Plant Biotechnology: Potential Impact for Improving Pest Management in European Agriculture

Rice – Herbicide-Tolerant Case Study
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Introduction

Rice probably reached the Mediterranean area after Alexander the Greats' expedition to Asia. Around the year 800, Arabs in Spain first grew rice. The Arabs and Spanish introduced it to the Italian peninsula, but the cultivated area remained limited until the 15^{th} century, when the crop underwent experiments within flooded areas of the Po Valley [26]. There is no clear information about when rice cultivation began in Greece, although it is known that it has been cultivated for the last two centuries [5]. The importance of rice as a food was very minor in Greece until after World War II. After then, rice cultivation became more intensive.

Four countries account for 97% of the European Union's production of rice: Italy, Greece, Spain and Portugal. Growers in these four countries produce 2.6 billion kilograms of rice worth €716 million on 395,000 hectares (see Table 1). The E.U. is 85-90% self-sufficient in rice.

Rice is grown in Italy, Spain, Greece, and Portugal, where a warm and dry growing season, characteristic of the Mediterranean climate, prevails. The normal growing season is from April/May to September/October depending on the temperature.

Rice is a unique crop in that, unlike all other field crops, it is grown in flooded fields. It is grown mostly on fine-textured poorly drained soils such as peat and clay soils and soils with impervious hardpans. The low water permeability of these soils enhances water use efficiency and provides standing water conditions that rice requires.

Rice planting in Europe involves direct seeding into flooded (water seeding) or dry soil (dry seeding). In water seeding, the pre-germinated seed is dropped directly from a centrifugal fertilizer spreader or a low altitude aircraft into flooded fields. In dry seeding, seed is drilled into dry ground and then the field is gradually flooded once plants develop two leaves. With both planting methods the floodwater is maintained until harvest. Water is drained from the field prior to harvest so the fields can dry and harvest equipment can pass through. Transplanting the seedlings into flooded fields has been abandoned in Europe since the 1960s, in view of enormous labor demand and drudgery associated with this practice [2].

Water seeding is the most predominant planting method in Europe. While water seeding is the only planting method used in Greece [5], Italy [15], and Spain [8], rice is both water and dry-seeded in Portugal [3]. Dry seeding is gaining popularity in Italy (10% planted currently) in recent years to overcome the problems of poor seedling germination and development due to low water temperature and aquatic pests associated with water seeding. Moreover, the flexibility to apply pre-emergence herbicides at the time of planting has made dry seeding an appealing technique in Europe, due to savings in time and labor. A major disadvantage to dry seeding is the increased level of weed problems. Whereas dry seeding promotes weed seed germination, water seeding prevents the germination of buried red rice and other weed seeds due to unavailability of oxygen for germination under flooded conditions. Since rice is pre-germinated and sits on top of the soil where there is a thin layer of oxygen, germination and emergence are not of concern in flooded fields, which hamper the germination of weeds.

Weed Problems in European Rice

Weeds are the most significant of the pest problems that affect European rice production. Prior to the development of herbicides, weeding was done by hand. In May, the rice fields had to be weeded to prevent the young rice from being choked by other vegetation. Hundreds of women known as *le mondine*, or weeders, arrived from all parts of Italy to perform the delicate task of rooting out the weeds while leaving the young rice in place [30].

Le mondine have become a nostalgic memory, immortalized by the famous film *Riso Amaro,* or 'Bitter Rice', featuring a young Silvana Mangano. It was a hard life for *le mondine,* living together in dormitories housing hundreds of women far from their homes. They had to work bent double, up to their knees in water under a blazing sun. They were bitten by mosquitoes, and the water teemed with wildlife. They worked in rows, moving backwards, controlled by an overseer who sat high on a chair like a tennis umpire. When the women approached the end of a field, the overseer called out for the central girls to move out in order to leave a space for the frogs (which had been driven back as they progressed down the field) to escape. It is said that the water seemed to boil as all the wildlife made a mass exit. As the women weeded they sang. One of the songs, *Bella Ciao,* was adopted by the Italian Communist Party to express the social injustice of the system [30].

Prior to 1965, the most significant weed, barnyardgrass, was controlled in Greece by maintaining a high water level in the rice fields and by removing the weed by hand. This method was then replaced by the herbicides molinate and propanil [5]. A key factor leading to adoption of herbicides by rice growers was a reduction in the availability of agricultural labor, which took place after 1960 [2].

