corresponded simulations (model and top boundary limits)

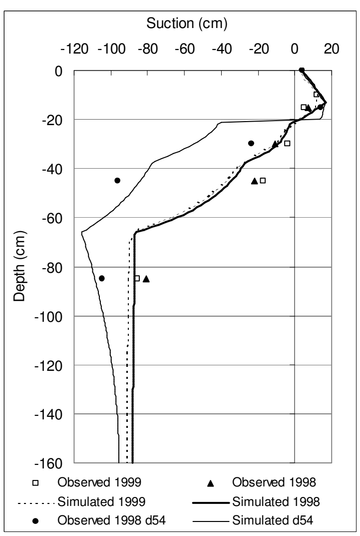
Crop Period	Year	DAHA	Model	Top Boundary condition
Continuous Irrigation Herbicide Application	1998	0-66	Coupled PCPF-SWMS	Time-dependent pressure head
	1999	0-63		
Mid Term Drainage	1998	66-83	SWMS	Atmospheric boundary
	1999	63-73		
Intermittent Irrigation	1998	83-114	SWMS	Time dependent pressure head
	1999	74-132		

Results and discussion

Hydraulic functioning

The hydraulic functioning of a paddy system is the main key to characterize the pathway of pollutant in both compartments (surface and soil). The monitored values started after application date (DAHA=0) until the beginning of mid term drainage (DAHA=66 and 63 for 1998 and 1999 respectively). The monitored pressure head showed (figures 2 and 3) a relative steady state due to the continuous irrigation (despite fluctuation of ponded water depth). However in 1998, the monitored data presented a drop in pressure value below hard pan layer

(45cm depth) after 40 days in the second period of the continuous irrigation stage. This decrease was due to the evolution of properties of hard pan layer. Indeed fine particle and clay dispersion might clog this layer to reduce the apparent saturated hydraulic conductivity. This phenomenon was not observed in 1999. The first objective was to determine the properties of hard pan layer, which is considered as the key layer to control percolation rate (Bouman et al., 1994). The soil profile properties (bulk density, saturated water content and hydraulic conductivity, cone penetrometer values) were synthesized to define hard pan layer between 17-23 cm depth, including a transition layer (17-21cm depth). Monitored pressure head values were used to fit the saturated hydraulic conductivity of hard pan layer. In 1998, the optimized values of Ksat were fitted to 0.08 and 0.03 cm/d for DAHA=0-40 and 41-63 respectively. In 1999, optimized Ksat value was 0.08cm/d for the first entire period (DAHA=0-66). These results were in agreement with previous studies (e.g. Bouman et al 1994; Chen and Liu, 2002). During the third period (intermittent irrigation), the fitted Ksat values were evaluated to 0.8cm/d and 0.3cm/d in 1998 and 1999 respectively. The joint action of drying cracks (due to mid term drainage) and rice root development explain this increase of Ksat between the first and the third periods. Moreover the inter-annual difference of percolation (0.8cm/d and 0.3cm/d in 1998 and 1999) was directly affected by mid term drainage duration: a longer period generated deeper cracks. We assumed to include this preferential flow into an increased Ksat. The pressure head profile showed a saturated zone from the surface to the middle of hard pan layer (all pores are connected together, which are defined as close system in Adachi and Sasaki, 1999). And it showed an unsaturated zone beneath the middle of hard pan layer (pores are not connected: open system). The new coupled model PCPF-1 / SMWS was first compared to the PCPF-1 results for the surface compartment. In the simple PCPF-1, percolation and seepage rates were fixed to constant values (0.2cm/d and 0cm/d). The simulated percolation rate remained almost constant in 1999, about 0.22 to 0.26cm/d. And in 1998 including evolution of hard pan layer, the average value was evaluated to 0.18cm/d in 1998. Improving the daily percolation values with the new coupled model increased the accuracy of the water balance of the model.



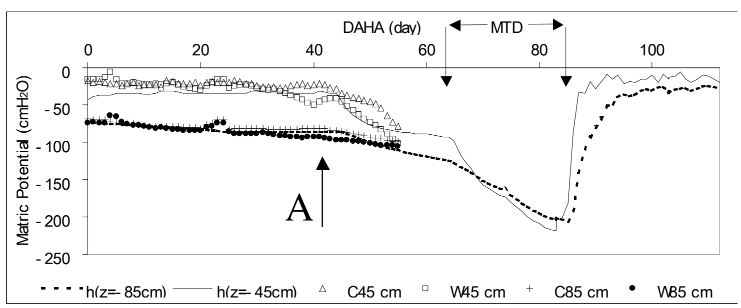
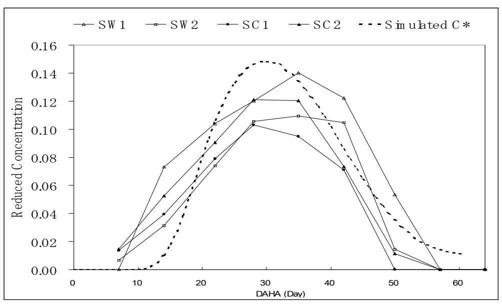


Figure 3 Measured matrix potential (15, 30, 45 and 85 cm deep) and ponded water depth versus day after herbicide application (DAHA), from May 13, till July 7, 1998. (Tsukuba, Japan). "A" indicates the change in Ksat parameter of hard pan layer.

Figure 2 Suction profile Observed 1998 and 1999 and simulated versus depth, optimized on Ksat, and suction profile d54 in 1998 (Tsukuba, Japan)

Tracer

Chloride was considered as an ideal tracer, determining the advective-dispersive pathway of a non-reactive pollutant. First we have to acknowledge that the choice of Chloride was not suitable due to high initial background concentration in soil profile (above 20mg/l). Nevertheless the monitored data were useful to determine the transport parameters of soil layers. The time repartitions for each monitored depth and the maximum concentration peaks served to calibrate the dispersivity parameter. With a dispersivity value fitted to 1cm, the simulated curves reproduced accurately the weekly measured concentrations for 1998 and 1999 series (figures 4 and 5). Leachate amount of chloride through paddy soil layer was estimated to 5.4% for both years. The surface drainage losses were calculated to 94.5% . The residential time in each layer for both year was estimated: in the puddled layer, the peak was maximum 24 DAHA in average for 1998 and 1999; in hard pan layer, after 41 DAHA. At the end of agricultural crop season, the concentration peak of tracer in soil profile was estimated to 61 cm deep, and the plume was distributed between 40 and 90 cm deep. Taking the final conditions of 1998 simulation as initial conditions of 1999 simulation, the tracer front reached the 80cm depth.



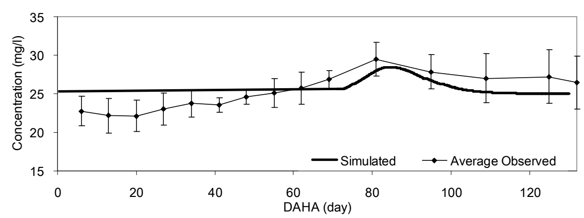


Figure 5 Simulated and Average Observed concentration at 45 cm depth in 1999 (Tsukuba, Japan)

Figure 4 Measured and simulated concentration at 15 cm deep versus day after herbicide application (DAHA), from May 13 till July 7, 1998 (Tsukuba, Japan)

Pesticide

Simulated pesticide in paddy water with PCPF-1 / SWMS model (figure 6) were similar to those simulated with PCPF-1 alone and were in good agreement with observations (Watanabe and Takagi, 2000b).

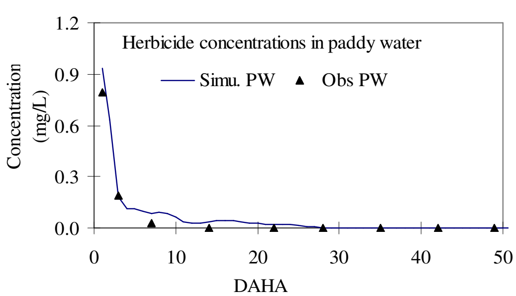


Figure 6 Observed and simulated concentrations of Pretilachlor (PTC) in paddy water (Tsukuba, Japan, 1998).

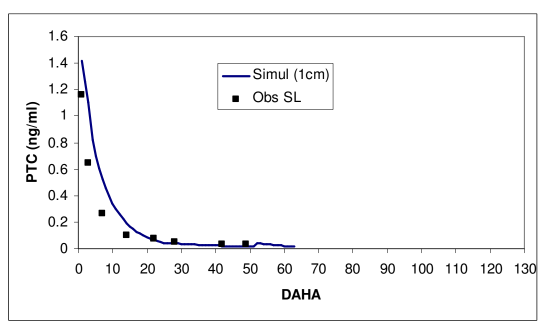


Figure 7 Observed and simulated concentrations of Pretilachlor in paddy soil at 1 cm depth (interface boundary limit between PCPF-1 and coupled PCPF/SWMS model), (Tsukuba, Japan, 1998)

The dissipation of Pretilachlor was specifically studied in paddy soils under laboratory oxidative and reductive conditions in a previous work by Fajardo et al. (2000). Their conclusion can be summarized as follow: it is necessary to consider a biphasic first order kinetics, i.e. two simple first order kinetics to describe the half-life in the reductive puddled layer: 6 days (0.114/day) for the 0-21 days phase, 23 days (0.03/days) for the 22-63 days phase. In this simulation, we treated 1-17cm puddle layer to be a reductive condition and 17-50cm subsoil layer to be an oxidative condition. Therefore soil partitioning coefficient and degradation rate constant reported by Fajardo et al. (2000) were used accordingly.

Observed concentrations were well reproduced with these parameters in puddled layer. In a first description of observation and simulation (figures 7, 8 and 9), when the maximum applied concentration reached 1.4 ng/ml in soil solution at 1cm paddy surface soil, due to degradation, sorption and dilution the maximum concentrations in underneath soil layers ranged to 0.06 ng/ml at 15 cm depth and 0.02 ng/ml at 45 and 85 cm depths. In the puddled layer, at 15 cm depth, the pesticide concentration peak occurred 29 days after application. The residential time in this layer is evaluated to 41 days. Below hard pan layer up to 50cm of subsoil layer, pesticide concentration ranged mostly below 0.02 ng/ml during the monitoring period. Simulated concentration underestimated the herbicide concentration in earlier period and overestimated later period. Simulated pesticide transport below hardpan layer was considerably small however measured concentrations were significant and it could be the herbicide residue concentrations from previous applications of 1996 and 1997. Also, the observed Pretilachlor concentrations at 85cm (not simulated) of volcanic ash soil ranged about 0.2 ng/ml, which was higher that those concentrations at 45 cm depth especially in the later period. We observed that PCPF-SWMS can simulate pesticide concentration with measured parameters up to 50 cm. Further application and investigation could improve simulation to more layer below 50cm.

In term of mass balance, surface runoff represented 52% of applied pesticide rate. Soil degradation amount was simulated to 42%, and occurred mainly in puddled layer. Then 6% remained in the soil below the hard pan layer at the end of crop season. The fact to apply pesticide under granular form increased the pesticide mass in soil, compared to tracer application (high dissolution) but decreased surface runoff losses.

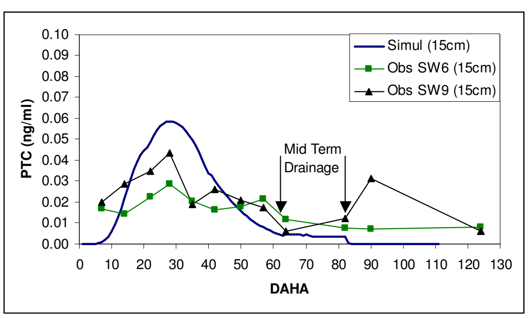


Figure 8 Pretilachlor measurements and simulation at 15 cm depth in 1998 (Tsukuba, Japan)

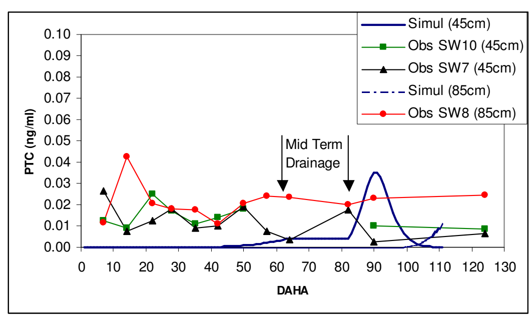


Figure 9 Pretilachlor measurements and simulation at 45 cm depth in 1998 (Tsukuba, Japan) (Kd=13l/kg and degrad=0.0026/day)

Conclusion

The new coupled PCPF / SWMS model is a good tool to understand the mechanism of degradation, sorption and transport of Pretilachlor. Preferential flow could be integrated in an apparent time-variable saturated hydraulic conductivity parameter of hard pan layer. The main drawback of this mixed lumped and deterministic model is the number of necessary parameters. However this model is useful to elaborate the basis of a simplified model and to be utilized as reference. It appears that, in Japanese paddyfield hard pan layer is the key layer controlling hydraulic functioning and puddled layer is the key controlling pesticide concentration in soil and particularly the capacity of pesticide biodegradation.

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MONITORING PESTICIDE FATE AND TRANSPORT IN SURFACE WATER IN JAPANESE PADDY FIELD WATERSHED

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Abstract
In order to investigate Best Management Practices (BMPs) for reducing pesticide runoff from paddy fields into surface water, a two-year project for monitoring hydrological balance, water management and pesticide concentrations in paddy fields and stream water was carried out in a paddy field-watershed of about 86 ha at Sakura-river basin in Tsukuba, Japan since April 2002. Most paddy plots within the watershed applied intermittent irrigation practice. However, height of drainage gate in paddy fields seemed to be set up not high enough to store significant amount of rainfall exceeding about 1.5 cm/day. Total of 15 herbicides were detected in stream water. Peak concentration occurred mostly from early to mid May followed by the pesticide application period. More than 70% of the total loss of detected herbicides from the watershed occurred from May 7 to May 22, corresponding to the herbicide application period. Significant daily pesticide losses occurred with corresponding peak flows after significant rainfall events. The BMPs for controlling pesticide runoff from paddy fields could be an application of intermittent irrigation scheme with surface drainage gate installed high enough to store rainfall water in the paddy field during significant rainfall events and holding paddy water about 2 weeks after herbicide application.

Keywords
Pesticide; paddy field; monitoring; watershed; best management practices.

Introduction
Japan is one of the major pesticide users worldwide, with the fact that more than 500 pesticides of different active ingredient were registered among more than 5000 commercial products (JPPA, 1998). About 98% of all the pesticide sold in Japan was applied for agriculture and forest, wherein 50% used to paddy fields (Nagafuchi, 1994). Extensive use of pesticide in company with inappropriate water management in rice fields has been caused pollution of public surface water. Monitoring studies for pesticide concentrations in river systems in Japan indicated that herbicides commonly used in paddy rice fields have been detected (Maru, 1985; Nagafuchi et al.,1994; Ishihara et al., 2003; Sudo et al., 2002a and 2002b). In 1998, Japanese Ministry of the Environment listed about 70 chemicals as suspected endocrine disrupter substances. Among them, about 40 chemicals are pesticide and about a half of those pesticides were registered (MOE, Japan, 1998). Therefore, public concern for surface water quality affected by rice pesticide is eminent.
The monitoring fate and transport of pesticide in surface water have been studied. The results had been used for development of simulation model of pesticide fate and transport (Watanabe et al., 2000a and 2000b; Inao et al., 2003; Capri et al., 2002, Williams, 1998) and for investigating the Best Management Practices (BMPs) for reducing pesticide concentrations in public surface water (Watanabe et al.,2000c and 2002; Kreuger, 1998 and 2001). However, monitoring fate and transport of rice herbicides along with detailed hydrological monitoring

in surface water for watershed scale is limited. In order to investigate the Best Management Practice or Good Agricultural Practices (GAPs) for reducing pesticide runoff in Japanese rice production, this study focused on evaluation of the pesticide fate and transport from paddy fields into river through the agricultural watershed monitoring.

Materials and methods

Study watershed description

The study area is a small watershed located in Sakura river basin in Ibaragi prefecture, about 50 km northwest of Tokyo, Japan (Figure1). The area of the watershed is about 97 ha including 86 ha paddy field and 11 ha upland field. The area of paddy plots ranges from 0.2 to 0.4 ha and their short side faces a farm drainage canal and an irrigation pipe line established along a farm road. Each farm drainage canal covers a farm block. Irrigation water was supplied to individual paddy plots through pipelines from pump stations. The surface water for the most of paddy plots was gravitationally drained through the drain-pipe to the drainage canal. The source of irrigation water supplied to watershed consists of two springs from Mt. Tsukuba and regional irrigation supply water from the lake Kasumigaura through pipeline (Figure1). Drainage water in the canals was reused for irrigation by pumping stations (P1 and P2).

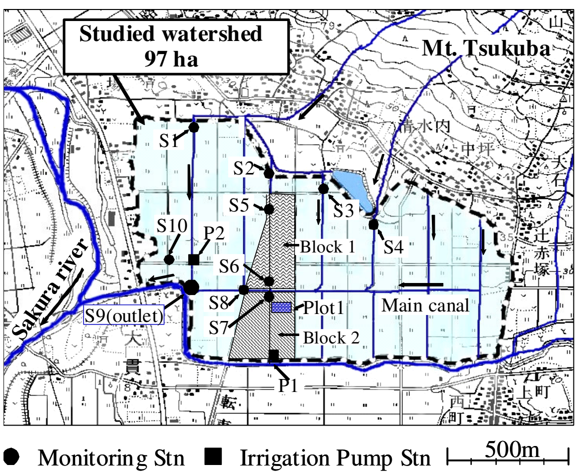

Figure 1 Studied watershed and monitoring stations

Watershed monitoring

Figure 1 shows monitoring stations in the studied watershed. Monitoring hydrological balance within watershed were conducted on paddy plot, farm block and watershed scales. A paddy plot (Plot1) and two farm blocks of 4.8 ha (Block 1) and 5.3 ha (Block2) were selected in order to represent the hydrological response of the watershed. The monitoring period was lasted about 45 days from rice transplanting (May 1st) to midterm drainage period (June 16th). In order to investigate water management practice, irrigation, drainage and ponding water depth were monitored at paddy plot 1 by using boxes with V-notch weir and water level sensors (HM–910, HI-NET Co., ltd) connected with data loggers in 2002. Precipitation was monitored by a rain gauge (No. 34-T, Ota keiki Co., ltd) at the site S10 of the watershed. Evapotranspiration (ETc) in paddy fields was estimated using FAO Penman-Monteith method (Allen et al., 1998) from meteorological data observed at a local meteorological station located about 5 km west of the study site. Percolation rate during monitoring period was computed from water balance data in paddy plot and percolation in paddy field was assumed to be the same in the whole study area during the monitoring period.

Table 1 shows monitoring stations, locations and monitored contents. The flows in the secondary canals was estimated by water depth-discharge curves obtained by continuous monitoring of the water depth of the canal with water level sensors (HM–910, HI-NET Co., ltd) and weekly measurements of average flow velocity with a flowmeter (AEM1-D, Alec Electronics Co., Ltd.). The discharge at the main canal of S8 and S9 was calculated by monitored water depth at each weir using equations described in Rao and Muralidhar (1963). Also, direct measurement of surface drainage from paddy fields into canal segments in Block 1 and 2 were conducted once a week to examine water balance in the canal segments associated with lateral seepage from paddy field. In 2003, the hydrological balance in the watershed was defined using observed data of inflows (at S1, S2, S3, S4) and out flow (at S9), and estimated water management in the paddy compartment of 86 ha.

