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POTENTIAL FOR GLUFOSINATE AS A SELECTIVE HERBICIDE FOR RED RICE CONTROL IN BAR-TRANSFORMED RICE

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

by

Sujatha Sankula
B.S., Andhra Pradesh Agricultural University, 1989
M.S., Andhra Pradesh Agricultural University, 1991
May 1997

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TABLE OF CONTENTS

ACKNOWLEDGMENTS .......................................... ii
LIST OF TABLES ........................................ vii
LIST OF FIGURES ........................................ ix
ABSTRACT ................................................ x

CHAPTER

1. INTRODUCTION ......................................... 1
   LITERATURE CITED .................................... 14

2. EVALUATION OF GLUFOSINATE HERBICIDE ON RICE (Oryza sativa L.) TRANSFORMED WITH THE BAR GENE AND ON NON-TRANSFORMED RED RICE (Oryza sativa L.).
   Introduction ......................................... 21
   Materials and Methods ................................ 24
   Results ad Discussion ................................ 28
   Literature Cited ..................................... 39

3. RESPONSE OF BAR TRANSFORMED RICE (oryza sativa L.) AND RED RICE (Oryza sativa L.) TO GLUFOSINATE APPLICATION TIMING.
   Introduction ......................................... 42
   Materials and Methods ................................ 44
   Results and Discussion ................................ 48
   Literature Cited ..................................... 55

4. GLUFOSINATE RESISTANT BAR TRANSFORMED RICE (Oryza sativa L.) AND RED RICE (Oryza sativa L.) RESPONSE TO GLUFOSINATE ALONE AND IN MIXTURES.
   Introduction ......................................... 58
   Materials and Methods ................................ 60
   Results and Discussion ................................ 63
   Literature Cited ..................................... 70

5. CROSS RESISTANCE OF GLUFOSINATE RESISTANT RICE (Oryza sativa L.) AND BASELINE RESISTANCE OF RICE CULTIVARS TO GLUFOSINATE.

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Introduction .........................................100
Materials and Methods ...............................104
Results and Discussion .............................109
Literature Cited ....................................119

7. SUMMARY .............................................123

VITA ....................................................127
LIST OF TABLES

2.1. BAR-transformed and non-transformed commercial rice varieties evaluated for response to applications of the herbicide glufosinate ................................25

2.2. Effect of glufosinate on transgenic Gulfmont and Koshihikari rice lines and commercial Gulfmont and Koshihikari rice varieties 21 DAT in Crowley,LA ..........29

2.3. Effect of glufosinate on grain yield of transgenic Gulfmont and Koshihikari rice lines expressed as a percent of their non-treated control .........................32

2.4. Red rice control 21 DAT as affected by flooding and glufosinate rates in the field .........................34

2.5. Red rice control in the greenhouse as affected by glufosinate rates and flooding depth 21 DAT ..........37

3.1. Injury and plant heights of BAR-transformed rice 14 DAT as affected by application timing of 2.2 kg ai/ha of glufosinate .........................................................48

3.2. Yield of BAR-transformed Gulfmont rice treated with glufosinate at 2.2 kg/ha as influenced by application timing.................................................................50

3.3. Red rice control 14 DAT as influenced by application timing of glufosinate at various rates...............52

4.1. Effects of glufosinate alone and in combination with other herbicides on injury, plant height, and yield of BAR transformed rice..................................................64

4.2. Red rice control, plant heights, panicle maturity and 100-seed weight as affected by glufosinate alone and in combination with other herbicides .........................68

5.1. Effect of trifluralin and metolachor applied preplant incorporated on injury to BAR-transformed Gulfmont rice, non-transformed Gulfmont rice, and red rice 8 WAT ......84

5.2. Effect of glyphosate, sulfoate, paraquat, and imazethapyr applied post-emergence on injury of BAR transformed Gulfmont rice, non-transformed Gulfmont rice, and red rice 4WAT .................................85

vii
5.3. Effect of trifluralin and metolachlor applied preplant incorporated on plant heights of BAR-transformed Gulfmont rice, non-transformed Gulfmont rice, and red rice 7 WAT ..........................................................87

5.4. Regression equations, correlation coefficients, and calculated I₅₀ values (50% visual injury 21 DAT) for U.S., Japanese, and red rice genotypes .................................89

5.5. Ammonia accumulation of commercial Gulfmont and Koshihikari rice varieties 5 DAT as a measure of baseline resistance compared to other rice cultivars ..........94