A survey of rice weeds in Italy showed that barnyardgrass is the most frequent weed followed by water plantain, sea club-rush, and duck salad [16]. Other weeds of minor importance in Italian rice production are narrow-leaved water plantain, flowering rush, various sedges, red rice, rice cutgrass, water foxtail, and European water clover. Whereas red rice is an important general problem in Spanish rice production, barnyardgrass and sedge species are the most significant weeds. Weeds common to rice fields in Greece include barnyardgrass, red rice, bulrush species, variable flat sedge, galingale, typha, Canada thistle and water finger grass [5][11]. While barnyardgrass, red rice, and Erva-serra or the perennial rice cut grass are the most frequent and damaging species in Portuguese rice fields, perennial knotgrass, sedges, water plantain, arrowhead, common duckweed, purple ammania, and false pimpernel are less frequent in their occurrence [3].

In general, barnyardgrass and red rice are the most predominant and serious weeds in Europe [2][4][5][16]. Barnyardgrass is the world's principal weed of rice and is a perennial problem in Greece, Italy, Portugal, and Spain. Barnyardgrass plants look very similar to rice from seedling to flowering stage and has similar ecological preferences as rice. Moreover, the intensive handweeding practices prior to the introduction of herbicides led to the evolution of barnyardgrass biotypes that mimic rice plants, as a result of which they escaped control measures.

The replacement of rice transplant by direct seeding offered weeds more possibilities for germination and growth. For example, in Spain and Italy, red rice is becoming an acute problem and a major production constraint due to the recent use of dry seeding methods. The use of red rice contaminated seed lots and introduction of less competitive semi dwarf rice varieties has further contributed to the increase in red rice infestations [17]. Among the 14 weeds of importance to Italian rice production, red rice ranked tenth in significance in 1988 [16]. However, by 1995, the frequency of red rice had increased dramatically in Italy. Currently, red rice infests about 70% of the rice acreage in Italy and 30% of the rice acreage in Greece [11][17].

Botanically, red rice is the same genus and species as cultivated rice and therefore is difficult to distinguish from rice until tillering. Characteristics that make red rice a weed are its prolonged period of dormancy, germination over a number of years, greater competitiveness, higher growth than cultivated rice plants, brittle seed, and red color of the seed coat. The red seed coat layer can be removed during milling process with an extra milling. As a result, commercial grain will be broken and grade is reduced. Chemical control of red rice is difficult as selective herbicides that do not cause injury to rice are not currently available.

One uncontrolled red rice plant can produce 1,500 seeds in one season, and this can result in 2.25 million seeds during the next season [12].

Weed control is critical in rice production as uncontrolled infestations cause both yield and quality impacts. In Greece, full-season interference of barnyardgrass at 10 plants/m^2 was reported to reduce rice yields by 20 to 30% [11][13]. Red rice at a density of 40plants/m^2 reduced rice yields between 63 to 79%, depending on the variety [12]. The presence of red rice in harvested cultivated rice reduces the commercial value due to the undesirable color of red rice and the difficulty in separating the two types of seed

Without weed control, rice crop losses were reported to be as high as 92% in Italy [27]. On average in Europe, the potential reduction in rice yields due to uncontrolled weeds has been estimated at 55-60% [20].

<u>Rice Weed Management</u>

Herbicide choice and weed management strategies in rice differ with the planting method (dry seeding vs. water seeding). Weed problems are more severe and weed control is more crucial in dry seeding as a lag period exists between planting and flood establishment. Thus, unlike other crops, weed management in rice is complex and is a common constraint shared by all European rice producing regions.

Unlike other row crops such as maize or soybean, inter-row cultivation for weed control is not feasible in rice as the crop is planted in narrow rows due to drilling or broadcasting. Rice varieties that are currently being used are not very competitive against weed infestations, especially against barnyardgrass [4]. Cultural tactics such as crop rotation and water seeding and mechanical methods such as plowing and harrowing are used routinely to control weeds such as red rice. Soil salinity and a shallow water table limit the use of rotations in countries such as Spain [8]. Though weed problems are less severe in water seeding compared to dry seeding, water seeding does not provide complete control of red rice and other weeds as a few seeds are always present on the soil surface where they can germinate through water, as noted in Italy and Spain [8][11]. Moreover, water seeding is not an effective tool for the control of aquatic weeds such as duck salad and rushes. An alternative technique being used by Italian rice growers involves delaying the planting of rice until red rice germinates so it can be controlled with chemicals such as dalapon [17]. A disadvantage to delayed planting is the shortening of growing season and consequent yield loss. Chemical control is the most commonly used and reliable method for controlling weeds in European rice.