Table 1 Monitoring stations

Monitoring station	Location	Content
First year (2002)		
Paddy plot 1		Irrigation, Drainage Poding water depth, Water Sampling
S5 *(inlet of farm block 1)*	Secondary canal	Flow measurement, Water sampling
S6 *(outlet of farm block 1)*	Secondary canal	Flow measurement, Water sampling
S7 *(outlet of farm block 2)*	Secondary canal	Flow measurement, Water sampling
S8	Main canal	Flow measurement, Water sampling
S9 *(outlet of watershed)*	Main canal	Flow measurement, Water sampling
S10 *(Raingauge)*		
Second year (2003)		
S1, S2, S3, S4 *(inflows of watershed)*	Secondary canal	Flow measurement
S7 *(outlet of farm block 2)*	Secondary canal	Flow measurement, Water sampling
S9 *(outlet of watershed)*	Main canal	Flow measurement, Water sampling
S10 *(Raingauge)*		Precipitation

Water sampling and chemical analysis

Water samples were taken once a week for irrigation water, paddy water (2002), stream water at each station in drainage canals (2002 and 2003). Immediately after sampling, water samples were filtered through a Whatman GF/F-0.7μm glass filter and pH of filtrate was adjusted to be 6.5. Herbicide concentrations in water samples were analyzed using a gas chromatography (GC/FTD, SHIMADZU) and a liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS, Applied Biosystems). For GC analysis, one litter of filtrate was passed through solid phase extraction cartridges (Sep-Park^R, tC18 for 2002 and PS-2 for 2003) and extracts were eluted by dichloromethane. This elute was concentrated in *vacuo* and dissolved in 2ml acetone. Then the pesticides in each sample were determined by GC/FTD. For LC-ESI-MS/MS, frozen filtered sample was thawed and well mixed with the same volume of acetonitrile, then analyzed directly. Above pesticide analysis was performed at National Institute of Agro-Environmental Sciences in Tsukuba, Japan.

Results and discussion

Water management and canal discharge

The water balance data in paddy plot 1 in 2002 is shown in Figure 2. Average evapotranspiration (ETc) during 46days of monitoring period was about 4.1 mm/day. Average value of vertical percolation and lateral seepage was estimated to be about 3.2 mm/day from the water balance in plot 1.

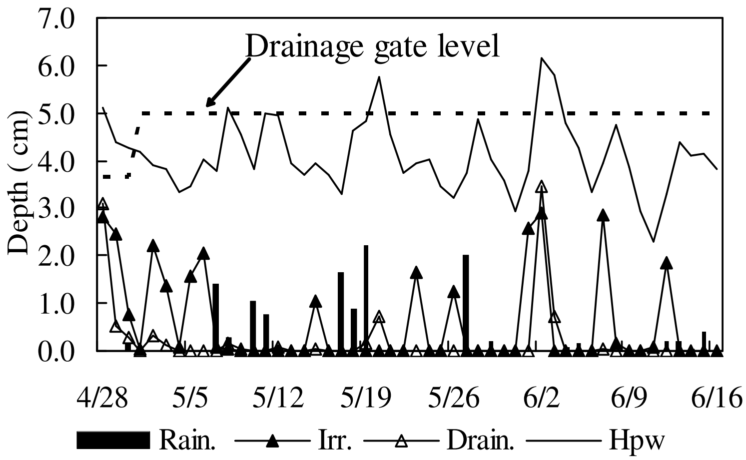

Figure 2 Water management in plot 1 (2002).

From our observation for two years, more than 90% paddy plots within the watershed applied intermittent irrigation scheme as performed in plot 1 (Figure 2). However, the drainage gate in these plots had been setup about 5cm from soil surface and paddy water depth had been kept about 4 cm in most of the monitoring period. This setup may cause high potential of pesticide runoff when significant rainfall events occur and in cases of strong wind if drainage located at the end of wind direction. Monitoring data showed that ponding water increased responding to rainfall events (Figure2).

Figure 3 shows surface drainage discharged from paddy field in block 2 and the paddy surface drainage significantly increased by responding to the rainfall events exceeding about 1.5 cm/day. In some periods, the surface runoff from farm blocks occurred was not very consistent with rainfall events.

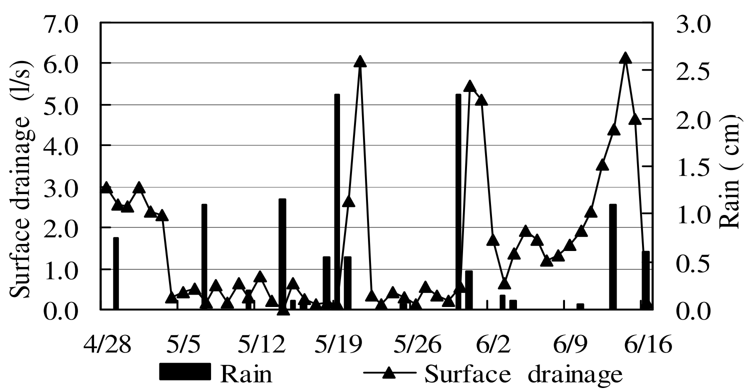

Figure 3 Surface drainage from farm block 2 (2003).

This is probably due to the water management that some paddy plots in the farm block applied continuous irrigation and overflow drainage scheme. This practice potentially causes large amount of pesticide losses from paddy fields (Watanabe et al., 2002). From June 9^{th}, surface drainage significantly increased since farmers started mid-term drainage in the fields. From our observation for both years, the mid-term drainage in the fields was completed around June 16th. Total runoff depth from farm block 2 at S7 in paddy block 2 was 26.7 cm and 32.5 cm, respectively for 2002 and 2003.

Discharge hydrograph of monitored stations at secondary canals was responded for observed rainfall pattern. Discharge and rainfall during monitoring period showed no great fluctuations between two years. Total rainfall during 46 day monitoring was 114 and 118 mm, respectively for 2002 and 2003. Some peak flows occurred following the significant rainfall events and the lag time of rainfall-runoff response was about 1 day. In earlier and later period, flow in main canal increased without rainfall input since paddy surface drainage increased during the soil preparation before rice transplanting and during the mid-term drainage period.

Pesticide concentrations in surface waters

A commercial herbicide, Thoroughbred RX flowable® containing 1.7 % of imazosulfuron, 18 % of dymron, 6.6% of clomeprop and 1.2 % oxaziclomefone was used in plot 1. The herbicide was applied on April 30 at the same day of transplanting. Concentration of the major active ingredient, dymron in paddy water in plot 1 is shown in Figure 4. Dymron concentration exponentially declined from 827 μg/l measured on May 2nd (2 days after herbicide application) and significant dymron concentration lasted during first two week after herbicide application.

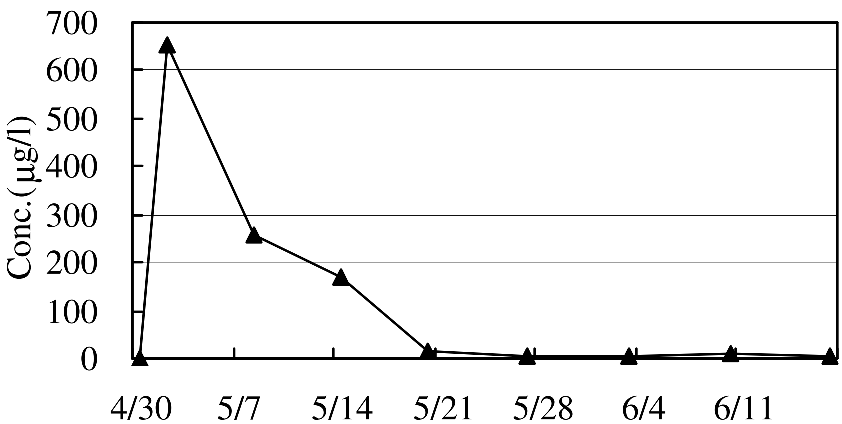

Figure 4 Dymron concentrations in paddy water at plot1 in 2002.

In the first year of 2002, total of 15 herbicides were detected in drainage water at stations as shown in Figure 5. Among these herbicides, bensulfuron-methyl, imazosulfuron, pyrazosulfuron-ethyl, dymrone were detected by LC-ESI-MS/MS analysis. The remains were detected by GC/FTD analysis. Dymron was frequently used active ingredient in the region and its application rate is relatively high as compared to other compounds hence it was detected with the highest concentration at all stations. The peak concentration of dymron was 63 μg/l at S7 and it ranged from 20 to 33 μg/l at remaining stations. Other herbicides detected with high concentration were mefenacet, imazosulfuron, and pretilachlor with maximum concentration ranging from 2 to 20μg/l.

In the second year, as the results GC/FTD analysis, 13 herbicides were detected as shown in Figure 6. However, LC-ESI-MS/MS analysis is still in progress. Beside detected compounds in previous year monitoring, two new herbicides, molinate and thiobencarb were found with peak concentrations ranging from 0 to 0.2 μg/l. Maximum concentration of mefenacet in secondary drainage canal was 2 to 3 time higher than water quality advisory level of 9.0 μg/l for mefenacet in surface water (MOE, 2004) issued by Japanese Ministry of the Environment. With farm block scale, peak concentrations of mefenacet were detected up to about 20 μg/l at S5 in 2002 and about 30 μg/l at S7 in 2003. With watershed scale, maximum concentration of mefenacet was from about 4 to 7 μg/l at S9 as being less than the water quality advisory level because of dilution by stream water.

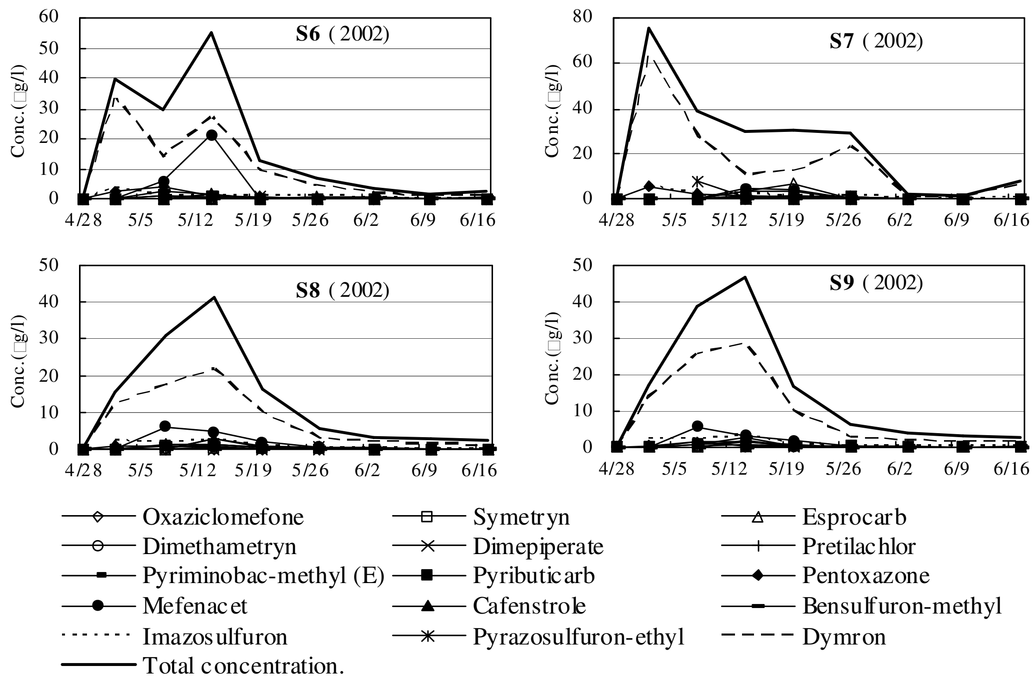

Figure 5 Herbicide concentrations in stream water at station S6, S7, S8 and S9 in 2002. *(by gas-chromatography and Liquid-Chromatograph analysis).*

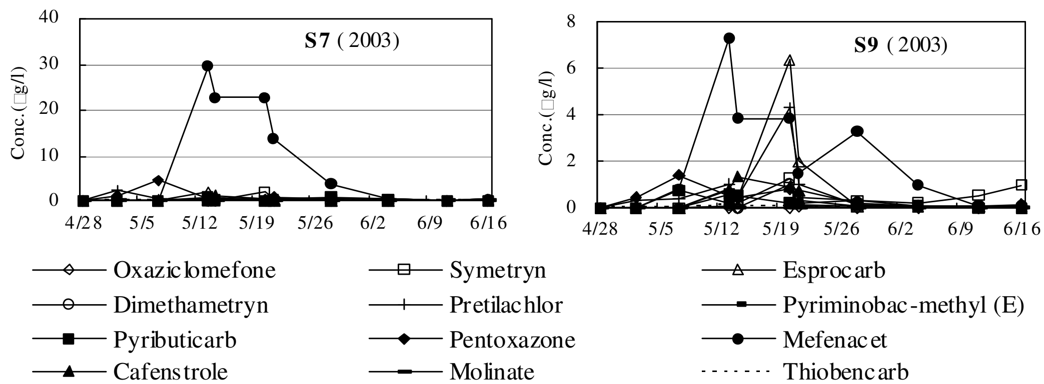

Figure 6 Herbicide concentrations in stream water at station S7 and S9 in 2003. *(by gas-chromatography analysis).*

The monitoring data for both years showed that maximum concentrations in streams occurred mostly during May 1st to 22nd corresponding to the period during and short time after the herbicide application. After that, pesticide concentrations rapidly decreased in spite of significant runoffs from farm blocks. This was explained with Figure 4 and in the study results of Watanabe et al. (2000b and 2002) that pesticide concentration in paddy water declines drastically during earlier periods after pesticides application. Times of the peak concentration were varied among monitoring stations. Within the same station, times and levels of the peak concentration were varied between herbicides. Also, those levels and times of peak concentration of one herbicide fluctuated year by year (Figure 5 and 6). This variation was explained by the variation of the timing of herbicide application among plots and farmers. In addition, communications with local farmers revealed that many growers chose the most suitable pesticide for their field and they change applied product each year for preventing from herbicide resistant problems. Actually for rice production in Japan, most of farmers start soil preparation in the late of April and plant in early of May. Depending on selected products, the time of application varies from right after rice transplanting to about 3 weeks after. Observed period of the herbicide treatment in the monitoring field lasted from early to mid May in 2004. Inao et al. (2002) conducted a surveying in 1996 and 1997 in a catchment of 2.71 km^2 having 338 paddy plot in the same prefecture (Ibaraki, Japan) and reported that distribution of treatment date followed normal distribution function with the most frequent application date was May 13 and standard deviation was 3.5 days.

Total concentration in stream water, which is summed values of concentrations of detected herbicides, mostly peaked on around May 14 (Figure 5). The shape of total concentration curve in Figure 5 was similar with those monitored in some other river in the region such as Sakura river (Ishihara, 2003) and rivers in Chiba Prefecture (Maru et al., 1985). Therefore, it can be said that the shape of curve in figures 5 and 6 indicated the typical rice herbicide concentrations in stream water in the region. However, values of peak concentrations in those rivers were smaller as few μg/l level since dilution by river water.

Herbicide runoff from watershed

Stream discharge and herbicide runoff monitored at outlet of watershed was shown in Figure 7. Cumulative discharge from watershed during monitoring period was $327x10^3$ m^3 for both 2002 and 2003. Monitoring results showed that discharge increased followed by significant rainfall events exceeding about 1.5 cm/day. From early June, the runoff from watershed increased even though there were no significant rainfall events. This was probably due to the mid-term drainage as mentioned above in Figure 3. Significant daily herbicide losses occurred with corresponding to peak flow after significant rainfall events. More than 70% of the total loss of detected herbicides was discharged from the watershed from May 7th to May 22nd responding to the pesticide application period. After that, in spite of significant water discharge, herbicide runoff was small. This was due to low herbicide concentrations in paddy water in this period as indicated in Figure 4. The key factors for pesticide runoff from this paddy field watershed were considered to be significant herbicide concentrations shortly after the herbicide application and paddy field drainage following significant rainfall events. For controlling herbicide runoff from paddy fields in monsoon area such as in Japan, the recommended Best Management Practice or Good Agricultural Practice could be an application of intermittent irrigation scheme with surface drainage gate installed high enough to store rainfall water in the paddy field during significant rainfall events and holding paddy water about 2 weeks after herbicide application. However in Japan, the directions of pesticide package, which is sole information of water holding period for most of rice farmers, recommend only 3 to 4 days of water holding. In Sacramento valley in California, water holding requirement of rice herbicide is obliged to rice growers such as 30 days for granule thiobencarb and 28 days for molinate (Newhart and Ceesay, 2001). However, appropriate water holding period for Japanese rice production should be recommended with further study.

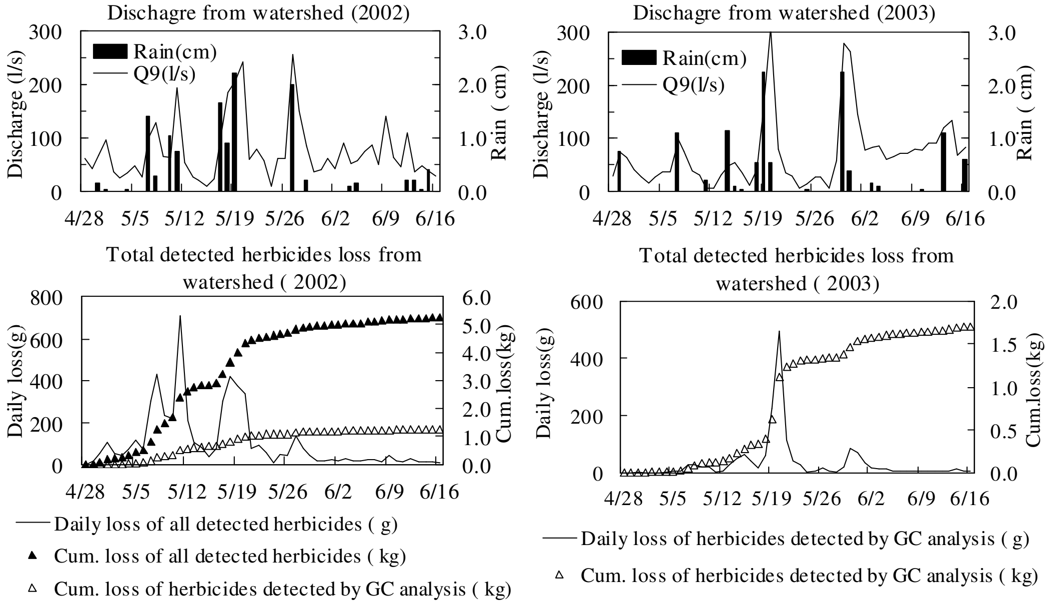

Figure 7 Discharge and pesticide loss from watershed into Sakura River.
(Herbicide concentration analysis for 2003 still is in progress).

Amount of herbicide losses from watershed in 2002 was about 5 kg. Sudo et al. (2002a and 2002b) reported that annual losses of herbicides from studied watersheds ranged from 0% to 13.0% depending on the herbicide. Total amount of active ingredients included in commercial herbicide used in the monitored farm block vary about a few % to roughly 20% which gives about 0.3kg to 2kg of total active ingredients per hector from the recommended application rate. Suppose those herbicide were applied to the studied watershed, total active ingredient that watershed received were from about 26 kg to about 172 kg. The monitored discharge of total active ingredients of 5 kg is compared to be about 20% to 3% of those value estimated above. However, detailed mass balance study should be accompanied with pesticide use survey in the future work.

Conclusions

The key factors for pesticide runoff from this paddy field watershed were considered to be significant herbicide concentrations shortly after the herbicide application and paddy field drainage following significant rainfall events. Holding paddy water during and short time after pesticide application is important since pesticide concentration in paddy water is high. However, specific data for water holding period need to be studied more in our future works. The recommended BMPs or GAPs for controlling pesticide runoff from paddy field could be an application of intermittent irrigation and drainage practice with the drainage gate setup high enough to prevent potential runoff from the paddy field when significant rainfall occur.