6.1. Segregation of glufosinate resistance in four F₂ populations based on glufosinate dip test and chi-squared probabilities for fit to single gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA .........................114

6.2. Segregation of glufosinate resistance in four F₂ populations based on glufosinate spray test and chi-squared probabilities for fit to single gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA ...............115

6.3. Segregation of glufosinate resistance in four F₂ populations based on ammonia assay and chi-squared probabilities for fit to single gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA .......................117
LIST OF FIGURES

6.1. Ammonia accumulation 4 and 8 days after treatment (DAT) in glufosinate tolerant F₁ plants in comparison to transformed and non-transformed parents and red rice ... 110

6.2. Frequency distribution of percent rice injury from glufosinate spray test of F₂ population resulted from crosses between transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RxTK) 113
ABSTRACT

A three year study was conducted to evaluate the herbicide glufosinate on BAR-transformed rice and on the weed red rice. Preliminary evaluations with 1.1 and 2.2 kg/ha glufosinate on the BAR-transformed Gulfmont and Koshihikari rice varieties showed that both were resistant, but there was more injury to Koshihikari. Glufosinate at 2.2 kg ai/ha injured BAR transformed Gulfmont rice more when applied to the 1- to 2- leaf stage (23-26%) than when applied to 3- to 4- leaf (13-19%) plants. The damage was least with boot stage applications (3-14%). Phytotoxicity to BAR transformed Gulfmont rice was greater when 2.2 kg/ha glufosinate was combined with 0.4 kg/ha triclopyr (59%) or 0.6 kg/ha acifluorfen (22%) than with glufosinate alone. Single applications of 1.1 kg/ha glufosinate to the 3- to 4- leaf stage of non-transformed red rice resulted in greater control (91%) than applications at the panicle initiation (74%) or boot stages (77%). Glufosinate efficacy was reduced when red rice was submerged in water to 25 to 50% of its height at application. Red rice was controlled 92% with either 3.4 kg/ha propanil or 0.6 kg/ha acifluorfen mixed with 0.6 kg/ha glufosinate, which was greater than for glufosinate alone and other tested combinations.
Greenhouse studies on cross resistance of BAR-transformed Gulfmont rice in comparison to non-transformed Gulfmont rice and red rice showed that injury due to metolachlor, trifluralin, glyphosate, sulfosate, paraquat, and imazethapyr was similar on all rice types. In baseline resistance studies, \( I_{50} \) values for visual injury of non-transformed Gulfmont and Koshihikari were 0.13 and 0.06 kg/ha, respectively. Ammonia accumulation was greater in Koshihikari than Gulfmont rice. Reciprocal crosses were made in the greenhouse between the BAR-transformed Gulfmont and Koshihikari varieties and non-transformed red rice, to assess the inheritance of the transgene. \( F_2 \) populations segregated as a ratio of 3 (resistant) : 1 (susceptible), confirming that the glufosinate resistance gene was inserted into a single chromosomal locus or closely linked loci.
CHAPTER 1
INTRODUCTION

Red rice is a noxious problem weed in Louisiana rice fields. Both red rice and commercial rice are forms of Oryza sativa L. Red rice differs from commercial rice in that the dehulled red rice seed is deep red to pink when compared with the light brown bran color of commercial rice (Hoagland and Paul 1978). At tillering and later growth stages, it can be distinguished from commercial rice by its pubescent, light green leaves, profuse tillering, taller and later maturing growth habit, long slender panicles, heavy shattering of grain and soft brittle grain that causes reduction in milling yields (Dodson 1898; Smith et al. 1977).

Red rice causes an estimated $50 million loss each year in the rice producing states of the southern United States (Smith 1979). Rice yield reductions from season-long interference of as high as 82% were reported in Arkansas (Diarra et al 1985). Four red rice plants per square meter caused an economic loss equivalent to about 20% of the potential value of a red rice free rice crop (Navarro 1985). Potentially damaging densities of red rice should not be permitted to compete for longer than 60 days after rice emergence if optimum grain yields are to be
realized (Kwon et al. 1991). Red rice densities of 1 to 3 plants/m² are adequate threshold infestations for implementing control practices to prevent yield and quality losses of rice grain (Smith 1988).