Rice herbicides are classified as preplant (PP), preemergence (PRE), or postemergence (POST) based on when the applications are made. Popular preplant rice herbicides are molinate and thiobencarb. Molinate is used in both dryland and water seeded systems while thiobencarb is used in water-seeded rice only. Besides applications as PP, molinate can be used up to 3 to 4 leaf stage of rice [3][15]. Molinate and thiobencarb are predominantly grass herbicides with excellent activity on barnyardgrass and some red rice suppression but limited activity against cutgrass, water finger grass, rushes, and some sedges [9][10]. Molinate and thiobencarb are active up to 8 and 20 days after application, respectively.

Available preemergence herbicides include, pendimethalin, molinate, and thiobencarb. Quinclorac controls annual grasses and some broadleaf weeds. Quinclorac and thiobencarb provide longer control of barnyardgrass and other weeds compared to molinate [14]. Pendimethalin, which is applied to dryland seeed systems, controls annual grasses and broadleaf weeds such as lambsquarters and pigweeds.

Common postemergence rice herbicides used in Europe are amidachlor, propanil, pretilachlor, thiobencarb, molinate, cyhalofop, fenoxaprop, azimsulfuron, quinclorac, bentazon, bensulfuron, cinosulfuron, propanil, and 2,4-D [1][5][9][10][14]. These herbicides are sometimes mixed in various combinations to achieve a broader range of weed control, depending on weed spectrum. Propanil is effective on barnyardgrass (1 to 4 leaf stage) and many other grasses and broadleaf weeds in rice fields. Pretilachlor and quinclorac were introduced for rice weed control at the same time, mainly to control barnyardgrass. Pretilachlor is effective against grasses, sedges, and broadleaf weeds. Cyhalofop is the newest barnyardgrass herbicide in Europe [14]. Azimsulfuron is a POST herbicide used for both grass and broadleaf weed control. Bentazon, bensulfuron, cinosulfuron, propanil, and 2,4-D provide broadleaf and sedge control [9][10].

Herbicide treatments for control of barnyardgrass doubled rice yields in Italian experiments [25]. In experiments in Greece, control of barnyardgrass led to a four-fold increase in rice yields [14].

Traditionally, weed control in European rice has been achieved by using two herbicide applications, one timed between planting and 3-leaf stage of crop and the second between 20 and 60 days after planting when the field is usually drained to ensure thorough coverage of weeds with the herbicide [1][3][10][14][15][22][23]. The first application is aimed at the control of grass weeds such as barnyardgrass and red rice, with which rice has to compete severely for resources. The second application is mainly aimed at control of sedges and broadleaf weeds [10]. Application of molinate as PP, PRE or early POST followed by a POST application of bentazon is the standard treatment for rice weed management in Spain and Portugal [10]. Herbicide programs in Spain use molinate, thiobencarb, amidachlor or fenoxaprop if red rice is the key problem [8]. While molinate is the most widely used herbicide, molinate plus bensulfuron is the most popular herbicide combination in Portugal [3].

Historically, Italian rice growers made two to three herbicide applications for rice weed management. The first application involved the use of molinate (applied from preplanting to 3 or 4 leaf stage) aimed at barnyardgrass control, followed by a tankmix of propanil plus MCPA or propanil plus bensulfuron to control escaped barnyardgrass and to provide broadleaf weed and sedge control [1]. A third application of bentazon was sometimes used for broadleaf weed control as needed. In recent years, with the increase in the duck salad problem, the program has been changed to include a preplant application of oxadiazon at 0.3 kg ai/ha, followed by a combination of molinate (3.75 kg ai/ha) plus bensulfuron or cinosulfuron (both at 0.070 kg ai/ha), or two applications of propanil at 4 kg/ha each about 20 days after planting [22]. In some cases, growers apply azimsulfuron alone for the second treatment. A third application with propanil may be needed to prevent contamination of rice by weed seeds such as barnyardgrass. In areas where red rice is a serious problem, use of dalapon at 12 kg ai/ha or pretilachlor at 0.63 kg ai/ha prior to planting is the norm as none of the standard herbicide programs provide selective red rice control. About 50% of the rice acreage in Italy receives dalapon applications for red rice control. Overall, Italian rice growers apply 1.6 to 16 kg ai/ha and spend €150/ha to €250/ha for weed management [21].