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A RICE HERBICIDE TIER 2 EXPOSURE ASSESSMENT FOR EUROPEAN RIVERS BASED ON RICEWQ/RIVWQ

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Abstract

The Med-Rice working group has provided a thorough guidance on Tier 1 (screening level) environmental exposure assessments for regulatory evaluation of rice pesticides in the European Union. However, only general recommendations were proposed for higher tier refinement of these assessments. For the rice herbicide bensulfuron methyl, a refined Tier 2 method was developed to predict the magnitude and duration of this compound in a conceptual watershed, representative of those that might be found in primary European rice production areas. An intensively cropped small watershed consisting of 2000 ha of rice paddies with associated ditches, canals, and a river was conceptualized. Agronomic practices such as sowing dates, drainage dates, and herbicide applications dates, were randomly varied within the watershed. Daily weather data for a 20-year period, representative of rice areas in France, Greece, Italy, Portugal, or Spain, were imposed on the watershed. Daily herbicide concentrations in the paddies and mass loadings to receiving ditches were simulated with RICEWQ. Daily herbicide concentrations in the ditches, canals, and the river were simulated with RIVWQ. Comparison of predicted paddy and river concentrations with available monitoring data suggest the Tier 2 method realistically depicts the magnitude and duration of bensulfuron methyl in surface water.

Keywords

RICEWQ; RIVWQ; Med-Rice; bensulfuron methyl

Introduction

The Med-Rice (2003) guidance was created to provide a standardized, accepted methodology for the Tier 1 assessment of rice pesticides in the European registration process. Equations were provided for the calculation of predicted environmental concentrations (PEC) in paddy water (PEC_{PW}), paddy soil (PEC_{SOIL}), receiving ditch surface water (PEC_{SW}) and ditch sediment (PEC_{SED}). Simplifying assumptions, such as instantaneous and irreversible chemical partitioning between the water and solid phases, were made to facilitate direct calculation. Some processes, such as degradation and wash off from the canopy and flowing water hydrology were excluded. Predictions were restricted to a generalized paddy and static receiving ditch. Therefore, while allowing rapid and conservative assessments appropriate for a Tier 1 screen, the Tier 1 methods offer limited opportunity for refinement.

In order to provide a more realistic representation of the complex chemical and hydrologic environment associated with rice production, a more complex tool must be employed. For the paddy environment, the simulation tool RICEWQ allows major hydrologic processes (e.g. precipitation, evaporation, seepage, irrigation, releases and overflows, controlled drainage) and chemical processes (e.g. foliar degradation and wash off, gradient driven reversible partitioning between water and solids, degradation in paddy water and soil) to be simulated on a sub-daily time step (Williams, et al., 1999). RICEWQ can be linked to RIVWQ, a related model designed to accommodate tributary systems (e.g. ditches, canals, river) with non-uniform flow and mass loadings at any point in the system (Williams et al., 1999).

A higher tier exposure assessment for the rice herbicide bensulfuron methyl was conducted using a linked RICEWQ-RIVWQ modeling system. Bensulfuron methyl is a sulfonylurea herbicide used for the control of broadleaf weeds and Cyperaceae (sedges) in European rice production.

Materials and methods

The simulation models RICEWQ 1.64 and RIVWQ 2.02 were used to conduct the simulations and generate PEC output. The model system was conceptualized as a small, intensively cropped watershed of 2000 ha, which was divided into 10 management blocks of 200 ha each. Paddies drain to ditches, then to canals, and then to a river. The timing of key agronomic activities, such as flooding, drainage, and bensulfuron methyl application were identical for each management block in relation to the date of rice sowing. However, the date of sowing was randomly varied among the blocks over a three-week period from late April to mid May, typical timing for post emergence application of bensulfuron methyl to rice. Daily meteorological data available from FOCUS (2000, 2001) for France, Greece, Italy, Portugal, and Spain were imposed on the watershed for a simulation period of 20 years each (Table 1).

Table 1 Average monthly rainfall and air temperature for the meteorological scenarios over a 20-year period

	France[1]		Greece		Italy		Portugal		Spain	
Month	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)	Rain (mm)	Temp (°C)
Jan	67.4	6.1	77.4	8.6	52.5	2.6	140.1	9.3	53.9	10.7
Feb	62.1	7.5	62.8	9.1	51.8	4.5	148.4	10.3	63.6	11.9
Mar	52.2	10.0	47.3	11.0	72.0	9.0	94.6	11.7	40.0	13.9
Apr	60.2	12.1	32.9	14.0	81.4	12.1	89.1	13.0	47.3	15.3
May	62.7	15.9	17.3	18.0	76.6	17.0	80.0	15.1	25.6	18.3
Jun	32.0	19.7	4.2	22.3	77.8	20.9	43.3	18.6	13.2	22.5
Jul	20.7	22.8	1.3	24.5	44.0	23.9	20.9	20.5	0.8	26.0
Aug	49.3	22.4	9.8	24.4	70.9	23.4	20.9	20.5	8.9	26.3
Sep	68.6	19.4	13.4	21.7	68.4	19.7	66.2	19.5	12.9	24.1
Oct	150.7	15.0	56.1	17.7	126.6	14.1	144.2	15.8	47.9	19.0
Nov	63.5	10.0	102.0	12.9	79.4	7.5	127.6	12.5	88.6	14.6
Dec	66.3	7.1	75.7	9.9	55.8	3.6	174.7	10.5	90.1	12.1
sum =	755.7		500.0		857.2		1149.9		492.8	

[1] *Weather file source: France = r4noirr.met distributed with the FOCUS (2001) surface water scenarios. Greece = tzz6.met, Italy = pzz6.met, Portugal = ozz6.met, and Spain = szz6.met distributed with the FOCUS (2000) ground water scenarios.*

A single post emergence, broadcast, ground spray application of bensulfuron methyl was simulated to occur 30 days post sowing (representative of BBCH 20) in each year. A foliar interception value of 50% was used, consistent with the recommendation of Linders et al. (2000) and with estimates based on regulatory field dissipation experiments. A total of 25% of the watershed (500 ha) received treatment, representing a high level of product adoption in a watershed of this size. Spray drift of 2.77% to ditches adjacent to treated paddies was assumed at the time of application (BBA, 2000). Soil and hydrology of the model system were based on information from the Med-Rice (2003) guidance document for the clay scenario, field notes and photos for European rice fields, and discussions with local rice experts. PEC_{SW} and PEC_{SED} values in the paddies were generated using RICEWQ. PEC_{SW} and PEC_{SED} in receiving ditches, canals, and the river were generated using RIVWQ. Daily PEC values from one treated paddy, one outflow ditch node, and one outflow canal node in each management block were selected for summarization. Daily PEC values for the river node at the exit of the watershed were evaluated.

Table 2 Summary of information used to derive RICEWQ inputs

Parameter	Value used	Source/rationale
Bensulfuron methyl parameters		
• application rate (g a.s./ha)	60	Product GAP
• number of applications per year	1	Product GAP
• K_{OC} (mL/g)	315	DuPont regulatory study
• water solubility (mg/L)	67	DuPont regulatory study
• paddy water DT_{50} (days)	4	DuPont regulatory study
• paddy soil DT_{50} (days)	12	DuPont regulatory study
• foliar DT_{50} (days)	10	Default FOCUS (2001) value
• foliar interception fraction (BBCH 20)	0.5	Linders et al., 2000; DuPont regulatory study
• foliar wash off rate per cm precipitation	0.5	Default FOCUS (2001) value
Agronomic activity (days relative to sowing)		
• initial paddy flood	-16	Generalized representative agronomic practice
• rice sowing	0	timing derived from information given in section 2
• first drain after flood to promote pegging	2	of the Med-Rice (2003) guidance and input from a
• resume irrigation after pegging	8	local EU expert (E. Capri, personal
• rice seedling emergence	9	communications).
• bensulfuron application (BBCH 20)	30	
• drain paddy after bensulfuron application	36	Representative timing of bensulfuron methyl
• resume irrigation	43	application taken from the product GAP.
• maximum crop canopy development	50	
• stop irrigation prior to final drain	85	
• final drain	90	
• harvest	100	
Hydrologic parameters		
• surface area of paddy (ha)	2	Med-Rice, 2003
• maximum paddy drainage rate (cm/d)	2.5	Conservative professional judgment
• irrigation rate (cm/d)	2.592	Med-Rice, 2003
• depth of paddy outlet (cm)	20	Conservative assumption for overflow
• initial depth of paddy (cm)	0	Depth on 01-January of each simulation year
• Seepage rate in paddy (cm/d)	0.1	Med-Rice, 2003 – rate for the clay scenario
• depth at which irrigation will begin (cm)	5	Assumed typical practice for the region
• depth at which irrigation will cease (cm)	10	Water level per Med-Rice, 2003
Paddy soil parameters		
• depth of active soil layer (cm)	5	Med-Rice, 2003
• soil bulk density (g/cm^3)	1.50	Med-Rice, 2003
• organic matter (% wt/wt)	3.0	Med-Rice, 2003
• field capacity (cm/cm)	0.42	Rawls & Brackensiek (1985), Med-Rice clay soil
• wilting point (cm/cm)	0.23	Rawls & Brackensiek (1985), Med-Rice clay soil
• soil moisture (cm/cm)	0.42	Field capacity
• mixing depth for partitioning to soil (cm)	0.1	Professional judgment
• suspended sediment concentration (mg/L)	45	Capri and Maio, 2002
• burial velocity (m/day)	0	not used
Crop Parameters		
• maximum crop coverage fraction	0.98	Professional judgment
• harvest flag	-2	straw disposal, residues removed with straw

First-order water and soil DT_{50} values derived from paddy field dissipation experiments conducted at three Italian sites over three years (nine experiments) were used to represent degradation in paddy water and soil (Molinari et al., 1999). First-order DT_{50} values from a sunlight irradiated water/sediment study and an anaerobic aquatic soil study were used to represent degradation in ditch/canal/river water and sediment, respectively. The average Freundlich K_{OC} was used to represent sorption. Default FOCUS values for foliar degradation and wash off were used to represent canopy behavior (FOCUS, 2001). A summary of input information is given in Table 2 for RICEWQ and Table 3 for RIVWQ. Monthly average rainfall and air temperature for the five meteorological scenarios is summarized in Table 1.

Table 3 Summary of information used to derive RIVWQ inputs

Parameter	Value used	Source/rationale
Bensulfuron methyl parameters		
• initial concentration in water (mg/L)	0	not used
• initial concentration in sediment (mg/kg)	0	not used
• K_{OC} (mL/g)	315	DuPont regulatory study
• water solubility (mg/L)	67	DuPont regulatory study
• ditch/canal/river water DT_{50} (days)	7	DuPont regulatory study; Anselmetti et al., 1999
• ditch/canal/river sediment DT_{50} (days)	118	DuPont regulatory study
• volatilization coefficient (m/d)	0	not used (bensulfuron VP $<3\times10^{-12}$ Pa at 25°C)
• Spray drift to ditches by treated paddies (%)	2.77	BBA, 2000; Med-Rice, 2003
Aquatic/sediment parameters		
• settling velocity (m/day)	2.0	Calculated for fine grained particles (Guy, 1977)
• mixing velocity (diffusion) (m/day)	0.01	Within range of 0 to 0.1 m/day (Arnold et al. 1991)
• suspended sediment concentration (mg/L)	45	Capri and Maio, 2002
• mixing depth for sediment partitioning (cm)	0.08	Professional judgment
• depth of active sediment layer (cm)	5	Med-Rice, 2003
• porosity of bed sediment (cm^3/cm^3)	0.43	Calculated from bulk density (Arnold et al., 1991)
• sediment bulk density (g/cm^3)	1.50	Med-Rice, 2003
Ditch parameters		
• Top width (m)	1.0	Field notes, photos[1]; Spain, Portugal, France maps
• Length between ditch nodes (m)	100.0	Paddy width
• Base depth (m)	0.5	Field notes and photos[1]
• QC coeff., stage-flow rating curve for depth	4.16	Calculated for 0.01 m/s velocity, 0.5 m base depth
• QD expon., stage-flow rating curve for depth	0.40	Average exponent value (Knighton, 1984)
• Longitudinal dispersion (m^2/s)	0.01	Fischer et al., 1979
• Muskingum-K coefficient	10000	Length divided by a velocity of 0.01 m/s
• Muskingum-X coefficient	0.20	Typically 0.1 to 0.3
Canal parameters		
• Top width (m)	2.0	Field notes, photos[1]; Spain, Portugal, France maps
• Length between canal nodes (m)	200.0	Paddy length
• Base depth (m)	1.0	Med-Rice, 2003; Field notes and photos[1]
• QC coeff., stage-flow rating curve for depth	3.08	Calculated for 0.03 m/s velocity, 1.0 m base depth
• QD expon., stage-flow rating curve for depth	0.40	Average exponent value (Knighton, 1984)
• Longitudinal dispersion (m^2/s)	5.0	Fischer et al., 1979
• Muskingum-K coefficient	6666.7	Length divided by velocity of 0.03 m/s
• Muskingum-X coefficient	0.20	Typically 0.1 to 0.3
River parameters		
• Top width (m)	25	Spain, Portugal, France maps
• Length between river nodes (m)	1000.0	Paddy block length
• Depth (m)	2.0	Med-Rice, 2003
• QC coeff., stage-flow rating curve for depth	1.05	Calculated for 0.1 m/s velocity, 2.0 m base depth
• QD expon., stage-flow rating curve for depth	0.40	Average exponent value (Knighton, 1984)
• Longitudinal dispersion (m^2/s)	5.0	Fischer et al., 1979
• Base flow (m^3/s)	5.0	Calculated based on a 0.1 m/s velocity
• Muskingum-K coefficient	10000	Length divided by a velocity of 0.1 m/s
• Muskingum-X coefficient	0.20	Typically 0.1 to 0.3

[1] *Field notes and photos from Waterborne Environmental, Incorporated.*

Daily PEC output values were summarized in two ways. First, the maximum daily PEC_{SW} and PEC_{SED} values were taken from each simulation year (20 years per scenario) for each sample point (10 for paddies, ditches, and canals; 1 for the river) for each meteorological scenario (5 scenarios). This resulted in 20 to 200 maximum daily values per meteorological scenario and 100 to 1000 daily maximum values in aggregate. The average of these maximum values was calculated to conservatively assess PEC magnitude in paddies, ditches, canals, and the river. Second, the number of days in each simulation year that the PEC_{SW} exceeded various acute ecotoxicological endpoints for aquatic species were taken for each meteorological scenario individually and in aggregate. Any acute endpoint for any aquatic species could be used in the assessment. As an example, an acute endpoint value of 1.45 μg/L was assumed. The average number of exceeding days was calculated to assess the duration of exceedence. Therefore, both the magnitude of the PEC_{SW} and duration of exceedence were determined using the higher tier method. Statistical analysis (ANOVA, Fisher's comparisons, correlations) was conducted using MINITAB release 13.32 (MINITAB, 2004).

Results

Observed and predicted concentrations of bensulfuron methyl in paddy water at the time of application and six days later, at the time of paddy drainage, are presented in Table 4. The concentration decreased from approximately 30 μg/L to 8 μg/L during this period. Observed and predicted concentrations were not significantly (α = 0.05) different for either measurement time. The average maximum concentration observed in six northern Italian rice area rivers and tributaries in 1998 is also given in Table 4. The monitoring data was taken from the work of Anselmetti et al. (1999) and Greppi et al. (1999) with additional data values provided by one of the authors (M. Greppi, personal communication, 2004).

Table 4 Comparison of observed concentrations of bensulfuron methyl from Italian field and river monitoring studies with predicted concentrations based on the Italian meteorological scenario

Measurement	Observed (μg/L)	Predicted (μg/L)	Significant difference?
Paddy water concentration on Day 0	N=9[1] 33.4±13.4	N=200 30.2±7.0	No P>0.05 (P = 0.208)
Paddy water concentration on Day 6	N=9 8.5±5.0	N=200 8.4±3.2	No P>0.05 (P = 0.965)
Maximum concentration in river water[2]	N=6 0.31±0.24	N=100 0.54±0.24	No P>0.05 (P = 0.052)

[1] *N = number of values used in the average and standard deviation calculation.*
[2] *Maximum observed concentration in six Italian rivers and tributaries in East and West Sesia during 1998 and the maximum predicted daily concentration at the exit of the conceptual watershed in the river.*

The observed average maximum, 0.31±0.24 μg/L, was slightly lower than the predicted average maximum at the exit of the simulated watershed, 0.54±0.024 μg/L. While the difference was not significant at the α = 0.05 level, it was significant at the α = 0.1 level, indicating the predicted values were somewhat conservative from a risk assessment perspective. The daily concentration pattern of bensulfuron methyl in the simulated river along with observed monitoring data from northern Italy is given in Figure 1. The minimum, average, and maximum daily predicted values are based on a 20-year simulation. The range of predicted values encompass the observed values in magnitude, and the temporal distribution co-occurs with the observed values. Predicted bensulfuron methyl concentrations in paddies, ditches, canals, and the river are listed in Table 5. Concentrations decreased by approximately two orders of magnitude from the treated paddies to the river node at the exit of the conceptual watershed.

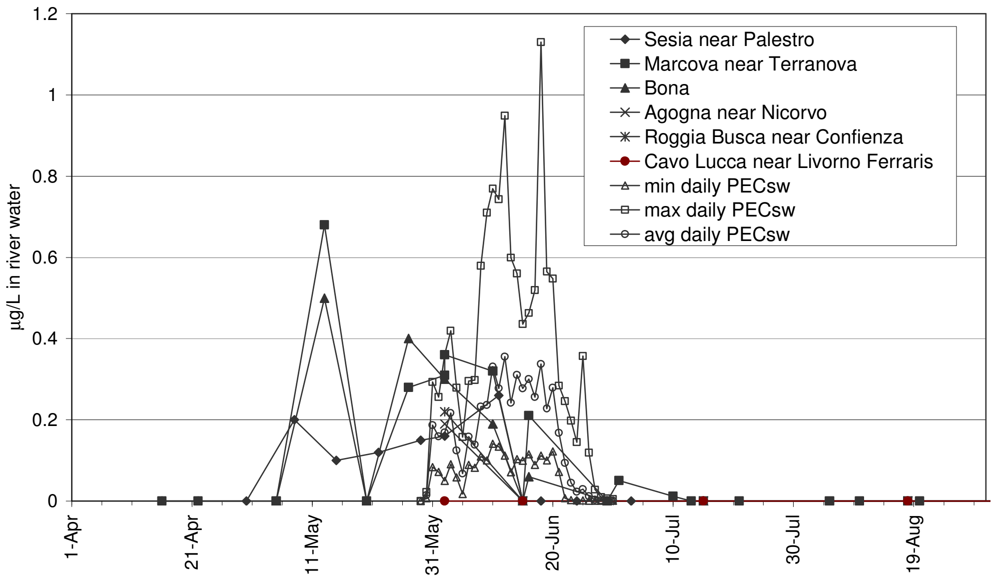

Figure 1 Comparison of observed bensulfuron methyl concentrations in six northern Italian rivers and tributaries in 1998 with daily PEC_{SW} values based on the Italian meteorological scenario (N=20 simulation years).

The average number of days that bensulfuron methyl concentrations exceeded the example acute aquatic endpoint is given in Table 6. While significant differences between meteorological scenarios are shown, this is primarily due to the large PEC_{SW} sample size that allows excellent statistical power. The actual difference in the number of exceedence days between the scenarios is small. The overall average exceedence time was 9 days in paddies, 3 days in ditches, and 1 day in canals. There were no exceedences in the river.

Table 5 Average daily maximum predicted bensulfuron methyl concentrations in treated paddies, receiving ditches, canals, and the river.