Prior to 1960, no specific research was conducted to document the impact of red rice on rice production. Even though red rice was widely known to pose problems in rice production, the consensus was that no easy solutions existed, nor could they be expected (Dunand 1988). Although there are still no easy solutions, methodology to reduce the severity of the problem is available.

Conventional tillage is the traditional system used for land preparation in rice. In southwest Louisiana, rice fields are generally tilled under flooded conditions. Conventional tillage followed by a continuous flooding after rice seeding has been shown to help keep red rice under partial control and is one of the reasons rice grown in this area is water-seeded more than drill-seeded (Bollich and Feagley 1994). Rice production in reduced tillage systems started gaining popularity in Louisiana in 1975¹ with a sharp increase in acreage in 1994. A limitation for this cropping system is the lack of many

registered herbicides for use in no-till rice (Bollich and Sanders 1993).

Apart from reduced tillage, several herbicides including glyphosate, glufosinate, sulfoate, paraquat, and thiobencarb used with different application timings have been used for red rice control but all of them resulted in only partial success (Anonymous 1994; Anonymous 1996; Dunand and Baker 1994; Hill 1978; Parker and Dean 1976; Smith 1981). Rice rotation with soybeans is another approach to reduce red rice populations in the rice/soybean rotation cycle (Griffin et al. 1986; Khodayari et al. 1987). As there is a maturity differential between commercial rice and red rice, a growth regulator like maleic hydrazide can be used to control red rice which may result in eventual depletion of the red rice seed bank in soil (Irwin 1996). However, no completely satisfactory means for red rice control is available so far. The use of transgenic rice has been the latest approach to alleviate the red rice problem (Braverman and Linscombe 1994).

Cosmetically modifying plants for resistance to broad spectrum herbicides would allow their selective use for crop protection (De Block 1987). Two approaches have been followed in order to achieve this goal. In the first, a mutant form of the target enzyme is produced, which is still active but less sensitive to the herbicide. In this
way, mutant plants producing the enzyme acetolactate synthase have been selected for resistance to sulfonylurea and imidazolinone herbicides (Chaleff and Ray 1984; Shaner and Anderson 1985). The second approach involves overproduction of the target enzyme. This has been demonstrated in the overproduction of 5-enolpyruvylshikimate-3 phosphate synthase that conferred glyphosate tolerance in transgenic petunia plants (Shah et al. 1986). An alternative strategy to engineer herbicide resistance in plants is expressing an enzyme that detoxifies herbicides. This concept has been used in the expression of the bialaphos resistance (BAR\(^2\)) gene in transgenic tobacco, tomato, and potato plants conferring resistance to the herbicide bialaphos and phosphinothricin (De Block et al. 1987). Similar techniques were used to transgenically alter rice to contain the BAR gene for glufosinate resistance (Christou et al. 1991).

Bialaphos is a tripeptide antibiotic produced by *Streptomyces hygroscopicus* (De Block et al. 1987). It consists of phosphinothricin (PPT), an analogue of L-glutamic acid, and two alanine residues. Upon removal of these residues by peptidases, PPT is a potential inhibitor of glutamine synthetase (GS) (E.C. 6.3.1.2). This enzyme

\(^2\)BAR, bialaphos resistance; GUS, galacturonidase; Hm, hygromycin; PAT, phosphinothricin acetyl transferase.
plays a key role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants (Skokut et al. 1978). GS is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, aminoacid degradation, and photorespiration. Inhibition of GS by PPT causes rapid accumulation of ammonia which leads to the death of plant cell (Tachibana et al. 1986). PPT is chemically synthesized (Basta®, glufosinate, Hoechst AG) while bialaphos is produced by fermentation of Streptomyces hygroscopicus (herbiace®, Meijia Seika Ltd.). The BAR gene, which is involved in the bialaphos biosynthesis pathway encodes a phosphinothricin acetyl transferase (PAT²), which acetylates the free NH₂ group of PPT and thereby prevents autotoxicity in the producing organism (Murakami et al. 1986).

Glufosinate, a phytotoxic metabolite of bialaphos, is a non-selective herbicide effective against a wide range of weeds (Blackshaw 1989; Tachibana et al. 1986). It acts more slowly than paraquat, but faster than glyphosate in controlling weeds (Tachibana and Kaneko 1986). Genetically engineered glufosinate resistant rice offers the possibility of selective control of red rice in commercial rice fields (Braverman and Linscombe 1994). The commercial rice varieties, 'Gulfmont' and 'Koshihikari' were
genetically transformed to contain the BAR gene for glufosinate resistance. The transgenic rice lines possess two vector plasmids, pWRG4517 and pWRG2426. Plasmid pWRG4517 contained the BAR gene for glufosinate resistance and the Hygromycin gene (Hm²) that confers resistance to the antibiotic hygromycin. The plasmid construct pWRG2426 had the galacturonidase (GUS²) gene in addition to the above two genes (Agracetus Inc. 1991).