A common treatment in Italy is to prepare the seedbed and stimulate weed growth with an early flood. This practice is known as a ‘stale seedbed’ or ‘false-seeding’. Weed seedlings are then killed with cycloxydim, glufosinate, glyphosate, or dalapon. A few days after the treatment, the field is planted [27]. In stale seedbeds, glyphosate applications reduced the red rice seed bank by 100 % in the top 5cm of soil, by 80% in the 5-10cm of soil, and by 85% in the 15-20cm of soil [28].

The rice herbicide program in Greece involves the use of molinate as PRE followed by an early POST application of propanil plus MCPA [22]. Molinate is applied to flooded fields, while propanil is applied when fields are drained. Dispersion of molinate occurs through water currents facilitated by wind [14]. This program is widely used in areas where barnyardgrass has not developed resistance to propanil. Rice growers apply 14.5 kg of herbicide ingredients at a cost of €130 per hectare in this approach. In fields where propanil-resistant barnyardgrass exists, the alternative strategy is to apply molinate (PRE) followed by a POST treatment with either quinclorac + azimsulfuron or cyhalofop. Typically, rice growers spend €200 to €220 per hectare for 4 kg of ingredients when herbicide-resistant weeds are a problem [22].

Even at high herbicide use rates, control of certain weeds in European rice, red rice in particular, has never been completely adequate with the use of available herbicides [3][8].

Repeated use of same herbicides in rice fields year after year has led to the development of herbicide-resistant weeds in Europe (Table 2). Five weed species have been reported to be resistant to various herbicides thus far. The first case of resistance in rice weeds was documented in 1992 in Spain in barnyardgrass to quinclorac [6]. Subsequently, resistance to acetolactate synthase enzyme-inhibiting herbicides such as bensulfuron, cinosulfuron, and azimsulfuron was reported in common water plantain, rice field bulrush, sea clubrush, and smallflower umbrella plant from all the key rice producing European countries. While ALS-resistant water plantain acres are stabilizing, rice field bulrush infested acreage has been rising in recent years in Italy. More recently, a propanil-resistant barnyardgrass biotype has been reported from Greece and Italy [11][19]. About 10% of rice acreage in Portugal is infested with bensulfuron-resistant weeds [29].

Combinations of herbicides have been recommended and are being used as tools to enhance the control of resistant weeds and to delay the development of herbicide resistance in susceptible plants. However, control of herbicide-resistant weeds has been difficult due to lack of effective control with the registered chemical alternatives. Non-selective herbicides such as glufosinate or glyphosate provide broad-spectrum weed control and, due to their alternative mode of action, could help in controlling weeds that have developed resistance to the traditional rice herbicides.

Weed control represented 5% of the total rice production costs in Greece fifteen years ago compared to the current 10% [5]. The increase in production costs may be attributed to weed resistance and expensive herbicide alternatives. For example, many Greek growers are facing €70-90/ha higher weed control costs due to herbicide-resistant barnyardgrass [22]. Some growers in Greece have been using propanil at two or three times their previous rates because of resistance problems in controlling barnyardgrass [11].

At the present time, most farmers are successfully managing herbicide-resistant populations with chemical solutions such as pre-sowing applications and increased postemergence treatments [18].

Transgenic Herbicide-Tolerant Rice

Two types of transgenic herbicide-tolerant rice are in development. Transgenic glufosinate-tolerant or LibertyLink rice has been developed by Aventis CropScience and glyphosate-tolerant or RoundupReady rice was developed by Monsanto. Liberty Link rice withstands the application of the non-selective herbicide, glufosinate while Roundup Ready rice can tolerate the applications of glyphosate. Herbicide-tolerant rice has not been approved for planting in the E.U. or the U.S.

Research in the U.S. suggested that single or sequential applications of glufosinate provided excellent control of red rice, barnyardgrass, and broadleaf signalgrass [32][33]. Researchers have reported excellent control (90% or greater) of 3 to 4 leaf red rice from sequential glufosinate rates as low as 0.27 lb ai/a compared to a single application of 1 lb ai/a [7]. In the U.S., it has been estimated that herbicide tolerant rice would lower weed control costs by $16/a [34].

The efficacy of POST non-selective herbicides glyphosate and glufosinate as PRE applications (to avoid injury on non-transformed rice) for red rice control was researched in Greece [12]. Both the herbicides provided control superior to conventional herbicides even under severe red rice pressure. No reports exist to document field evaluations of herbicide-tolerant rice in Europe.