Meteorological scenario	Paddies, N=200[1] (µg/L)		Ditches, N=200 (µg/L)		Canals, N=200 (µg/L)		River, N=20 (µg/L)	
France	33.8±12.0	A[2]	4.4±1.9	A	1.5± 0.6	A	0.35±0.12	A
Greece	33.4±6.8	A	3.2±1.2	B	1.1± 0.4	B	0.23±0.07	B
Italy	34.9±24.7	A	5.3±2.5	C	1.8± 0.6	C	0.54±0.24	C
Portugal	33.1±20.4	A	4.6±1.5	A	1.6± 0.5	A	0.39±0.12	A
Spain	34.7±7.6	A	3.5±1.3	BD	1.2± 0.5	D	0.31±0.17	AB
All scenarios combined	N=1000 34.0±16.0		N=1000 4.2±1.9		N=1000 1.4±0.6		N=100 0.37±0.2	

[1] *N = number of simulation years per scenario. The maximum daily value from each simulation year was used to calculate the average and standard deviation.*

[2] *For meteorological scenarios, means with a letter in common are not significantly (α = 0.05) different.*

Table 6 Average number of days per simulation year that PEC_{SW} exceeded the example acute aquatic endpoint of 1.45 µg/L

Meteorological scenario	Paddies, N=200[1] (µg/L)		Ditches, N=200 (µg/L)		Canals, N=200 (µg/L)		River, N=20 (µg/L)	
France	9.7±12.3	A[2]	3.0±2.3	A	1.0±1.1	A	0	A
Greece	8.2±1.1	B	1.9±1.4	B	0.3± 0.7	B	0	A
Italy	10.2±2.3	C	3.4±2.2	C	1.6±1.2	C	0	A
Portugal	10.1±2.1	AC	3.2±1.7	AC	1.2± 1.2	D	0	A
Spain	8.8±1.8	D	2.2±1.2	BD	0.5±1.0	BE	0	A
All scenarios combined	N=1000 9.4±2.1		N=1000 2.7±1.9		N=1000 0.9±1.2		N=100 0	

[1] N = number of simulation years per scenario. The number of exceeding days in each year were used to calculate the average and standard deviation.

[2] For meteorological scenarios, means with a letter in common are not significantly (α = 0.05) different.

Discussion

The similarity of observed and predicted concentration values at the paddy level and at the river level indicate that the selected hydrology and chemical inputs provided a realistic representation of bensulfuron methyl behavior in the European rice environment. The model system was not calibrated *per se* to the observed results since the goal was not to represent a specific paddy or river system. Rather, the goal was to generate realistic distributions with respect to magnitude and time.

The predicted mean concentration values in ditches, canals, and the river were linearly correlated with the average monthly rain total during and following the application period of April through June (correlation coefficient = 0.916 to 0.987). This is consistent with the river monitoring data from northern Italy (Anselmetti et al, 1999; Greppi et al. 1999). In 1997, the total rainfall received in Vercelli, Italy, the center of the monitoring area, was 191 mm during April, May, and June (ISC, 2003). Bensulfuron methyl was not observed in any river samples collected in 1997 (detection limit 0.01 µg/L). In 1998, the rainfall total for this period was 318 mm, with observed concentrations as given in Table 4 and Figure 1. Comparison of the Vercelli rain values with the Piacenza, Italy long term meteorological rain values indicated 1997 was a 31st percentile year while 1998 was an 82nd percentile year for the April through June period.

The predicted exceedence data demonstrates the transitory nature of bensulfuron methyl in the watershed system. The number of days the example acute endpoint was exceeded were of short duration in ditches and canals (1-3 days). No exceedences were associated with the river.

Conclusions

The higher tier RICEWQ-RIVWQ modeling system provided a realistic representation of both the magnitude and temporal distribution of bensulfuron methyl in surface water. The method generated exposure and exceedence values throughout the conceptual watershed system in paddies, ditches, canals, and the river. This refined exposure assessment may be used to further refine the risk assessment for aquatic organisms within rice growing areas of Europe.

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FERTILIZATION MANAGEMENT OF PADDY FIELDS IN PIEDMONT (NW ITALY) AND ITS EFFECTS ON THE SOIL AND WATER QUALITY

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Abstract

The fertilization management of the rice crop in Piedmont was analyzed, and the agronomic and environmental sustainability of the actual fertilization strategy of rice was evaluated through the analysis of its effect on the soil and water quality, at a regional scale.
Data collected in 2001 showed that, on average, a total amount of 127 kg ha^{-1} of N, 67 kg ha^{-1} of P_2O_5 and 161 kg ha^{-1} of K_2O were supplied to the rice crop. Extreme N applications (exceeding 200 kg ha^{-1}) were always associated to stocking farms.
In most cases N and P fertilization was rather well balanced with crop removal. The N balance was in the range ±50 kg for 77% of the surface. The low concentration of N in the groundwater reflected the small N surplus. P fertilization resulted to be smaller than removal for 53% of the surface. Nevertheless, the soil extractable P was very high, probably because of former higher inputs, and this resulted in a high concentration in water courses and aquifers. The K fertilization was excessive (surplus >100 kg ha^{-1}) for 53% of the surface, but most soils showed a low K content. K is probably contributing to nutrient leaching to a great extent.
The average soil organic matter content of paddy fields was higher than that of normally-cultivated soils in Piedmont, and the C/N was higher, owing to the low mineralization rate in waterlogged conditions. The SOM content was in relation with the management of the crop residues, as the tradition of burning straw after harvest was still widespread on 65% of the paddy surface.
The fertilization management of rice in Piedmont can be improved, but agronomic and environmental indicators show that the contribution of paddy fields to water pollution is limited.

Keywords

fertilization; nutrient balance; nutrient efficiency; rice; water quality.

Introduction

Although the relationship between nutrient supply and water quality can be studied at a small scale in experimental fields, not necessarily all processes that act at a wider scale can be understood or evidenced in few square meters. At a regional scale, the agricultural practices that farmers actually adopt on their fields must be monitored to relate the crop management techniques to the resulting quality of soil and water. This kind of studies provides regional stakeholders with a tool to plan land use, to assess the efficacy of policy measures and the need to introduce new ones, to forecast the effects of a change in the policy or in prices, and to discriminate the pollutant load of agriculture from urban or industrial activities.

A specific territorial survey is the only way to acquire information on the actual agricultural practices in Italy, as official data such as Census or commercial registers cannot be georeferenced and are not sufficiently detailed in terms of single crops.

This paper presents results on the nutrient amounts supplied to crops and the nutrient balance, and their effects on the soil and water quality, in a regional study that was conducted on the rice crop. The reference area was the paddy area of the Piedmont Region (NW Italy).

Materials and methods

The study was conducted on wide, continuous and unconfined territory that extends across the provinces of Vercelli, Novara, Biella and Alessandria, known as the paddy area of Piedmont because rice represents 83% of the farmland, for a total of 103503 ha.

The crop management practices were investigated through an interview to farmers that were considered as representative within homogeneous typical areas. The homogeneity of typical areas was assessed through the following agronomic aspects: soil type and fertility (IPLA, 1982), source of irrigation water, most diffused agricultural system, types of farms. A total of 67 typical areas were outlined, 105 farmers were interviewed and 298 cases of fertilization techniques applied to rice were listed (approx. one every 347 ha of paddy fields). A weight coefficient was calculated, which represented the diffusion of each technique over the typical area. The amounts of N, P_2O_5 and K_2O spread through fertilization, those removed with the yield, and their differences (balances) were calculated and weighted over each typical area. More detailed information on the method of investigation is reported in Zavattaro *et al.* (2004).

The analysis was conducted in winter and spring 2002 and the techniques were those that had been used in 2001. Any variability between the years was not considered in this study.

Soil analyses reported in this study were performed on the first horizon of paddy fields during the period 1993-2003, for a total of over 3200 cases (Regione Piemonte, LAR of Torino - Banca Dati dei suoli Agrari del Piemonte). The concentration of nutrients in rivers and groundwater was measured in the years 2000-2003, for a total of almost 15000 cases (Regione Piemonte, Direzione Pianificazione delle Risorse Idriche - Rete di Monitoraggio delle Acque Sotterranee).

Results and discussion

Nutrient spreading: totals and splitting

The fertilization strategies adopted by the farmers resulted to be rather variable across the paddy area. However, the average fertilizer supply to the rice crop was very similar for rice in monoculture farms and in farms where other crops were cultivated. Table 1 reports the average amount of nutrients spread on fields, using different types of fertilizers.

On average, a total amount of 127 kg ha^{-1} of N was spread on the rice crop, with a high dispersion of data. No cases of zero distribution were encountered. The amount of N spread ranged from 25 to over 300 kg ha^{-1}, but 45% of the paddy surface was fertilized with 90-120 kg ha^{-1}.

Total amounts of N greater than 170 kg ha^{-1} were spread over 15% of the farmland. This is interesting because 170 kg ha^{-1} is in fact the maximum amount of organic N that can be supplied to fields in Nitrate Vulnerable Zones (Nitrates Directive 91/676/EEC and Regione Piemonte 2002, DPGR 9/R): in most of the farms, fertilization was under that threshold, even as far as total N supply (and not only organic) is concerned.

Table 1 Average amount of nutrients spread on paddy fields through different types of fertilizers, top dressing amount, and diffusion of products.

		amount spread kg ha^{-1}	top dressing fraction % of total amount	surface where spread % of total paddy surface
N	inorganic	98	74	96
	calcium cyanamide	55	0	11
	slowly available	50	68	7
	organic	32	0	34
	manure	91	0	6
	organomineral	27	0	29
	total	127	58	100
P_2O_5	*total*	67	71	71
K_2O	*total*	161	58	98

Extreme N applications (exceeding 200 kg ha^{-1}) were always associated to stocking farms. The application times of the N fertilizer, before sowing or top dressing, were also very variable. Generally, a slightly greater amount of N was given as top dressing than at sowing (73 *vs* 63 kg ha^{-1}). The percentage of fertilizer spread before sowing had no relation to the total amount that was spread. The highest amount of N at sowing was associated with the spreading of manure, in those farms that still use it (91 kg ha^{-1} of N). Traditional inorganic fertilizers (mainly urea), calcium cyanamide or slowly available N fertilizers were used at sowing to supply 55-68 kg ha^{-1} of N. When more expensive commercial organic or organomineral fertilizers were used, the amount of N supplied at sowing was minimal (27-32 kg ha^{-1}). Inorganic and slowly-available N products were the only sources of N to be used as top dressing (72 and 34 kg ha^{-1}, respectively).

Phosphorous fertilizers were widely utilized in paddy fields, but 29% of the surface did not receive any supply in 2001. It is possible that distributions are not repeated every year. The total distribution was quite variable, ranging from 0 to 240 kg ha^{-1} of P_2O_5 with an average of 67 kg ha^{-1}. However, less than 60 kg ha^{-1} were spread on 79% of the paddy surface. P was distributed entirely before sowing on 61% of the surface, and only as top dressing (before flowering) on 6% of the paddy surface, but very seldom at both moments.

Potassium fertilizers supplied 161 kg ha^{-1} of K_2O, thus ranging from 100 to 250 kg ha^{-1} on 80% of the surface. In some exceptional cases even more than 300 kg ha^{-1} were distributed, when KCl was added to a distribution of manure: farmers in Piedmont tend to underestimate the nutrient content of manure (Grignani and Zavattaro, 1999). The fertilizer was spread mainly before sowing (>60% of the total), on 18% of the surface; half as basic fertilization and half as top dressing, on 21% of the surface; it was spread totally as top dressing on 33% of the surface. Contrary to other farmers in Piedmont, rice farmers recognize that potassium is a soluble compound, that can efficiently be spread as top dressing. In the majority of cases, K was supplied through inorganic products. However, a certain amount of K was supplied to the crop as a part of manure, or as other organic (especially distillery washes), or organomineral products.

Further information were reported by Zavattaro *et al.* (2004).

Commercial fertilizer products used in paddy fields

A great variety of commercial products were utilized in rice fertilization: 11 inorganic products with one of the three primary nutrients, 17 inorganic products with two primary nutrients, 16 inorganic products with all three primary nutrients, 5 with slowly available N, 16

organic products that included manure, and 10 organomineral products. The total amount of commercial fertilizers spread on rice in the paddy area added up to 76120 t.
Inorganic fertilizers were complessively used on almost the whole surface, 64% of the land at sowing, and 76% top dressing. A wider variety of products was used at sowing than top dressing. A total of 39% of the surface was treated at sowing with simple inorganic products, while 25% was treated with composite products containing more than one element in the mineral form. The use of composite products was more widespread as top dressing (55% of the surface), with a strong dominance of those containing N and K.
Of the inorganic products with a N slow release effect, calcium cyanamide was still more widely used than more recent fertilizers. Its use was limited to sowing time. The same was observed for methyleneurea, while coated urea and ammonium sulphate were spread as top dressing only, and nitrification inhibitors were used either at sowing and top dressing. Calcium cyanamide, when used, supplied about half of the total N.
Organic compounds were widely used in rice fields (about 40% of the surface), and they generally supplied one third of the total N. Farmyard manure was distributed over only 6% of the surface, mainly located in the provinces of Biella and Novara. Distillery washes were spread over a limited territory (3% of the total land, mainly in the province of Novara). Commercial organic fertilizers were spread over the rest of the surface, namely 32% of the total. Most of them were derived from animal slaughtering residues. This indicates that even in the absence of farmyard manure, rice farmers are very interested in using organic compounds and are likely to buy them, even though these commercial products are usually characterized by a low nutrient content and a high cost per nutrient unit. Organomineral products were almost as important as commercial organic ones, as they were used on 29% of the surface and supplied about 30% of the total N. Farmers tend to confuse organic and organomineral products, even though the availability dynamics and efficiency can be different, depending on the amount of mineral N that is added and on the type of organic matrix.
The strategy of using organic or organomineral fertilizers together with inorganic products was the most widespread (55% of the paddy surface). Organic and organomineral products were used jointly on less than 3% of the surface.

Management of the crop residues
Paddy rice straw is traditionally chopped and left on the soil after harvest, in Piedmont. The field is set to fire during winter, hence most of the straw and part of the standing stubbles are burnt. The reasons for this are that farmers fear that i) the decomposition of organic matter in spring could cause toxic effects to the small plants, and ii) partially-decomposed straw could accumulate in the soil and then float during irrigation, due to the low mineralization and humification rates in waterlogged conditions. Table 2 shows that this practice is still widely used over 65% of the paddy surface. These data are likely to be quite different from year to year, as a consequence of the autumn rainfall: if the autumn is wet, straw is burnt on a smaller percentage of land.

Table 2 Fate of straw in the paddy area in the different provinces.

province	removed	burnt	buried	*area*
Alessandria		45%	55%	*7%*
Biella		95%	5%	*4%*
Novara	21%	29%	50%	*31%*
Vercelli		86%	14%	*58%*
total	*6%*	*66%*	*28%*	*100%*

The northern part of the paddy area (the province of Novara) was characterized by a relevant amount of surface where straw was removed and used for cattle bedding, but the total importance of this practice was rather limited. Burning straw was mostly diffused in the central part (the province of Vercelli) and the north-western part (the province of Biella) of the paddy area. Straw was buried on about half the surface of Alessandria (south-eastern part) and Novara (northern part). No clear relation was noticed between the management of crop residues and the use of organic or organomineral products, thus indicating that the role of straw as a source of carbon to the soil is often neglected by farmers.
Considering that the overall average yield was 6.72 t ha^{-1}, with a harvest index of 50% on the dry matter, the straw potentially affected the nutrient balance with about 53 kg of N, 29 kg of P_2O_5 and 163 kg ha^{-1} of K_2O.

Nutrient balance
The nutrient balance, calculated as the difference between supply and removal, is often used as an indicator of the fertilization efficiency of a system. It was here intended as a simple indicator of potential losses (Öborn et al., 2003). Figure 1 shows the fertilization balance for rice fields in Piedmont.

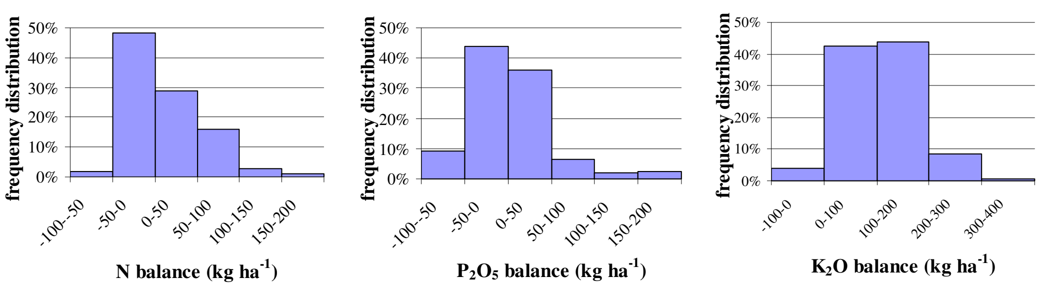

Figure 1 N, P_2O_5 and K_2O balance.

The N balance was in the range ± 50 kg on 77% of the surface. The surplus exceeded 100 kg on only 4% of the surface, and the deficit was never greater than 80 kg ha^{-1}. In most cases fertilization was rather well balanced with the crop removal. Rice farmers are probably conscious of problems related to N over-fertilization: waste of money and time, lodging, blast disease and sterility of rice.
If the whole area is considered, the average excess of N was only 14 kg ha^{-1}. In a system in equilibrium, this would suggest that losses are almost negligible. Grignani *et al.* (1997) measured the N balance at the field scale in a site near Vercelli (Tab. 3). The net amount of N supplied through irrigation water was 60-130 kg ha^{-1} of N (at a concentration of 1-11 mg l^{-1} of total N), namely almost as high as fertilization, but with a high variability in the two years of measurement. Leaching below the depth of 1 m was calculated as 95-160 kg ha^{-1}, a remarkable amount, and its concentration ranged from 1.2 to 28.4 mg l^{-1} of total N. However, this nitrogen might be re-used, as both runoff and drainage contribute to downstream irrigation. The fertilization balance ranged between 50 and 100 kg ha^{-1} of surplus, like 16% of the paddy surface (Fig. 1), and the overall balance indicated an excess of 50-80 kg ha^{-1} of N; this surplus could be immobilized in the field, or lost by denitrification. The paddy area behaves like a whole system where inner imbalances are smoothed through surface and subsurface water flows, which connect wide areas through cascade irrigation from one field to another.

Table 3 N balance measured at the field scale (after Grignani *et al.,* 1997). Data are expressed in kg ha^{-1}.

		in				out					*Balance*
		fertil.	irrig.	rainf.	*Total*	grain	leach.	volat.	runoff	*Total*	
winter	1993	0	0	16	*16*	0	27	0	0	*27*	*-11*
summer	1993	150	213	12	*375*	87	135	8	85	*315*	*60*
winter	1994	0	0	17	*17*	0	45	0	0	*45*	*-28*
summer	1994	173	78	14	*265*	80	50	8	19	*157*	*108*
year	*1993*	*150*	*213*	*28*	*391*	*87*	*162*	*8*	*85*	*342*	*49*
year	*1994*	*173*	*78*	*31*	*282*	*80*	*95*	*8*	*19*	*202*	*80*

The P fertilization resulted to be smaller than the removal on 53% of the surface, while the P balance exceeded 50 kg ha^{-1} of P_2O_5 on 11% of the surface.
The K fertilization resulted to be much higher than the removal in several cases: the supply was approximately balanced in the range ± 50 kg ha^{-1} on only 12% of the surface, and the crop was greatly over-fertilized (>100 kg ha^{-1}) on 53% of the surface. It is possible that farmers exceed with the K supply because i) soil analyses have indicated a deficit in almost all the fields (Tanaka et al., 1973; Regione Piemonte, 2000); ii) rice does not suffer from an excess of K; iii) K increases the resistance to lodging and to some diseases (Moletti, 1989); iv) the cost per fertilizer unit is quite low. No information could be found on K runoff or leaching in paddy fields, but recent studies suggest that losses could be remarkable (Askegaard et al., 2003).
Apparently, the supply of nutrients was in general not modulated to the crop removal. Nevertheless, the excess of nutrients was relatively limited, thus showing that interviewed farmers consider the nutrient recovery to be higher than the value of 20-40% which is typical of Asian environments (Vlek and Byrnes, 1986).