Application of POST herbicides at 3- to 4- leaf stage or tillering stage is a widely followed practice in rice weed management, especially for red rice control (Kwon et al. 1991). However, there are no postemergence herbicides that would provide adequate control of red rice. Red rice control in fields is further complicated due to germination and emergence of red rice over a longer period of time than cultivated rice. When flood water is drained off fields before herbicide application, some lower sections in field may still have standing water, or may collect water after a rain. Based on red rice height and water depth, this may leave some red rice covered either partially or totally with water, which may reduce the ability of a herbicide to control red rice.

Pantone and Baker (1992) reported that the tolerance of rice to bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy] acetic
acid) was dependent on growth stage. Rice is tolerant to 2,4-D [(2,4-dichlorophenoxy)acetic acid] during the late tillering to early jointing stages, but may be severely injured prior to tillering or in the boot stage (Smith et al. 1977). While previous research noted the tolerance of BAR-transformed rice to glufosinate (Braverman and Linscombe 1993; Braverman and Linscombe 1994), there has not been any information on how BAR-transformed rice and non-transformed red rice would respond to application of glufosinate at different growth stages.

Historically, glufosinate has been used to control annual and perennial weeds in noncrop land areas and as a non-selective contact type herbicide prior to crop emergence in minimum tillage systems (Haas and Muller 1987). The efficacy of glufosinate in controlling grasses and other weeds may be enhanced by combining it with other herbicides. Although glufosinate controls a wide spectrum of broad leaf-weeds, it is less effective on grasses (Anonymous 1993). Thus, supplemental grass control may be necessary. Combinations of contact herbicides with other herbicides having foliar and soil residual activity can enhance initial weed control, provide residual weed control, and reduce the number of trips across the field (Bruce and Kells 1990; Bruff and Shaw 1992a, 1992b; Lanie et al. 1994). Combining herbicides also may be beneficial
because this practice tends to delay the appearance of resistant weed biotypes, which is an important threat in monocultures (Hatzios and Penner 1985).

Deep water may reduce the effectiveness of glufosinate under flooded rice conditions. Weeds should be well above the flood water so that they will be exposed to glufosinate spray. Drained rice fields often are subject to standing water in lower sections especially after a rain. Therefore, other herbicides which have activity in the flood water may be needed for effective weed control. This is especially true with the control of aquatic weeds such as ducksalad [*Heteranthera limosa* (Sw.) Willd.] and alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], which are not controlled effectively with glufosinate in rice fields of Louisiana3.

Herbicide resistance is a heritable trait in the population, not a transient, phenotypic response to an environmental condition which might allow a plant to escape herbicide effects (LeBaron and Gressel 1982). Repeated use of the same herbicide or herbicides with the same mechanism of action will select for resistance in the population by killing the susceptible biotype while the resistant biotype will survive, reproduce, and pass the herbicide trait to

next generation (Gressel and Segel 1982). Rice is predominantly a self pollinated crop. But, chances of open pollination and hybridization still exist and can range from 1% in Lemont to over 50% in the Nortai variety (Langevin 1988). So, the possibility for the development of glufosinate resistant red rice exists if the BAR gene is transferred from a transformed commercial variety to red rice by outcrossing. Also, red rice characteristics can be transferred to transformed plants of a commercial variety by outcrossing with red rice pollen. Furthermore, glufosinate resistant rice as a volunteer crop could be a weed in another variety. Cross resistance of BAR transformed rice to other herbicides has not been investigated. If cross resistance exists, the magnitude of the problem of controlling glufosinate resistant red rice would escalate.