Experiments in Italy have utilized glyphosate in a wiping bar to the top of red rice plants above the height of the rice crop. The glyphosate application resulted in a germination decrease of more than 90% [28].

Impacts

A major potential impact of herbicide-tolerant rice in the E.U. would be a reduction in overall herbicide use. Since glufosinate and glyphosate are broad-spectrum in activity, no additional herbicides are needed.

A recent report estimated that herbicide-tolerant rice varieties would be economically advantageous for planting on 35% of the E.U.'s rice acreage due to improved weed control and/or lower costs of weed control [24].

The substitution of two applications of glyphosate for the current herbicide use in rice production would lower herbicide use by 83% (see Table 3).

In Greece, Spain, and Portugal, the substitution of two applications of glyphosate plus a technology fee of €50 per hectare would lower weed control costs by 50%, while in Italy, the reduction in costs would be 58% (see Table 4).

It is assumed that the biotech varieties would be planted on 35% of the E.U.'s rice area, implying an overall reduction in herbicide use of 1.2 million kg and an annual overall savings of €13.5 million/yr.

Since no experiments have been conducted in Europe with the herbicide-tolerant biotech rice, it is assumed that yields would be unchanged.

Table 1a: Rice Production			
	Area (000 HA)	***Production (million kg)***	***Value (€ million)***
Greece	20	147	41.2
Italy	218	1273	356.4
Portugal	25	147	45.6
Spain	113	888	248.6
Total E.U.	395	2557	716.0
U.S.	1326	9785	925.1

Source: [31]

Table 1b: Rice Production			
	Area (000 A)	***Production (million Lbs)***	***Value ($ million)***
Greece	50	323	41.2
Italy	545	2801	356.4
Portugal	63	323	45.6
Spain	283	1954	248.6
Total E.U.	988	5625	716.0
U.S.	3314	21527	925.1

Dollars and Euros assumed equivalent

Table 2: Cases of Herbicide Resistance in Rice Weeds in Europe			
Herbicide	*Weed*	*Country*	*Year resistance noted*
ALS inhibitors (bensulfuron-methyl, cinosulfuron)	Common water plantain	Italy	1994
ALS inhibitors (bensulfuron-methyl)	Common water plantain	Portugal	1995
ALS inhibitors (bensulfuron-methyl)	Common water plantain	Spain	2000
ALS inhibitors (azimsulfuron, bensulfuron-methyl, cinosulfuron, ethoxysulfuron)	Rice field bulrush	Italy	1994
ALS inhibitors	Sea clubrush	Spain	1997
ALS inhibitors	Small flower umbrella plant	Spain	1997
Propanil	Barnyardgrass	Greece, Italy	2000
Quinclorac	Barnyardgrass	Spain	1992

Source: [6] [18] [19]

Table 3: Potential Impact of Glyphosate-Tolerant Rice on Herbicide Use						
	Acreage (000 ha)	***Rate (Kg ai/ha)***		***Total herbicide use (million Kg)***		
		Current	*Biotech*	*Current*	*Biotech*	*Change*
Greece	20	14.50	1.92	290	38	-252
Italy	218	8.50	1.92	1,853	418	-1,435
Portugal	25	15.62	1.92	390	48	-342
Spain	113	15.62	1.92	1,765	217	-1,548
Total	395			4,298	721	-3,577

Source: [21] [22]

100 % adoption assumed.

Portugal and Spain assumed to use the average of Italy and Greece. It is assumed that two applications of glyphosate at 0.96 kg ai/ha each would replace conventional herbicide programs in all countries.

Table 4: Potential Impact of Glyphosate-Tolerant Rice on Weed Control Costs					
	Acreage (000 ha)	***Weed Control Costs (Euros/ha)***		***Savings (Euros/ha)***	***Total Savings (million Euros/yr)***
		Conventional	*Biotech*		
Greece	20	170	85	85	1.7
Italy	218	200	85	115	25.1
Portugal	25	170	85	85	2.1
Spain	113	170	85	85	9.6
Total	395				38.5

100% adoption assumed.

Herbicide costs were calculated based on [21] [22]. Portugal and Spain are assumed to have costs identical to Greece. Assumptions made for weed control calculations with glyphosate-tolerant rice: Cost of glyphosate = €18 per kg (1.92 x 18 = 34.56); Technology fee = €50 per ha. It is assumed that two POST applications of glyphosate at 0.96 kg ai/ha each would replace 2 applications of conventional herbicides.

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