Soil organic matter and nutrient content
Soil analyses performed on the first horizon in paddy fields of the study area during the period 1993-2003 were averaged and reported in Table 4.

Table 4 Soil organic matter, total N, available P and exchangeable K in the paddy area. Average data 1993-2003. Source: Regione Piemonte, LAR of Torino - Banca Dati dei suoli Agrari del Piemonte.

province	SOM (%)	total N (%)	C/N	Olsen P (mg kg^{-1})	exchang. K (mg kg^{-1})	num.
Alessandria	2.22	0.134	9.7	36.3	64.7	99
Biella	2.26	0.112	11.6	32.9	67.3	99
Novara	2.74	0.139	11.5	43.6	61.6	1365
Vercelli	2.40	0.121	11.4	26.0	58.9	1639
total	*2.53*	*0.129*	*11.4*	*34.0*	*60.5*	*3202*

The average soil organic matter content of paddy fields was 2.53%, higher than what was observed in normally-cultivated soils in Piedmont (Regione Piemonte, 2000). In general, waterlogging determines an overall reduction in the net mineralization rate, and consequently an increase in the total soil organic matter content. Therefore, the need to preserve the soil organic matter content through conservative agricultural practices in paddy fields is less imperative than in the case of other crops. If we exclude the province of Alessandria, the SOM content was in relation with the percentage of surface where straw was buried (Tab. 2).

Soils were richer in organic matter where manure was distributed, namely the province of Novara. The C:N ratio was high, indicating that the SOM was scarcely humified. Soils from the province of Alessandria were generally coarse-textured, and this might be the cause for the higher mineralization which is indicated by the lower SOM content and the lower C:N ratio of these soils. Along the period 1993-2003 the average SOM content was rather constant over years, except in Alessandria where a decrease was noticed.
The soil extractable P was very high, probably partly because of a high natural release of P in waterlogged soils (due to the solubilization of Fe, Al or Ca compounds), and partly because of a build-up caused by excessive supplies in the previous decades. Apparently, modern rice-growing in Piedmont is now depleting such high soil reserves, and this explains why P is not limiting yields although supplies are frequently smaller than removal.
The soil exchangeable K was low if compared with reference tables which are used as guidelines for fertilization. These tables should probably be revised for the waterlogged soil conditions in NW Italy, however the real effect of K fertilization on yield should be investigated in our conditions. Wihardjaka *et al.* (1999) and Hu and Wang (2004) reported that non-exchangeable K can be an important source for rice.

Deep and surface water quality
The nutrient concentration of the groundwater in the paddy area, measured in about 250 wells every year, is reported in Table 5 as an average of years 2000-2003. The low concentration of N in the groundwater (3.00 mg l^{-1} of NO_3-N + 0.04 mg l^{-1} of NH_4-N in the first aquifer) reflects the small amount of N leached from fields to the groundwater which resulted from the territorial nutrient balance. The pollution of deep aquifers was negligible. Orthophosphate P in the first aquifer (0.08 mg l^{-1}) was instead high if compared with literature thresholds of eutrophication (0.02-0.035 mg l^{-1} of TP following Brookes *et al.*, 1997), and with the value of 0.05 mg l^{-1} of TP that what was measured in intensively-cropped areas in the south of the Piedmont region (Zavattaro *et al.*, 2003). Potassium concentrations, 3.42 mg l^{-1} on average, cannot be compared with any legal threshold because of the absence of reference data.

Table 5 Water concentration of nutrients in the paddy area in the years 2000-2003, average and standard deviation. Data are expressed as mg l^{-1}. Source: Regione Piemonte, Direzione Pianificazione delle Risorse Idriche - Rete di Monitoraggio delle Acque Sotterranee.

water	unit	NO_3-N	NH_4-N	Total N	PO_4-P	TP	K	num.
deep groundwater	average	0.87	0.06		0.07		1.48	296
	S.D.	1.41	0.06		0.02		2.30	
shallow groundwater	average	3.00	0.04		0.08		3.42	437
	S.D.	2.89	0.03		0.07		12.63	
surface (rivers)	average	1.79	0.32	3.06	0.08	0.12	n.d.	14157
	S.D.	2.06	1.39	2.82	0.14	0.22	n.d.	

Surface water was measured every year in about 4700 rivers within the paddy area and results, reported in the same Table 5, indicate that the average concentration of mineral N (nitrate+ammonium) was slightly higher that that of the groundwater (2.11 mg l^{-1}), orthophosphate P concentration was very similar to that of the groundwater, but the total P (including particle-bounded P) was high, 0.12 mg l^{-1}, which is exactly the reference concentration for Piedmont rivers as it was set by the river basin authority (Autorità di Bacino del fiume Po, 1999). Owing to the low P balance, probably the rice fertilization in Piedmont is not contributing to P loads in water courses, but waterlogged conditions probably promote the release of P from stable compounds. This suggests that it would be agronomically and environmentally interesting to pilot the soil P content to lower values.

In all cases the dispersion of data around the mean was remarkable, as it is indicated by the high standard deviation reported in the table.
No trends in concentrations were observed between 2000 and 2003.

Conclusions

The variability in fertilization and partitioning between sowing time and top dressing reflects a great variety in commercial products with different release times. The management of crop residues did not seem to reflect the real intention of the farmer to exploit straw as a source of carbon for the soil, as the fate of straw was not related to the widespread use of commercial organic or organomineral products. However, the soil organic matter content was related with the prevailing straw management technique at the province scale.
As far as N and P are concerned, there was a general balance between fertilization and crop removal (+14 kg ha^{-1} for N, -2 kg ha^{-1} for P). Consequently, only small amounts of nutrients were prone to environmental losses. This seems to be in contrast with literature field-scale data which report that the recovery rate of fertilizers in waterlogged conditions is very low and consequently losses are remarkable. Nevertheless, territorial data of the soil and water nutrient contents indicated that N leaching to the groundwater was not important in the Piedmont paddy area, and that P runoff towards surface water courses was instead rather high. The continuous surface and subsurface water flow between fields, which transports nutrients, could be the key-process that determines an inner balance within a large area and smoothes local variations. The fertilization management of rice in Piedmont can be improved, but agronomic and environmental indicators show that the contribution of paddy fields to water pollution is limited, especially if compared to other crops in the area.
Instead, K is probably contributing to nutrient leaching to a great extent, but the environmental impact of this has not been highlighted, yet.
Linking different data on the crop management and soil and water quality is possible at a field scale but was not easy at a territorial scale, owing to the different sampling strategies. The possible effects of the crop management on the environment should be investigated also considering the future evolution of cropping systems: rice management, in particular, is a very dynamical agricultural sector.

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Session 4

ENVIRONMENT AND ECOLOGY

Abstracts

IMPACT OF CONVENTIONAL AND ORGANIC FARMING ON ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED TO RICE PLANTS

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Arbuscular mycorrhizal fungi (AMF) play a crucial role in nutrient acquisition and soil fertility. These obligate mutualistic symbionts colonize the roots of the vast majority of plants, including most crop plant. In the last years some researches has been focussed on the effect of conventional and low-input agriculture on arbuscular mycorrhizal root colonization and molecular diversity of AMFs. The objective of our study is to verify if different crop management practices could affect the AMF communities and their root colonization on rice. Two different agroecosystems were considered: one site represented organic farming with 5-year crop rotation, and one site represented high-input continuous rice monocropping. Morphological and quantitative analyses were performed. After about 40 days from sowing, the conventional rice resulted not mycorrhized, in contrast the plant grown under organic agriculture system showed a typical mycorrhization pattern, with intercellular and intracellular hyphae. To evaluate the mycorrhizal potential in the soil as well as the AMF biodiversity, soil samples representative of the two agrosystems were used as inocula for AMF trap cultures. In these experiments the rate of root colonization by AMF was high with both types of inocula. Molecular and phylogenetical analyses on ribosomal genes demostrated the presence of species identified as Glomaceae. Our results demostrated that the colonization rate of rice roots is enhanced as well as rice productivity by a low-input organic cropping in respect to conventional management practices.

Keywords
Arbuscular mycorrhizal; rice; organic farming

WATER CHALLENGES FACING RICE PRODUCTION IN CALIFORNIA

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Abstract

Rice production in California is uniquely situated in the heart of the state's largest watershed. Over seventy percent of the precipitation falls in the northern one-third of the state. The rice production area is flanked to the south by a rapidly expanding urban area and co-occupies a region with the few remaining native salmon fisheries in the state. Consequently, water resource management decisions must attend to the needs of urban users, wildlife habitat, and agricultural production. Far removed high population urban areas, such as Los Angeles and San Diego, are located areas with very low rainfall. The population concentration allows these areas to substantially influence water policy throughout the state. The demand of domestic and industrial water is rising exponentially and is accompanied by increased pressure to sell agriculture water to these areas. Yet it is not quantity of available water alone that drives the debate, but also the quality, expressed partly in terms of temperature. Species of fish protected under the U.S. Endangered Species Act require cold water temperatures of adequate flow during a period that coincides with the majority of the rice growing season. Water used for irrigation is often diverted from rivers where water temperatures are controlled to optimize fish habitat by releasing water at selected depths from reservoirs. The water temperatures are frequently sub-optimal for rice production. Water 'export' to the population centers and the need to meet the biological requirements of wildlife is forcing agricultural communities to adapt new technologies and public policy to maintain an adequate supply of water to meet the needs of rice production.

Keywords

Water; water use; rice; California.

DEGRADABILITY OF THE HERBICIDE CINOSULFURON

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Cinosulfuron is a sulfonylurea herbicide largely used in the extensive cultures of flooded rice in North Italy. Its biodegradability has been investigated in laboratory experiments using selected mixed microbial cultures, and freshly collected rice field water. The degradation rates were very low and mediated mainly by chemical hydrolysis due to the acidic pH of the cultural broths. Two degradation products resulting from the cleavage of the sulfonylurea bridge have been identified by LC-mass spectrometry. The dissipation of cinosulfuron has been determined in a flooded sediment simulating the paddy-field environment. The degradation in the aqueous phase followed a pseudo first-order kinetics with a half-life time of 24.1 days. Part of the applied herbicide was transferred to the sediment in which the highest concentration was reached after 21 days then slowed down rapidly. The curve of the total amount of cinosulfuron versus time attests for the progressive degradation of cinosulfuron which occurred through a pseudo first-order kinetics with a half-life time of 35 days. The dissipation rate of cinosulfuron was higher than expected by chemical hydrolysis according to the pH of the system, indicating the involvement of the soil microflora.

Keywords
Cinosulfuron; biodegradation; paddy field.

Appendix

The EU-India Project RICE_NET

RICE_NET: EU-INDIA *RICE* DISTRICTS *NET*WORK PROMOTION THROUGH AGRO-ECONOMICAL, CROSS CULTURAL, AND TECHNICAL ACTIONS

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Abstract

Main objective of the project is to foster a district to district awareness and cooperative network between India and EU, namely in the sector of rice through agronomical, economical and cross cultural studies and actions to raise awareness of India in the EU and vice-versa.

Both the areas have the concept of rice district: European areas form an homogeneous "EU rice macro-district" and their agricultural, economical, historical, and geographical peculiarities have driven the development of earlier rural areas. The rice production in India has affected districts cultural inheritance (folkloristic issues, culture, food, music, dance, film, etc) and gained commercial and technical interest from rest of the world.

RICE_NET (www.ricenet.org) will study both similarities and differences of districts from several points of view, through studies, research and joint activities also in media and communication sectors. Main objectives are the strengthening of agro-technical dialogue, cultural information exchange through geographical and other matters, increasing of enterprise networking in the rice sector.

Introduction

Project funded by European Commission under the Europe-India Cross Cultural Programme (ECCP) activities started. The Government of India and the European Union are committed to the importance of raising public awareness on India-EU links and to the need of increasing mutual understanding between the two regions. Mobilising civil society channels is recognized as being vital in achieving this objective. Important milestones are the first EU-India Summit in June 2000, where cultural cooperation and fostering civil society links were identified as priority areas. At the second EU-India Summit in November 2001, the commitment was taken to resume the EU-India Economic Cross Cultural Programme (ECCP). At the third EU-India Summit in October 2002, the continued progress in the enhancement of the dialogue between civil society in India and the EU was welcomed.

The key objective of the ECCP is to promote and support links and joint ventures between civil society organizations in India and the European Union in the fields of media, enterprise and university. The programme was first launched in 1997. In the framework of the ECCP the Department of Agronomy, Forestry and Land Management was successful in getting a financing from the European Commission of the RICE_NET project, with the starting date on March 2004 and ending date on March 2006. RICE_NET

Main objective of the project is to foster a district to district awareness and cooperative network between India and EU, namely in the sector of rice through agronomical, economical and cross cultural studies and actions to raise awareness of India in the EU and vice-versa.

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Objectives of RICE_NET

First objective is the study and comparison of technical (i.e. agro-economical), cultural and historical aspects influencing the European and Indian rice districts. This study will give a first opportunity to create synergies among research partners, exchange research results, rise knowledge sharing on un-conventional approach (i.e. folklore, food, social issue, etc.) and establish an Indo-European rice link. Moreover they will lead to new academic discussions and research collaborations.

Second and main goal is to foster the social and economic cooperation dialogue between districts by promoting awareness through dissemination material like publications, video and CD-Rom, participation to local initiatives and organisation of public events. The cooperation will lead to a better understanding of needs and characteristics of each area and to a wider knowledge sharing on cultural and social issues also through folkloristic events participation and public seminars on rice cross cultural themes like heritage, food, festivals.

Third objective is to support SMEs in their process of finding concrete business opportunities starting from a better knowledge of the rice supply chain in India and Europe. Before achieving this goal a focused research on market and business state-of-the-art will be made in order to help companies and entrepreneurial associations in their internationalisation process. The mutually business opportunities will also carried out by means of access to new technologies, transfer of know-how and knowledge of quality management, technical trainings in Europe and India, business missions opportunities. Some on-site visits to selected companies will be arranged both in Europe and in India to achieve this objective.

Fourth objective is to prepare a variety of documents and publications to support the better knowledge of European rice districts in India and Indian ones in Europe. The book series will be general and describe the evolution and characteristic of rice districts according the research results along with a photographic publication that will be distributed and presented to international events to facilitate the cross cultural exchange and cultural awareness. A final video and documentary will be the main result and the best vehicle to boost the social, cultural and historical links and describe the EU-Indian rice scenario from heterogeneous points of view.

Fifth objective is the creation of an EU-Indian rice network that, starting from the project consortium, can play an innovative role in the rice arena, interacting with agronomical, humanistic, social and technical issues as well as participate to facilitate the match-making between the business cooperation and technological/know how needs of those specific SMEs which operate in the rice supply chain. The network aims is to be as much variegate as possible in order to satisfy all entities and organisations in need of deepening their mutual understanding and willing to promote the rice district to district methodology.

Sixth objective is to promote RICE_NET research results and innovative approach on rice districts study also within the international year of rice events promoted by world wide organisations like FAO (Food and Agriculture Organisation) and IRRI (International Rice Research Institute). The European Union is active member of the “2004: year of rice” committee while India is one of the targeted countries. The cultural and social aspect of the rice districts will not be addressed by the international events therefore the RICE_NET project will represent the first and innovative experience that could join research, entrepreneurial activities and media and communication outputs.

Partners

All the partners participating in the RICE_NET project are listed in Table 1

Table 1. Institutions participating in the RICE_NET project

ITALY (www.agroselviter.it)	University of Torino – Department of Agronomy, Forest and Land Management - Ente Nazionale Risi - Edizioni Mercurio - Università del Piemonte Orientale
ITALY (www.centroestero.org)	Centro Estero Camere di Commercio Piemontesi (Torino)
SPAIN (www.uv.es)	Universitat de València – Department de Geografia
FRANCE (www.cirad.org)	Centre de coopération internationale en recherche agronomique (Montpellier)
GREECE (www.ekato.org)	Pan Hellenic Consumer Organisation (Thessaloniki)
INDIA	Participatory Rural Development Foundation (Gorakhpur; Uttar Pradesh)
INDIA	Maharastra Centre for Entrepreneurship Development (Aurangabad)

All partners are actively involved in the project. They will also play important role in migrating RICE_NET activities to their countries, participating in the supervision of the research and dissemination activities coming from their areas. University of Valencia, CIRAD, EKATO and Participatory Rural Development Foundation will act as project referent in Spain, France, Greece and India respectively. Centro Estero Camere Commercio Piemontesi and MCED will be in charge of the entrepreneurial part of the project in Europe and India, in collaboration with Ente Nazionale Risi and other international institutions. They all will be also involved in editing of leaflets, updating website content, events organisation. Moreover the department of agronomy (applicant) has great interest in establishing solid relations on the research and institutional side with Indian universities in the state of Maharastra (Akola, Nagpur) and Uttar Pradesh (Gorakhpur) to increase the cooperation level and the future collaboration opportunities. The Indian partners will be directly involved to exploit the research activities and guidelines developed in Europe, in India and in particular within their rice districts.

Research partners

The research group is composed by seven important public institutions (in partnership or subcontract) with recognized researchers and consultant staff.

The Department of Agronomy, Forest and Land Management (Agroselviter) of the University of Turin has the coordination of the project. This Departmente is actively involved in the international rice scenario studies and actions.

Agroselviter has experience in research as well as project coordination and at present involved in FAO network “Medrice” (www.medrice.unito.it) for Europe, Mediterranean and Middle East countries rice activity as leader through the director, Prof. Ferrero.

The inter-department collaboration inside the University will include also economical and statistical components.

Agroselviter collaborates this project in with a wider and heterogeneous group composed by:

- **University of Piemonte Orientale** (www.unipm.it): based in Vercelli, the department of humanistic studies "A. Avogadro" is the most active research group on rice distric of north Italy. The university of eastern Piedmont was established as an autonomous institution (separated from University of Turin) in 1998 and it has taken its rightful place as scientific and cultural hub among Italian universities. The aim of this small but promising university is (among others) to enhance the correspondence with local community through economical and social know how and studies related local business, mostly centred on rice cultivation. The constant dialogue with students, professionals and local authorities have lead the university to became a disseminator of technological education and innovation as well as humanistic outcomes.
- **Ente Nazionale Risi** (National Agency for Rice www.enterisi.it): public-economic agency subordinate to the vigilance of the Ministry of Agriculture, carrying out an intense activity aiming to the protection of all the field linked to the rice sector: it promotes the rice " made in Italy " with campaigns of information and competitions, it supplies technical assistance to the farmers and service of analysis and leads actions focused to the improvement of the production. Next to the actions of which over, an other task of great relief is represented from the development of the organism activity payer of the aids and communitarian participations on behalf of the European Union. ENR has moreover a Rice Research Center that collaborates with various Italian and international institutions.
- **Edizioni Mercurio** (www.edizionimercurio.it): this media and communication company was established twenty years ago with the aim and mission of becoming the "cultural engine" and the cross sectorial actor between humanistic science and new media. Literature, sociology, philosophy, archaeology, history are some of the fields in which Mercurio has worked and gained experience and professionality. High level publication and media documentary published at national and international level. Since new communication tools were discovered, Mercurio has started activities to stimulate readers and web surfers with initiatives like web art (www.arschannel.it), e-contents, historical video, shorts, interactive CD-Rom. At present a strong partnership has been realised with University of Piemonte Orientale and Italian Geography association to provide media and communication assistance for publication and content management.

The dissemination of the results of the project will require to be well known and deep-rooted in the districts; on the other hand Italy, Spain and France are three relevant countries for the rice production in Europe (Italy in the first place): Agroselviter is coordinating all these aspects with the collaboration of project consortium as well as providing its knowledge, contacts and experience in events organisation.