Cross resistance is the increased resistance to one herbicide as a result of a selection pressure from another herbicide and is more likely to occur if the herbicides possess a similar mode of action (Gressel 1979). There have been cases of cross resistance to herbicides of unrelated modes of action. Heap and Knight (1986) showed that diclofop methyl (±-2-[4(2,4-dichloro phenoxy) phenoxy]propanoic acid) resistant annual ryegrass (Lolium

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*rigidum* Lam.) was cross resistant to fluazifop butyl [(R)-2-[4-[[trifluoromethyl]-2-pyridinyl] oxy]phenoxy] propanoic acid], chlorsulfuron [2-chloro-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino]carbonyl]benzene sulfonamide] and DPX-T6376 (methyl 2-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl]amino] carbonyl] amino] sulfonyl] benzoate). This was the first report on cross resistance and these results were confirmed by Matthews et al. (1990). Similar results were reported by Smeda et al. (1992) that trifluralin resistant green foxtail (*Setaria viridis* (L.) Beauv) was resistant to the structurally unrelated herbicides DCPA (dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate) and terbutol (2,6-di-tert-butyl-p-tolyl methylcarbamate). In a soybean-rice rotation, which is the predominant cropping system in southwest Louisiana (Griffin and Robinson 1989), metolachlor and alachlor [2-chloro-2'-6'diethyl-N-methoxy methyl]acetamide] are commonly used to control red rice in soybeans (Griffin and Harger 1986; Khodayari et al. 1987). Control of red rice often depends upon the herbicides used in rotational soybean (Huey and Baldwin 1980). Acetanilides such as metolachlor (Griffin and Robinson 1989), dinitroanilines such as trifluralin, and imidazolinones such as imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinoline
carboxylic acid) are commonly used in the control of red rice in soybeans (Anonymous 1994; Khodayari et al. 1987). Therefore, it is important to determine if BAR-transformed rice is cross resistant to any herbicides used in a rotational soybean crop. In addition, no-till rice production involving burndown herbicides is becoming increasingly popular in Louisiana (Bollich and Sanders 1993). Glyphosate, paraquat and glufosinate are non-selective herbicides that give rapid burndown of preplant vegetation in rice (Mendt and Braverman 1995). Therefore, cross resistance to these burndown herbicides that have a similar use pattern is of major concern.

Another important aspect of cross resistance is negative cross resistance. Negative cross resistance occurs when herbicides, other than the one to which a plant biotype has developed resistance, become more toxic to the resistant biotype than to the susceptible biotype (Hall et al. 1996).

Earlier field studies on the evaluation of glufosinate on BAR-transformed Gulfmont and Koshihikari rice suggested that transformed Gulfmont was more resistant to 2.2 kg/ha glufosinate than transformed Koshihikari (Braverman and Linscombe 1994). Studies by Baker et al. (1988) and Griffin and Baker (1990), suggested that tolerance of different varieties of rice (Lemont, Tebonnet and Mars) was
different to fenaxoprop, \([±-2-(\text{-4-}(\text{-6-chloro-2-benzoxazolyl})\text {-oxy})\text {-phenoxyl})\text {-propanoic acid}]\) and that the Mars variety was particularly susceptible to fenaxoprop. Further, Pantone and Baker (1992) reported that Lemont rice was more susceptible to triclopyr than either Mars or Tebonnet. Thus, inherent varietal differences in resistance of non-transformed rice may be reflected in the differential response of BAR transformed Gulfmont and Koshihikari rice to glufosinate.

A major benefit from herbicide resistant crops is the opportunity for new strategies and or increased flexibility in the management of problem weeds (Wilcut et al. 1996). Herbicide resistant crops also facilitate the addition of conservation tillage crop production practices because of more effective post-emergence treatments (Wilcut et al. 1996). Development of crop cultivars with resistance to post-emergence herbicides will encourage crop producers to use economic weed threshold predictions in making their weed management decisions (Coble and Mortensen 1992). In addition, herbicide resistant crops will potentially allow the use of more environmentally benign herbicides and lower rates of herbicides than many soil applied herbicides (Burnside 1992; Knake 1992).