University of Valencia was established in 1499 and is one of the earliest universities in Europe. The department of geography was established in 1960 and has a long tradition in the *Cuadernos de Geografia* publication and research on territory and social issues like turism. Department of geography will be involved in the project as well as department of history in order to fulfil the RICE_NET research needs from both side. An agronomical expert from Istituto Valenciano de Investigaciones Agrarias (IVIA) will collaborate directly with the department of geography and with the applicant's research group to exploit agro-economical research in appointed Spanish rice districts (www.ivia.es).

Participatory Rural Development Foundation is collaborating with several institutes and universities in many rice related activities. One of this is the department of sociology of University of Gorakphur (http://mycgiserver.com/~priyadarshi786/about.html) headed by prof. Shiv Bahal Singh. The university was established in 1957. The department of sociology has several research units operating in the rice territory and addressing social studies like social change and modern India, sociology of development. The main contribution to RICE_NET initiative will be focused to entrepreneurship and social changes, as well as anthropology of rice territory and historical features (in collaboration with department of history).

Maharashtra Centre for Entrepreneurship Development management board is composed by representatives of agricultural universities. Two of them will collaborate as subcontractors in the research WP, the College of Agricultural Engineering of Akola and University of Nagpur (social science department) both in Maharastra State. The "Dr.Punjabrao Deshmukh Krishi Vidyapeeth college" in Akola is one of the Indian leading universities involved in international agro-research project and included in national research committee. University of Nagpur (www.nagpurdarpan.com/main/index.htm) was established in 1923 and the anthropological department as well as the art and social science group are focused on rice districts of state of Maharastra and will provide contribution to the humanistic studies coordinated by the European group.

The **Centre de coopération internationale en recherche agronomique pour le développement** (CIRAD) is a French scientific organization specializing in agricultural research for development for the tropics and subtropics. It is a State-owned body, which was established in 1984 following the consolidation of French agricultural, forestry and food technology research organizations for the tropics and subtropics. CIRAD's mission is to contribute to the economic development of these regions through research, experiments, training and dissemination of scientific and technical information.
The Centre employs 1770 persons including 900 senior staff, who work in about 50 countries. Its budget amounts to approximately Euros 152 millions), more than half of which is derived from public funds. CIRAD is organized into seven departments : CIRAD/CA (annual crops), CIRAD/CP (tree crops), CIRAD/FLHOR (fruit and horticultural crops), CIRADS/EMVT (animal production and medicine), CIRAD/FORET (forestry), CIRAD/TERA (territories, environment and people) and CIRAD/AMIS (advanced methods for innovation in science). CIRAD (www.cirad.fr) operates through its own research centres, national agricultural research systems or development projects and will act an important role in the European rice district agronomical research jointly with the applicant. Moreover it will be the reference point for activities in France and will contribute to the international training and technical activities (at present 3 researchers are working in India).

Entrepreneurial partners
In order to demonstrate the need of commercial awareness in rice sector in Europe and India it has been chosen to experience the project on the entrepreneurial scenario. Within UNITO the participation of Ente Nazionale Risi will provide a consistent support to the entrepreneurial activity as well as contribution of CECCP on dissemination an business meetings organisation.

Centro Estero Camere di Commercio Piemontesi (The Piedmontese Consortium for Foreign Trade - CECCP) was founded in Torino (Italy) in 1976 aiming at creating international economic relations for SMEs of Piemonte and Valle d'Aosta and promoting their internationalisation assisting participants in managing international situations.
As a non-profit organization it groups up more than 30 members: they are small, medium and large-size companies from any economic fields, as well as public and private organizations.
Its main institutional aim is the provision of incentives for companies: inform them on the characteristics of foreign markets, train the personnel dedicated to international relations, assist them in legal, fiscal and customs procedures and promote their business abroad. CECCP are also willing to accommodate and satisfy requests for information and contacts that come directly from foreign enterprises and organizations to facilitate international relations and foster development of local enterprises and institutions. Main objectives are related to:

- Enterprises (domestic and international), organizations and associations interested in developing their relations with foreign companies; foreign companies and organizations interested in learning about and entering into contact with the north Italian economic system;
- Training activities through technical seminars, presentations of countries, in-depth courses on the theme of international business to allow a deepening of knowledge about the Italian economy and comparisons with local economic system; CECCP has created in 1982 a Consortium for Training in Foreign Trade which aims at design, promote and implement training activities to foster international development of local enterprises and institutions;
- Promotion activities singling out channels, searching for partners, assistance for participation in international trade fairs and business missions, receiving delegations from abroad and market research;
- Technology transfer in connection with the major European networks and promotion of partnerships between Italian and foreign companies

CECCP has a specific department devoted to international cooperation and it has expressed a specific request to work and develop project within the RICE_NET consortium to understand the expectations and values of Indian market with the goal to bring benefit to Indian and Italian/European enterprises and to its territory by means of the outcomes and follow up the project would produce. This project will supply an indispensable flow of information useful for Italian, Indian and European companies working in the whole rice supply chain.

Maharashtra Centre for Entrepreneurship Development was set up and still promoted by Government of Maharashtra (India). Main mission is facilitate the creation of entrepreneurial and managerial capabilities in individuals and organisation. MCED, having its core activity as Entrepreneurship Development training is devoted to this task since 15 years. MCED conducts variety of training programmes to fulfil its objectives which can be listed as: develop entrepreneurship through systematic training; spread entrepreneurial culture also in agricultural areas; disseminate information and data regarding all aspects of entrepreneurship; conduct research in entrepreneurship. The spectrum of training courses, therefore, covers the wide range of different strata in the society. MCED expressed specific interest in the project, seeking the opportunity to further develop its network of relations in India and in Europe, and willing to deepen the relations with the European Chambers of Commerce and commercial associations thanks also to the overall view of Indian rice enterprises willing to start business with Europeans they may provide. MCED has already experienced an ECCP activity under entrepreneurial framework.

EKATO is a non-profit non governmental organisation, which was established in 1999. The seat of the organization is in Thessaloniki and has branches operating in Athens, Florina, Grevena and Patras. EKATO is committed through its membership, to foster and maintain integrity of business in dealings with consumers, to encourage and promote effective communication and understanding between businesses, government and consumers. EKATO pursues excellence in achieving objectives like: conduct, encourage and coordinate meetings, conferences, seminars and workshops for the professional development; serve as a 'clearing house' for data and information; encourage liaison among consumers, educators, business, government and the media starting from Thessaloniki territory; assist in the development of educational and training programs on the greek districts.

Participatory Rural Development Foundation main mission is to improve the well being of present and future generations of rural people by means of knowledge dissemination, sustainable and environment friendly technologies adoption and upgrade. The activities are carried out (among others) through sharing of multi-media information, technical research (i.e. farmer based on-farm testing of technology), scientific and agronomical integrity, R&D. Scope of the foundation involves agriculture, farmers' related research and development institutions (government, non-government and autonomous), and private enterprises with similar focus and mission. PRDF provides services also in term of: development consultancy; agro-exim and marketing; technology testing; data analysis; quality control; on-site and off-site training.

In particular, with reference to the "2004: year of rice" initiatives, both UNITO (as coordinator of MEDRICE) and CIRAD are active members of the organising committee.

Conclusions

Beside the technical objectives of the project, RICE_NET is the first attempt to create an EU-India network to promote and rise awareness of both areas using as best fitting vehicle the rise districts. The different targets and activities ranging from agronomical studies to general purpose publication aims at creating a kaleidoscopic repository to which public, enterprises, policy makers and researchers can access. The hope is that this experience could lead to extensive follow up and continuation of cross linking activities between Europe and India.

RICE CROP SYSTEMS IN VERCELLI AREA: PEDOLOGICAL TRAITS, LAND USE CAPABILITY AND SOIL FERTILITY

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Abstract

Information on soil characteristics is essential to implement more rational and efficient agronomic practices; although this concept should be always highlighted in agriculture, the importance of soil information increases if the crop management, like in the case of rice, is particularly complex and involves a careful use of water resources. The Vercellese rice production area that includes Vercelli, Saluggia and Santhià can be considered particularly important and representative of rice production in Piemonte and covers plain soils which show a significant variability from the point of view of geomorfology, land use capability and potential protection for groundwaters. A comparison between the main sources of information on Vercellese soils allowed to understand better their characteristics and to compare their chemical pattern with their origin and evolution level.

Introduction

Information on soil characteristics is essential to analyse crop systems in a specific area; as a matter of fact, knowing soil means to understand better agronomic orders and to suggest choices for the best exploitation of agricultural potentials. Moreover it helps to focus possible limitations to crops and risks related to environmental impact of agriculture.

In the area that includes Vercelli, Santhià and Saluggia municipalities, soil characteristics will be examined, considering that this area is highly representative and constitutes the main part of the rice cultivated land in the Vercelli province, with a significant pedological variability.

In the Vercellese area rice-growing covers a fluvioglacial plain, which is attributed to Riss and Wurm glaciations (adapted from Arduino et Al., 1983 and from Ajmone Marsan et Al., 1988). In this area, water streams, caused by fusion of glaciers, originated large and flat fluvioglacial cones, which constitute the level of the present main plain. Soils show a significant variability, caused by the fact that some areas have escaped alluvial events since a long time, while others are still (like those close to Elvo river) subjected to periodic submersion, or were subjected until rather recent periods. Furthermore, some remnants of ancient terraces and fluvial river beds increase pedological variability.

This area has been classified according to the land use capability, an international classification of soil suitability to agro-forestry uses, which defines eight classes of capability with increasing limitations to cropping from the first to the eighth (Klingebiel and Montgomery, 1961). Land use capability is a term which intends soil aptitude to give shelter to cultivated and spontaneous plant species. In the Vercelli province rice crop soils range from the first to the fourth class of land capability (Table1); therefore from very fertile soil, without or nearly without limitations and suitable for a wide selection of crops, to soils subjected to several limitations, which are suitable to a few crops and demand a particularly careful management. On the other hand, some soils are subjected to limitations (slow internal drainage, texture with silty topsoils on clayey subsoils) which are unfavourable to several crops, but favourable to rice growing.

Table 1 Main characteristics of the land use capability classes which can be found in the Vercellese rice crop soils (adapted from IPLA, 1982)

Land Capability Class	
I Land with very minor or no physical limitations to use	Soils are usually deep, well-drained, with good reserves of moisture or with suitable access for roots to moisture; never subjected to damaging floods; they are either well-supplied with plant nutrients or responsive to fertilisers. Sites are level or gently sloping and climate favourable. Drainage intervents can be locally necessary. A wide range of crops can be grown.
II Land with minor limitations that reduce the choice of crops and interfere with cultivations	Limitations may include, singly or in combination, the effects of 1) moderate or imperfect drainage, 2) less than ideal rooting depth, 3) slightly unfavourable soil structure and texture, 4) slight previous erosion, 5) periodical damaging floods. The climate is favourable to a wide range of crops.
III Land with moderate limitations that restrict the choice of crops and/or demand careful management	Limitations may result from the effects of one or more of the following: 1) imperfect or poor drainage, 2) restrictions in rooting depth, 3) unfavourable structure and texture, 4) moderate slopes, 5) moderate to strong previous erosion, 6) frequent damaging floods 7) moderately unfavourable climate. The limitations affect the timing of cultivations and range of crops; whilst good yields are possible limitations are more difficult to overcome.
IV Land with moderately severe limitations that restrict the choice of crops and/or require very careful management practices	Limitations are due to the effects of one or more of the following : 1) poor drainage difficult to remedy, 2) shallow and/or very stony soils, 3) moderately to strongly steep gradients, 4) strong vulnerability to hydric erosion, 5) moderately severe climate. Climatic disadvantages combine with other limitations to restrict the choice and yield of crops and increase risks.

Materials and methods

In order to examine soil characteristics in the area included among Vercelli, Santhià and Saluggia, the main available sources of information were singled out and consulted; the present document should therefore be considered a survey of soil knowledge in Piemonte, with regard to Vercellese rice production area.

The interested area was chosen on the basis of bibliographic information regarding the presence of rice in Piemonte; the area among Vercelli, Santhià and Saluggia appeared to be particularly important and representative for rice production; successively, the area was delimited by mean of modern GIS (Geographical Information Systems) softwares and the raster CTR (Regional Technical Cartography) was used as first layer. Once delimited the area, the following soil information was overlaid:

a) Soil Map of Piemonte (scale 1:250.000)
b) Soil Potential Protection for Groundwaters Map (scale 1:250.000)
c) Land Use Capability Map (scale 1:250.000)
d) Georeferred soil chemical analysis data from the BDRTA (Regional Database of Agricultural Soil)

A comparison between the information at points a), b) and c) was carried out; it allowed to produce an overall description of Vercellese soils, from the point of view of geomorphology, agricultural use and environment protection. Afterwards, soil chemical analysis were subjected to a descriptive statistic study, grouping them on the basis of the soil types they were related to and observing the values frequencies for several significant agrochemical quantities in different soil types. The following agrochemical parameters were considered: soil reaction (pH), phosphorous assimilable by plants, organic matter, C/N rate, Cation Exchange Capacity. The statistical analysis results were then discussed taking into account the effects of agronomic management and the information provided by the other cartographic layers.

Results and discussion

Pedological characteristics

A pedological study on the area was carried out by I.P.L.A. (Istituto per le Piante da Legno e l'Ambiente), at a 1:50.000 scale, as a study window used by a methodological approach for the production of the Soil Map of Piemonte at the 1:250.000 scale. The soil legend given by this map indicates the following soil types according to the Soil Taxonomy (USDA, 1999):

1. Along water streams, Fluvent can be found: they are formed by alluvial deposits (sands and gravel); the soil profile, which can be considered representative of this soil type, is located on the bed of Elvo (eventualmente indicare l'area di esondazione)torrent, in the northern part of the surveyed area. It's probably right to think that several characteristics attributed to the soils of the river bed of Elvo torrent can be considered as valid as for other areas very close to water streams, like Dora Baltea, Sesia and their tributaries. In these areas, which are periodically flooded, rice growing is sometimes present, although the land use cover can be also constituted by riparian vegetation, while poplar plantations are sporadic; the presence of rice in such a vulnerable environment should highlight the importance of research to improve the efficiency of crop management and to control the impact of agriculture on ecosystems Frequent floods, events that sometimes repeat within a very short period, strongly affect present soil characteristics, which don't show any evolution trend as a consequence of continuous deposits and removals of sediments. The soil profile shows frequently an alternation of sandy sediments and gravel; on soil surface a thicker or thinner layer, characterised by a high rate of organic matter content, can be observed. Organic matter accumulation in the first pedological layer is due to decomposition of vegetation residues and to organic fertilisation. There are essentially two pedological types which can be individuated in areas close to water streams: the first, very close to the stream, has a high content in gravel and is subjected to severe limitations for agricultural purposes; the second, more distant from the river, has a high content in sand and is currently used for agriculture. Textures are very coarse, rich in sand and gravel of medium size, soil reaction is sub-acid at the surface and shows a trend toward neutrality in deeper layers (IPLA, 2004).

2. In the plain area which extends from Vercelli to S.Germano Vercellese and Olcenengo, toward W and NW and in the zones of Ronsecco and Stroppiana, in the Southern part of Vercellese, Inceptisols (Anthraquic Eutrudept) can be observed. They are not very much evolved, with an alteration layer (cambic) more or less recognizable according to the pedogenetic development. These soils are situated on middle plains, currently not subjected to water flooding; the pedological survey attibutes them to the main plain and consider them similar to the Alfisols without limitations which can be found on the ancient terraces along the Elvo torrent. Vercellese main plain is formed by flat or slightly undulated surfaces formed by sandy sediments and gravels from the Dora Baltea river, when it flowed far northern than its current stream, originating from the morainic range situated further down the Viverone lake; secondarily, sediments were originated also by floods of Elvo torrent, but in areas very close to the water stream only. These areas, very far from the present river bed of Dora Baltea, are not subjected to floods of Elvo torrent since long time; consequently, soils show some evolution. Rice growing is the main factor affecting soil physical and chemical characteristics: water saturation in topsoil layers during several months causes evident iron reduction and a greyish colour (Antraquic pedoclimatic regime according to Soil Taxonomy). Considering this plain area, two main soil types are recognisable, characterised by different potentials for agriculture:

   Inceptisols (Anthraquic Eutrudept) is the most representative soil type in the area, with a loamy texture and sand more or less in the same amount as silt; gravel at 70 – 130 cm deep; soil reaction is acid in surface layers, directly affected by water saturation, showing a trend toward neutrality in deeper layers.

   Alfisoils can be found along the stream of Elvo torrent, on the hydrographic right of the basin, on a surface slightly raised on the main plain; these Alfisols (Anthraquic Hapludalf), more evolved than Inceptisols, are rich in silt and clay and without gravel till 150 – 200 cm below the surface; soil reaction is acid in surface layers, directly affected by water saturation, showing a trend toward neutrality in deeper layers.Within these soil typologies, in the plain of Cigliano – Livorno Ferraris – Santhià, soils are classified in the first Soil Capability class and can be considered without or nearly without limitations, suitable for a wide range of crops; these soils are formed by fluvial-glacial sediments and their surface is flat or slightly undulated. In Saluggia plain, soils are classified in the second Soil Capability class and show some moderate limitations, which affect crop yield: inadequate thickness (generally between 25 and 50 cm); excessive stoniness; fast internal drainage (which demands more frequent irrigations); these soils are formed by fluvial and fluvial-glacial sediments, their origin can be considered variable from relatively ancient to rather recent; their surface is flat or slightly undulated. In Lamporo – Ronsecco plain, soils are also classified in the second Soil Capability class and show some moderate limitations which affect crop yield: inadequate thickness (generally < 50 cm); excessive silt rate; slow internal drainage (which causes some delay in soil ploughing and harrowing after rains); hydromorphy caused by a water-table fluctuating between 30 and 90 cm of depth; they can be considered relatively recent alluvial sediments and their surface is flat (IPLA, 2004).

3. Montarolo and Tricerro fluvial-glacial terraces are typical Alfisols and they are classified in the third Soil Capability class. They are different from the previous typologies because they are terraces formed by ancient fluvial sediments, they are sub-flat or undulated and they show also more recent loess or alluvial covers; these soils are subject to some limitations which narrow the range of suitable crops and affect crop yield: inadequate thickness (generally between 25 and 50 cm); excessive silt rate at the surface

associated with deeper clayey or clayey – like layers; poorly or very poorly internal drainage; seasonal hydromorphy caused by a water-table fluctuating between the surface and 30 cm of depth. Frequently these soils are excessively moist even if drained and they have superficial compact layers, consequently, rooting is limited and seasonal water stagnation occurs; limitations to agriculture operations timing (IPLA, 2004).

4. A plain belt departing from Ivrea morainic amphitheatre, where the Viverone lake is situated, has been recognised as an ancient river bed of Dora Baltea; the track of this ancient course is very evident to the West of Santhià, were an elongated depression, a remnant of the old river course, can be observed, interrupting the flat plain surface. Further East this depression becomes less accentuated in the neighbourhood of Santhià, fading away into the rice growing area. Anyway, pedological assessments allowed to track and to map the whole ancient river course, thanks to the abundance in gravel at the soil surface; for ages the ancient river course has crossed S. Germano Vercellese, heading toward Vercelli, long time before their foundation. The abundance in gravel at the surface is at the same time the more evident characteristic of these Inceptisols (Anthraquic Eutrudept) and their main limitation; the abundance in skeleton causes fast drainage and wear machinery applied for soil tillage, which leads to classify these soils at least in the third Soil Capability class.Intensive rice growing has had a great influence on these soils: soils cultivated with rice show chemical and physical characteristics which are different in comparison with soils not subjected to periodic flooding; in rice growing area to the East of Santhià, soils, very abundant in gravel, show a grey-brown colour due to an evident reduction caused by periodic water saturation. Organic matter content is relatively high in connection with slower mineralization. Soil texture is abundant in sand and poor in clay; reaction tends to be acid in the waterlogged superficial layer and is close to neutrality in deeper layers. In the western part of the Vercellese plain, were rice growing is absent, soils are very abundant in gravel and show a brown colour; organic matter content is relatively high, texture is abundant in sand and poor in clay; reaction tends to be subacid in the topsoil and variable between subacid and neutral in deeper layers (IPLA, 2004).

5. Another particular soil typology (Inceptisols classified as Typic Endoaquept) is developed on a plain with resurgences; it's a surface flat or slightly undulated and lowered, formed by sandy sediments and gravel of Dora Baltea river, when it flowed further north, originating from the morainic range situated further down the Viverone lake. In this area resurgences and springs are very frequent: water pours from several important depressions interrupting the main surface, heading East, at first in natural channels, later in canals for rice irrigation. This area since a long time is not any more subjected to flooding by rivers and consequently soils show some evolution. Periodic soil flooding and the presence of a water-table close to soil surface influence water cycle causing a periodic water saturation at the surface and partially also in deeper layers; continuous presence of water is the first factor for soil evolution. Like in the main plain soils, this area is classified in the first Soil Capability class and had an intermediate evolution. Reaction tends to be acid at the surface and neutral in deeper layers; gravel is absent in the first meter of depth. Texture is loamy, with on average the same content of sand and silt. In comparison with soils of the main plain, drainage is lower: in fact, deeper layers are affected by water resurfacing from the water -table (IPLA, 2004).

6. Ancient terrace of Carisio, situated in the furthermost North-Western part of Vercellese plain, rises above the main plain for about 10 m and shows typical incisions and

undulations. Local soils, very ancient, can be defined Alfisols (Typic Fragiudalf); they are abundant in silt and clay and their profile shows a shift from a light to a red-orange colour, which is typical of soils that evolved in outmoded climates. Gravel is absent in the topsoil and can be found at a depth of some metres only. Reaction in the topsoil is acid or subacid and the texture is very rich in silt and very fine sand; deeper in the profile, layers are more rich in clay and poor in silt and sand and reaction tends more and more to subacid. Very altered gravel emerges in the incisions. Limitations to crop development are essentially similar to those regarding Montarolo and Tricerro terraces (IPLA, 2004).

7. In this overview of Vercellese soils it's ought to mention some soils, which probably originated from ancient fluvial-lacustrine or marsh sediments; their surface is flat and they are situated in a circumscribed area to the south of Carpeneto, in a small portion of plain to the north – east of Crescentino and in a third area to the south – East of Bianzè; they are classified in the fourth soil Capability Class and therefore are affected by several limitations which narrow the suitable crop range: texture excessively rich in silt and clay; very poorly obstructed internal drainage and considerable hydromorphy, caused by a permanent water-table fluctuating between the surface and 30 cm of depth (IPLA, 2004).

Soil potential protection for groundwaters

Soil pedological characteristics affect its potential protection of groundwaters from surface pollutants, which can derive from human activities; pedological characteristics were considered very important in a recent monitoring carried out by Regione Piemonte in order to define the areas more vulnerable to agricultural nitrogen pollution of groundwaters; the area situated between Santhià, Saluggia and Vercelli presents a Potential Protection that on average can be considered moderately low (IPLA, 2004). This classification is due to the presence of one or more of the following characteristics: presence of skeleton at a rate between 30 and 60 %, sandy - loam texture, presence of irreversible fissures in the topsoil, permanently reduced layers at a depth between 50 and 100 cm. Anyway, some areas should be considered apart from this definition.

- In areas close to river courses and in particular to Elvo, Sesia and their tributaries and also along the ancient river bed of Dora Baltea and in the sites of the ancient fluvial-lacustrine basins of Lamporo, soil presents a low Soil Potential Protection, due to one or more of the following characteristics: presence of skeleton at a rate greater than 60%; sandy – loam or sandy texture, presence of fissures in the topsoil or in the subsoil; layers permanently reduced until a depth of 50 cm.
- In ancient terraces of Montarolo and Carisio, soil presents high Soil Potential Protection due to absence of skeleton or little content of skeleton, texture from clayey loam to clay or loam, absence of reversible or irreversible fissures, absence of hydromorph layers till a depth of 150 cm.
- In a wide part of plain situated to the west of the river Sesia and between Vercelli, Santhià, Tronzano Vercellese and the ancient terrace of Carisio Soil Potential is moderately high, thanks to the presence of soils with the following characteristics: skeleton content between 16 and 35%, loam, loamy-silt, loamy-sandy-clay or clayey-sand, presence of reversible fissures in the topsoil, permanently reduced layers between 100 and 150 cm of depth.

Topsoil chemical pattern

From the point of view of soil chemical characteristics, the Regional Agrochemical Laboratory of Piemonte provided a large dataset of soil analysis, which have been carried out in rice fields of Vercellese between 1991 and 2003; this collection does not contain so many information about texture, which, on the other hand, has already been fully investigated during IPLA soil surveys; yet, it contains many georeferred data related to the most superficial layer, until about 30 cm of depth, which is interested by agronomic practices; these data regard soil reaction, cationic exchange capacity, exchangeable bases, assimilable phosphorus and organic matter. Data related to the most superficial layer, which is subjected to agronomic practices, cannot be considered totally representative of a soil, because they are affected to a certain extent by agronomic practices (adapted from Bourlot and Del Vecchio, 2002); in spite of this limitation, it has been decided to carry out a statistical analysis in two steps:

- Assigning each soil analysis to a soil typology, following their georeferentiation (Figure 1)
- Aggregating data for each soil typology and carrying out a statistical analysis; in fact soil chemical and physical characteristics, in particular parameters less affected by agronomic practices, are thought to be rather typical within each soil typology; to individuate precisely where the different soil typology are situated, the Piemonte Soil Map (scale 1:250.000) has been used.

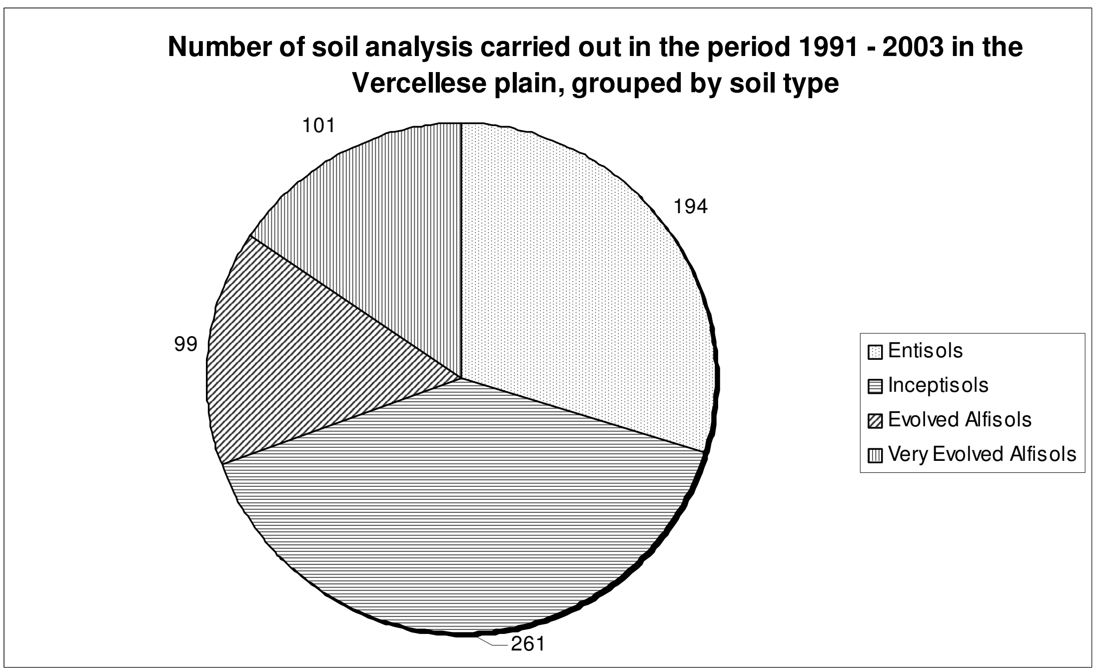

Figure 1 Soil analysis from the Regional Database of Agricultural Soils, grouped by soil type.

Soil types which have been considered in order to aggregate soil analysis data and to carry out the statistical analysis were: Entisols, Inceptisols, evolved Alfisols of ancient cones, very evolved Alfisols of ancient fluvioglacial terraces. Out of the available chemical information, four parameters have been chosen as particularly interesting form a pedologic and agronomic point of view, according to similar previous experiences carried out in Piemonte (Bourlot and Del Vecchio, 2002): soil reaction, organic matter content, cation exchange capacity and the rate of phosphorus that is thought to be assimilable by plants. On the basis of the statistical analysis and after a comparison with the pedological descriptions, the following considerations can be derived:

- Soil reaction is usually constant and not related to agronomic practices, with the exception of abundant and regular calcium additions; yet rice growing keeps soil in water saturation and reduction conditions, which according to the IPLA survey in the Vercellese plain caused a certain acidification. Monitoring of soil reaction data for different soil typology under rice allowed also to observe that very evolved Alfisols of ancient fluvial-glacial terraces are slightly more acid than other soil typologies. Soil analysis confirm an information which had already been achieved by IPLA by means of pedological assessments: on the whole, Vercellese rice soils can be considered sub-acid or acid (Figure 2).

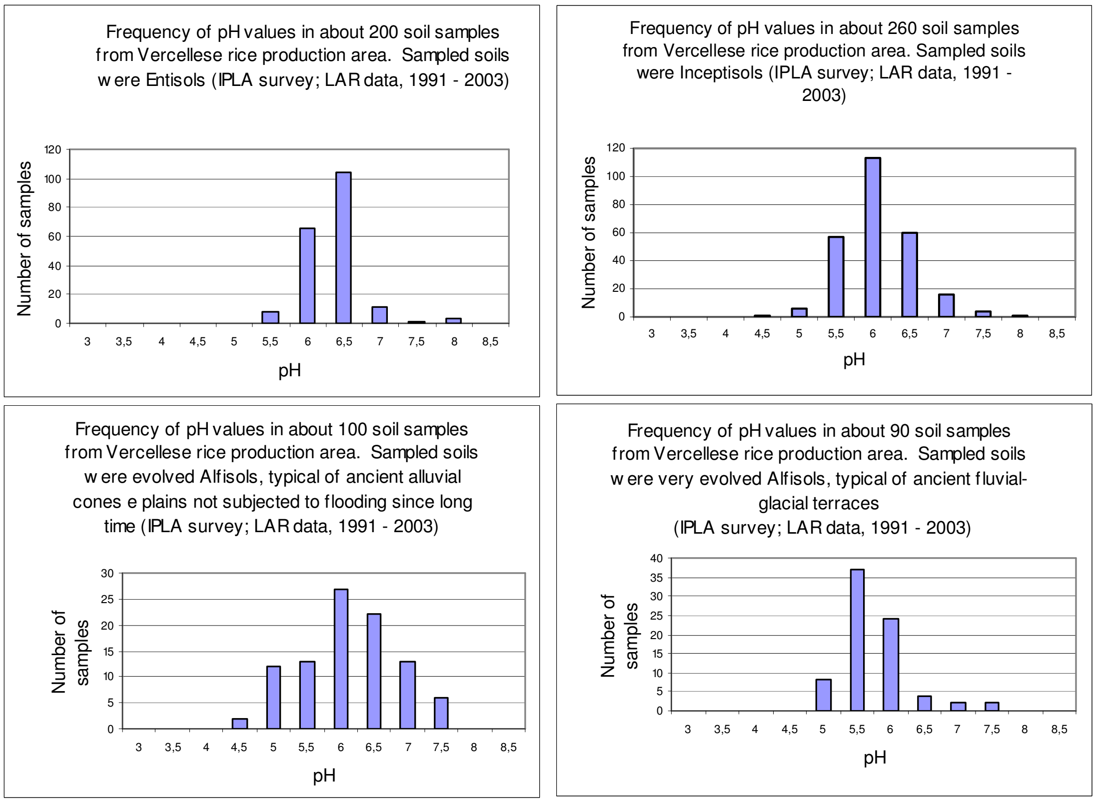

Figure 2 Frequency of pH values in different soil types of the Vercellese rice production area.

- Cation Exchange Capacity in Vercellese rice production area shows middle or middle-low values of about 10 – 12 meq/100g, with no great differentiations between different soil typologies. These values, rather common in agricultural conditions, can be considered as a result of both a high content of organic matter and a sandy or silt-sandy texture.
- Organic matter content in the Vercellese plain rice soils is fairly good, especially if compared to lower values which can be found in the alluvial plains of neighbouring Canavese area; a reason for this trend can be individuated, at least partially, in the fact that rice fields are subjected to particular conditions of prolonged submersion and partial anaerobiosis, which reduces mineralization processes and promotes accumulation of variably matured organic matter. C/N ratio, anyway, presents, on average, values less higher than 10, suggesting a fairly good organic matter maturation.
- Assimilable phosphorous rate in soils from Vercellese rice production area is definitely high, often too high, which can be attributed to intensive fertilization reiterated for several decades and to limited mobility of phosphorous through the soil.

Conclusions

Comparing different information sources on soil, which focus on different quantities and different points of view is a successful approach in order to understand better this natural resource and to improve land and crop management; in the case of rice, soil survey is even more necessary, to improve also water management and minimize the impact on the environment.

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CLIMATIC CHARACTERIZATION OF RICE CROP SYSTEMS IN THE NORTH-WEST ITALY

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Abstract

The climatic characterization of an agricultural area is a basic tool that can give important indications to farmers and agricultural advisors in order to apply the best and the most correct cultivation practices and obtain the best productive results. This study gives some historical and territorial informations about the climate of the agricultural area located in the north-west Italy, where a particular agro-ecosystem, the rice-growing system, has been developed.

Introduction

Agricultural territory in north-west of Italy presents a wide range of diversified environmental systems which originates from the great variability of its components, like territory, climate and vegetation.

Rural territories of the Vercellese and Novarese plain experienced a particular agricultural development, which led to rice production, that can be considered absolutely anomalous at these latitudes; anyway, thanks to human wits and to soil and climate characteristics this unusual crop system showed to be so efficient that it constitutes till today a model perfectly integrated in the modern agriculture.

Soil characteristics and the abundant water availability, once the regime optimised and the distribution carefully attended, made rice adaptation possible even under very different conditions from those regions where the crop originated.

Rice crop production area can be considered as an agro-ecosystem by itself, in the context of a diversified climatic system; this territory, in fact, extends from properly "Padana" plains to nearly piedmont areas, where proximity of alpine valleys affects also thermal-pluviometric characteristics. Rice field, as an agro-ecosystem, can also influence, within limits, climatic trends in the area; permanent submersion, traditionally practised, plays a role in modifying the air thermal-igrometric values.

Considering the available historic thermal-pluviometric data, it is now possible to describe the mean climatic characteristics of rice production area, carrying out a territorial representation of the main meteorological parameters.

Materials and methods

Considering the main meteorological parameters and carrying out data processing it's possible to perform correctly the climatic characterization of a territory. The more numerous the historical data series available, the more significant and stable are the considerations about meteorological trends and their evolution.

Such considerations can be carried out with regard to one or more sites on the territory. The second choice highlights trends and differences throughout the territory and in time; collected data and their elaboration can be successively displayed on thematic maps, using modern cartographic representation techniques.

Density of meteorological sites with long historical series of climatic data is usually too low to carry out a detailed, territorial analysis, although in some cases valuable and ancient databases are available. In fact, in agriculture the importance of methodical meteorological assessments has not been recognised until the ‘80s – ‘90s and consequently reliable data for detailed climatic characterization purposes are available since recent years only. Available historical series are probably not sufficient to provide informations for detailed considerations and to guarantee stability in time; anyway they can be considered very useful in order to analyze differences of the main meteorological quantities on the territory. On the basis of these considerations, the present study has been carried out.

Thanks to the activity carried out since 1987 by the technicians of the Agriculture Service of Vercelli Province, which carried out observations in collaboration with the Centre for defence against hail and atmospheric calamities of Vercelli Province, sufficiently precise and continuous climatic data are available for Vercellese rice production area.

Some meteorological stations, equipped with mechanic instruments, were in function during the whole year, while others during the farming period only. In spite of these differences, it is possible to collect several very interesting informations about the main meteorological parameters and related indexes for the period 1987 – 1998; the availability of this database allows to overtake the single assessment site point of view and to perform a territorial representation of phenomena. In order to carry out the present study, data until 1995 have been used; in fact, in this period it was possible to collect data from a greater number of meteorological stations, providing a better territorial coverage.

Since 1999 the Phytosanitary Service of Piemonte Region layed the foundations for a modern agrometeorological network, based on electronic stations continuously in function and equipped with information tele transmission. From this data source, which represents ideally a continuation of the first mechanical station network, information regarding the main meteorological parameters during the whole year can be collected, extending also territorial validity to Novarese rice production area. These data are now the basis to achieve new territorial representations, from the variable values and from indexes that can be elaborated; they allow also to carry out specific agrometeorological applications related to the crop development and to rice crop system. The territorial representativeness of meteorological stations allows also detailed and reliable studies regarding climatic risk, in particular referred to specific events, which characterised some years, affecting yields (BENINCASA et al. 2001).

Results and discussion

1. Thermal values

Rice production areas in Pianura Padana (the vast plain formed by the Po river) are characterised by a thermal minimum during winter, specially in January an February and a very high thermal maximum during summer, usually in July or August. (Figure 1).

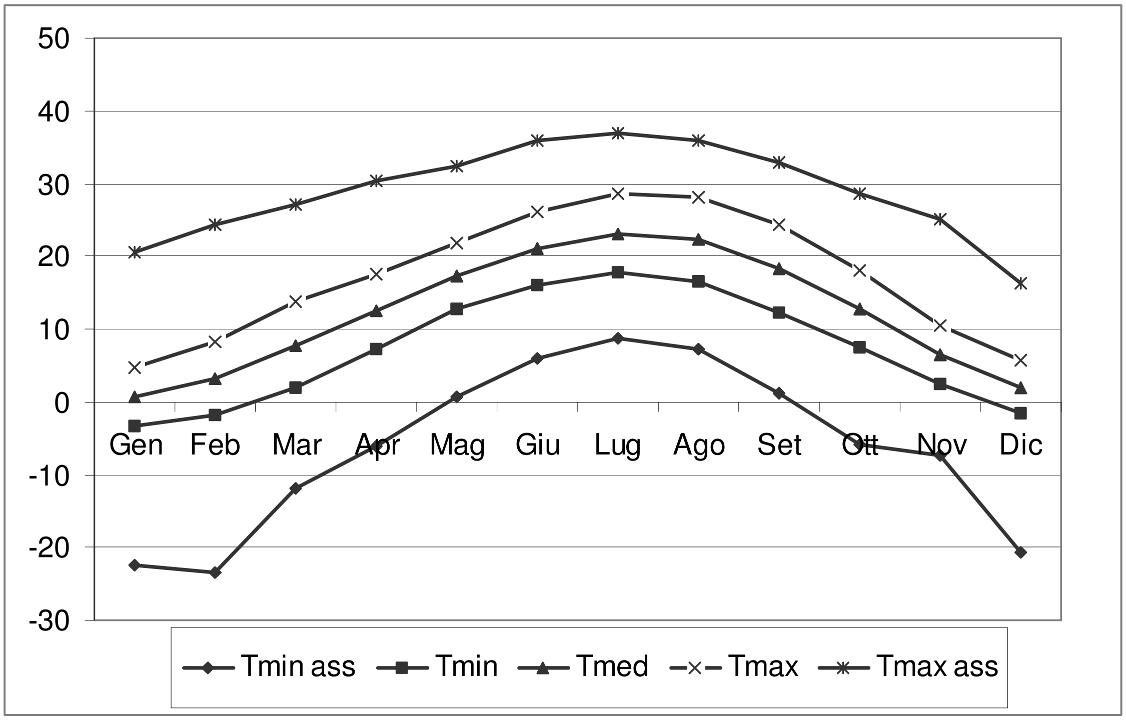

Figure 1 Vercelli – monthly temperatures 1932 – 2003.

Exceptional absolute maximum and minimum in June and December are, anyway, rather frequent. In these areas, all over the year, a minimum thermal mean of 7,8 °C and a maximum thermal mean of 19,0 °C can be recorded. Territorial distribution of minimum and middle values shows a net gradient toward north – west, from Vercellese and Novarese plains to piedmont areas.

Sharp thermal contrasts can occur during the farming season: drops in temperature can result from cold air streams or night irradiation or particularly significant storms during summer. Drops in temperature occurring during the first months of the growing season are not likely to damage rice seriously, thanks to the thermostatic function of flooding water, which protects seedlings from damages to vegetative organs.

Serious damages from flower sterility and caryopsis abort or poor filling up can result when, on the contrary, drops in temperature occur during particularly vulnerable growth stages, like flowering. This kind of thermal drop depends frequently on combined effect of cold advection and irradiation. Drops in temperature due solely to irradiation are usually well managed by the thermostatic function of flooding water.

The influence of flooding water affects also maximum thermal values. Evaporation from a free expanse of water and evapotraspiration from plant vegetative organs can reduce thermal daily values of some degrees, or some tenths of degree.

Thermal values were translated in growing degree days for rice growth, in order to compare different periods and sites. This index can be used to assess local thermal potentials and how they meet the crops needs (BACCI 1994). Growing degree days for the April – September period recorded values of about 1880 °C, varying from 1750 to 2020 °C depending on the assessment site. Thermal values show that southern areas have greater thermal potentials, while northern areas bordering the Biellese piedmont area and the vine growing hills have decreasing thermal potentials.

2. Precipitations

On average, about 976 mm of precipitations can be recorded yearly in the Vercellese and Novarese plains. Annual values, anyway, can vary on a wide range; in the short period considered from 1987 to 2003 very different amounts of precipitations were observed, from a minimum of 520 mm during the year 2003 to a maximum of over 1800 mm during the year 2002. These precipitations are scattered on average on 76 rainfall days. In the last years autumnal precipitations have been more intense than spring precipitations, as amount and also as number of events. Precipitations minimum occurs during the winter season, while during summer storms assure a sometimes consistent water supply, though frequently associated to hail storms. (Figure 2)

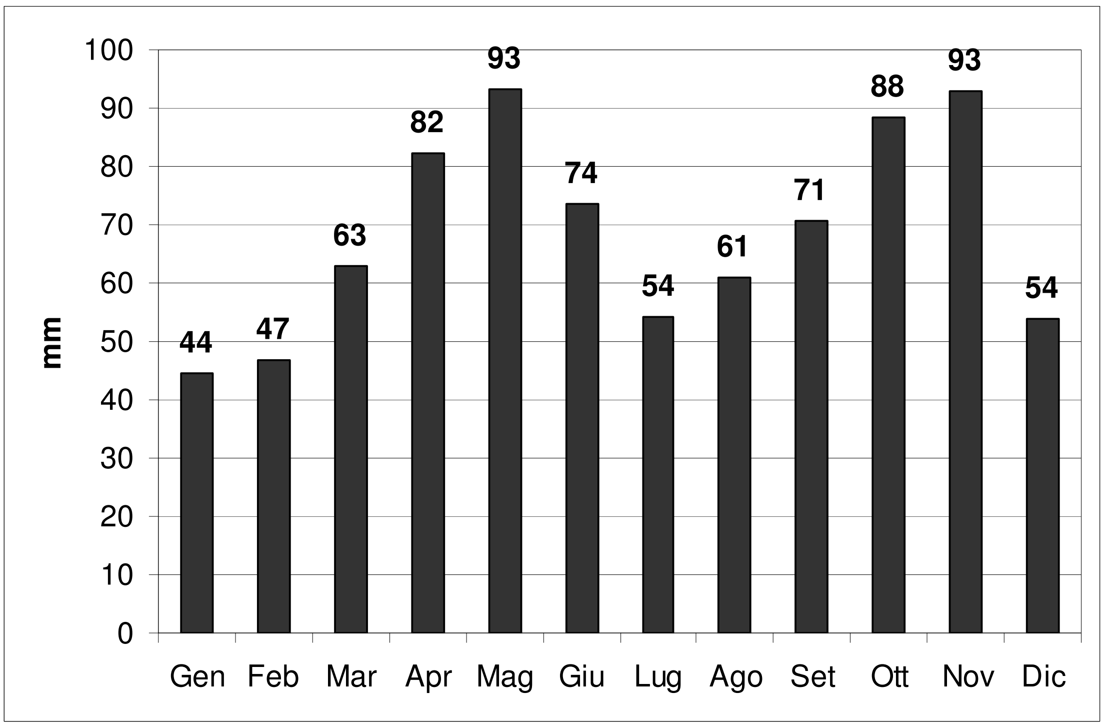

Figure 2 Vercelli - Monthly precipitations 1927-2003.

A gradient for precipitations can be observed passing from the south to the north, that is from the borders with the Asti, Alessandria and Torino Provinces to the vine-growing hills which mark the border between the plains and the alpine valleys. (Figure 3) The same trend can be observed also for the rainy days, though in a smaller proportion, suggesting that in these areas the amount of precipitations per event is greater.

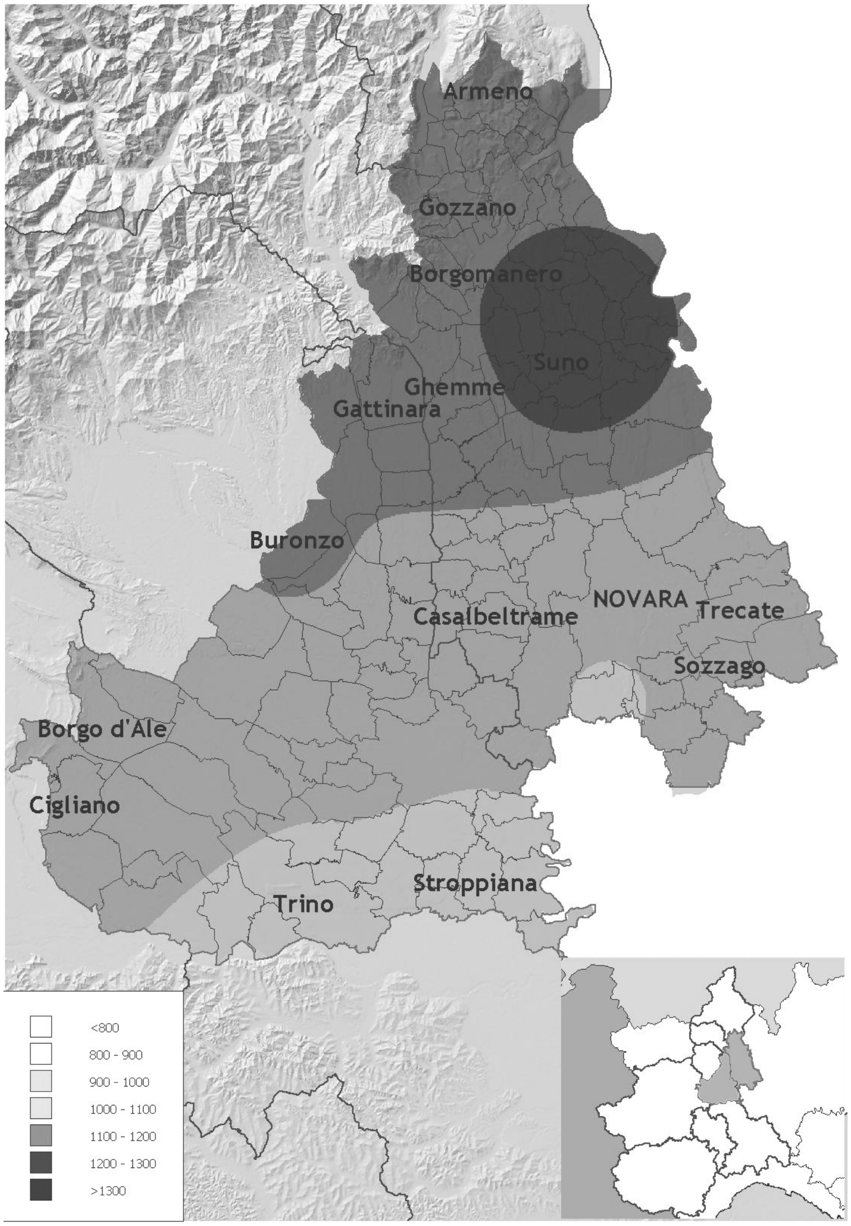

Figure 3 Precipitation gradient in the Vercelli and Novara Provinces

With regard to rice growing season, mean precipitations amount consists of 533 mm, scattered on average in 35 – 45 days depending on the assessment site; geographic gradients show the same trends observed on the whole year.

Conclusions

Potentials of an agricultural production area can be better understood carrying out studies on climatic conditions, from the point of view of their evolution in time and regarding the spatial distribution of meteorological phenomena. These researches can provide very useful and valuable information, in order to implement a rational use of the territory. A correct use of climatic data should also lead to set up new instruments, aimed at supporting farmers in improving a more efficient management of their crop systems (BOUMAN et. al. 2001)

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OUTLINE OF THE ITALIAN RICE FARM STRUCTURE

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The Italian rice area is mainly located in the Po Valley at the 45° N and is by 93% out of the total cultivated surface (220,000 ha) included in the Piedmont and Lombardia regions, in the north-west of the country. This area is caracterized by uniform climatic conditions and different types of soils ranging from sandy- to fine-textured.
The total rice production during the past ten years has been about 1.3 millions of tonnes of paddy, with an average yield of 6.2 – 6.5 t/ha.
Rice is not a staple food in Italy, even in the area where it is traditionally cultivated. Its consumption dropped from 20 kg during the 1920s to 5 kg/capita nowadays. Rice is mainly cultivated as a cash crop and 65% of the production is exported, mainly in the European area.
The choice of the varieties that has to be cultivated is usually taken according to the market request; usually every farmer grows several varieties to reduce the risks of cultivation.
Rougly all the Italian rice farms are equipped with their own grain drying plants and facilities to store the harvested grain. Paddy is usually sold dry and clean to the milling factory, all year round according to the economic convenience of each farmer.
The Italian rice cultivation system is characterized by two main elements: the reactivity to the market demand an the trend to the monoculture.
The spread of *indica*-type varieties, to be exported to the north European countries , is a good example of the reactivity to the market demand. In the last ten years the surface of the *indica*-type rice reached the 25% of the total cultivated area.
The mechanization of rougly all operations of rice cultivation and the spread of monoculture led to the increase of the average farm dimensions. This trend is still continuing, driven by the reduction of the crop profitability, the increase of the working capacity per ha and the cost of the machinery.

Table 1 Rice area and percentage on total surface of farms grouped in classes in relation to the rice cultivated area per farm, in 1983-2003 period (From Ente Nazionale Risi, 2004).

Year	< 10 ha		11-25 ha		26-100 ha		> 100		Mean	Total
	ha	%	ha	%	ha	%	ha	%	ha	ha
1983	17038	9,2	32116	17,4	93796	50,9	41328	22,4	20,9	184278
1985	15255	8,1	29976	16	98352	52,5	43603	23,3	22,7	187187
1990	12625	5,9	29176	13,5	113987	52,9	59653	27,7	28,8	215442
1995	9045	3,8	25581	10,7	125132	52,3	79501	33,2	37	239256
2000	6228	2,8	19453	8,8	120627	54,7	72969	33,1	43,3	220348
2003	5559	2,5	17752	8	118512	53,9	78164	35,5	45,6	219986

The data reported in Table 1 show that, during the last twenty years, the total area cultivated with rice increased by about 20%, while the number of farms dramatically diminished (-54%). In the same period the mean surface per farm raised from 20,8 ha to 45,6 ha.
It is also interesting to point out that the surface within the class of the farms with dimensions ranging from 26 to 100 ha, is 53 ha and within the class with more than100 ha is 157 ha. Currently about 90% of rice harvested in Italy is cultivated in farms with more than 50 ha..

During the last 20 years, the number of farms with less than 10 ha and of those with 11-25 ha diminished by 78% and 46%, respectively (Table 2), and the contribution to the total rice surface of the same type of farms went down from 9.2 to 2.5% and from 17.4 to 8%, respectively (Table 1). The farms with a size ranging from 26 to 100 and those with more than 100 ha increased in the last 20 years their number by 14 and 79%, respectively, with a raise of the contribution to the total surface of 3 and 13%, respectively. The number of farms with an average surface lower than 25 ha rapidly decreased in the last 20 years, as most of them were annexed to larger farms.

The full rice market liberalisation, that will be toroughly into force in the EU starting from 2009 (Ferrero & Nguyen, 2004), will speed up the trend to increase the dimensions of the farm, as it is estimated that only the farms with more than 150 ha will be economically sustainable.

Table 2 Number of farms grouped in classes in relation to the rice cultivated area per farm and their variations, in 1983-2003 period (From Ente Nazionale Risi, 2004).

Year	< 10 ha		11-25 ha		26-100 ha		> 101 ha		Total
	No.	Variation %	No.	Variation %	No.	Variation %	No.	Variation %	No.
1983	4681	0	1933	0	1940	0	277	0	8831
1985	4074	-13	1816	-6	2048	5	297	7	8235
1990	3023	-36	1750	-10	2317	11	391	41	7481
1995	1937	-59	1538	-21	2475	13	515	86	6465
2000	1239	-74	1181	-39	2312	12	473	71	5206
2003	1054	-78	1044	-46	2223	14	497	79	4818

Farm size is in general related to the dimension of the area that can be cultivated by a single worker. The complete mechanization of all cultural operations and the efficient chemical weed control reduced the request of labour to 20-25 h/ha. The area managed by a single worker can range from 40 to 60 ha and the farm size is then usually a multiple of that value.

The level of mechanization and cultivation technology applied in a small farm is not much different from that applied in a big farm, because of the high he cost of labour and shortage of manpower.

Italian rice farmers have on average a good education level. Many farmers, aged from 25 to 45, are graduated. The good returns obtained from rice cultivation during the period ranging 1970s to the 1990s encouraged the young farmers to continue their parent activity.

In these conditions the introduction of technical innovations have been remakably facilitated. In the past ten years, for example, there has been a wide spread of many newly introduced equipments, such as the laser controlled levelers for basin grading, the plants for grain cooling in bins or storage facilities, to avoid insect damages in summer.

Certain rice varieties (e.g. Carnaroli, Vialone nano) had a good appreciation on local markets , thanks to the promotion of their quality, through the attribution of DOP (Appellation of Protected Origin) and the direct selling of rice by the farmers, who directly process their production in the farm with small rice milling plants.

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THE CONTRIBUTION OF THE GEOGRAPHICAL PERSPECTIVE TO THE STUDY OF RICE CULTIVATION AND CULTURE

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Introduction

Geographical studies can contribute to the study of rice cultivation and of the related cultures. Though rice is a nearly universal food, in each country one can observe different systems of cultivation, different uses of the product, different cultural expressions related to rice. The spatial component, at different scales and from different points of view, is at the center of the geographers' attention. Many are the fields of research that are related to the geographical approach.

The study of rice within the "geographies of taste"

The "geographies of taste" vary in time and space, are related to cultural differences, and are the result of their long-lasting interaction (Atkins et al., 2001). In this perspective, one can think at ethnic restaurants, which are now common in Italy, and also to the so-called processes of "creolization" of Italian cuisine in foreign countries. The French geographer Jean Robert Pitte, in his famous work (2001) significantly entitled *La géographie du goût, entre mondialisation et enracinement local*, thoroughly analyses the relationships between nutrition, environment, and cultures; he also studies the alimentary aspects of globalization and their effects on the standardization of food consumption. The author considers the taste (geography of alimentary consumption) as strictly associated to the diversities that enliven the territory, both in the past and in the present (Millstone et al., 2003). The geographer Douglas Porteous, in his book *Landscapes of the Mind. Worlds of Sense and Metaphor* (1990) explained how alimentary experience contributes to shape our perception of the world. In this perspective, geographers can give their valuable contribution to the understanding of how the regional identity has been shaped by food and by alimentary traditions. As David Bell and Gill Valentine, paraphrasing the traditional saying "we are what we eat", write in their book *Consuming Geographies* (1997): "we are where we eat". The place and modes of consumption strongly contribute to shape the "*genius loci*" of a place and the sense of belonging of its inhabitants (Griffiths et al., 1998).

The identification and definition of "rice districts"

A "rice district" can be defined as a geographical area whose economic life and/or social structure and/or cultural life are characterized by and structured upon the cultivation of rice. It is important to identify the extension, the geographical characteristics and the population distribution in each rice district. Both quantitative data (about the area, the crops, the techniques adopted in rice growing) and qualitative data (physical and human landscapes associated to rice, role of rice cultivation in cultural identity, etc.) contribute to provide a multifaceted portrait of the area. The comparative study of rice districts, both within a country and in international perspective, is a useful tool to understand the specificities of each geographical context (Jackson, 2004). The same pattern of rice districts distribution can be seen at the European level and at the Italian national level. As in Europe there is a "productive core" (Italy, with 1,371,000 tons of production), accompanied by some secondary productive areas (Spain, Greece, France, Portugal, Hungary), in Italy the "rice triangle" made by the provinces of Pavia, Novara and Vercelli is surrounded by a series of secondary rice districts in the provinces of Alessandria and Biella (in Piedmont), Milan and Lodi (in Lombardy), Ferrara and Reggio Emilia (in Emilia-Romagna), Verona and Rovigo (in Veneto), Siena and Grosseto (in Tuscany), Oristano (in Sardinia). The large number of areas of cultivation creates a lively dialectical relationship between the local production and the commercial and cultural values associated to rice. The rice cultures of the areas of the above mentioned Italian provinces provide an interesting example of how an agricultural specialization (rice growing) has had several cultural outcomes (popular traditions, festivities, celebrations, literary descriptions, artistic works) which emphasized the regional identities and the sense of non-institutional, but rather economic, social and cultural "rice regions" within the larger context of the Italian nation.

Images and spaces of rice

Geographical studies have approached, with growing frequency in the last decades, immaterial dynamics of space, investigating the social and psychological constructions of spatial images. The discipline has become, for instance, more and more sensitive to the production of territorial images in advertising and publicity. The images that are conveyed through television commercials, together with newspapers or magazine ads, contribute to shape our ideas of the territories, be it the region in which we live or a far country we are dreaming to visit (Holmes, 1991). The role of geographically inspired images (landscapes photographs and paintings, maps) in the promotion of rice and its by-products is an important field of research for geographers. The presence (or absence) of the territory in the rice marketing helps to understand the way rice is conceived and sold as a product of a specific geographical environment (Nestle, 2002). Geography has also become more and more sensitive to the creation of "mental landscapes", of virtual images that affect our perception of the environment and our behaviors (the so called "geography of perception"; Geipel, et al., 1980). Rice cultivation and commercialization can play a role in the tourist as well as in the marketing promotion of the territory, at different scales (village, county, region, nation) and in different perspectives (tourist promotion, investment attraction, advertising, etc.). The presence (or absence) of images of rice in the land promotion helps to understand its visibility and its presence in the "geographical imagery" of a community. Tourism promotion is one of the most common ways in which a geographical imagery is conveyed to large masses of people. The tourist imagery is full of stereotypes and simplifications, but nevertheless it has a strong popular appeal. The study of the image of rice and rice districts in the tourist promotion is an important tool to verify the impact that this agricultural product has on the general geographical imagery associated to a region.

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Appendix: The Eu-India Project Rice_Net