In contrast to these advantages, the main concern of introducing transgenic herbicide resistant crops into
agriculture is the spread of the engineered gene(s), particularly by pollen, to related weed species (Keeler 1989; Williamson 1991). A possible negative environmental impact of this sexual transfer of engineered genes to related wild plants by natural hybridization is the evolution of more aggressive weed genotypes based on a few gene polymorphisms (Keeler 1989; Hoffman 1990). Also, if crop-weed hybrid seeds were formed, and seeds were dormant (a trait often found in weeds), some hybrids would establish with the weed at a similar time and hybrids may continue to cross, leading to a stable introgression (Jorgensen et al. 1996). Furthermore, gene exchange between a crop and a weedy relative may increase the adaptability of the weed, making it even more weedy. This type of added adaptability was noticed in weeds like wild beets, Beta vulgaris (Boudry 1993), red rice, Oryza sativa (Arnold and Hodges 1995) and wild lettuce, Lactuca sativa (Williamson 1993). Rice is predominantly a self-pollinated crop. But, chances of cross-pollination and hybridization between rice and red rice still exist and can range from 1% in Lemont to over 50% in the Nortai variety (Langevin 1988). There are several reports of introgressive hybridization (the morphological convergence of weeds similar to crop plants) between rice and its weedy
relatives (Oka and Chang 1959; Morishima et al. 1961). So, the potential for the inheritance of the glufosinate resistance and creation of glufosinate resistant red rice exists if a transgene movement occurs. Herbicide resistance in many cases can be achieved by the transfer of a single gene (Schulz et al. 1990). Gene expression varies with genetic background. Epistasis, linkage, and pleiotropy are examples. Therefore, even if the BAR gene is inherited, it can be difficult to predict how the genetically engineered gene will be expressed in a related weed species (Colwell et al. 1985).

LITERATURE CITED


Bruce, J. A. and J. J. Kells. 1990. Horseweed (Conyza canadensis) control in no tillage soybeans (Glycine max) with preplant and preemergence herbicides. Weed Technol. 4:642-647.

Bruff, S. A. and D. R. Shaw. 1992a. Early season herbicide applications for weed control in stale seedbed soybean (Glycine max). Weed Technol. 6:36-44.


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Griffin, J. L., J. B. Baker, R. T. Dunand, and E. A. Sonnier. 1986. Red rice control in rice and soybeans in

Griffin, J. L. and T. R. Harger. 1986. Red rice (Oryza sativa) and jungle rice (Echinochloa colo um) control in solid seeded soybeans (Glycine max). Weed Sci. 34:582-586.


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Navarro, M. A. 1985. Effect of stand density and composition between red rice and the cultivar 'Mars' under field conditions. M.S. Thesis. Louisiana State University, Baton Rouge, LA. p. III.


CHAPTER 2

EVALUATION OF GLUFOSINATE HERBICIDE ON RICE (Oryza sativa L.) TRANSFORMED WITH THE BAR GENE AND ON NON-TRANSFORMED RED RICE (Oryza sativa L.)

INTRODUCTION

Weed red rices were first documented in commercial rice in 1846 in North and South Carolina (Craigmiles 1978). Historically, red rice is the most difficult weed to control in Louisiana rice fields (Dodson 1900). Yield reductions from season-long competition with red rice can be as high as 82% (Diarra et al. 1985). The weediness of red rice is attributed to its competitiveness with cultivated rice (Diarra et al. 1985). In addition to contaminating the rice seeds, red rice has poor milling quality, shatters, and lodges, making commercial rice harvest difficult (Diarra et al. 1985).

The ability to control red rice in rice has always been a desired goal in U.S. rice production (Craigmiles 1978). Despite moderate successes in controlling red rice pre-emergence (Kwon et al. 1991; Smith 1981), efforts to control red rice postemergence have been unsuccessful. A promising alternative may be the creation of a herbicide resistant commercial rice through biotechnology. Recently, it has become possible to confer agronomically useful traits to crops by molecular transformation (Saito et al. 21).
1992). Herbicide-resistance traits are an important target of genetic engineering of crop plants and may offer an excellent opportunity for using postemergence herbicides such as glufosinate in rice (Droge et al. 1992).

Glufosinate was found to be the active phytotoxic metabolite of bialaphos, produced by the actinomycetes Streptomyces viridochromogenes and S. hygroscopicus. Synthetic glufosinate is used as a herbicide in orchards and as a preharvest desiccant (Duke and Lydon 1987; Kishore and Shah 1988). Glufosinate is effective against a wide range of weeds (Blackshaw 1989; Tachibana et al. 1986). It acts on weeds more slowly than paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) but faster than glyphosate [N-(phosphonomethyl)glycine] (Tachibana and Kaneko 1986). An adverse effect of using this herbicide in rice is its non-selective broad spectrum activity (Duke and Lydon 1987). Genetically engineered herbicide-resistant rice offers the possibility of direct application of glufosinate in rice for the selective control of red rice (Braverman and Linscombe 1994).

Recently the commercial rice cultivars, Gulfmont and Koshihikari have been altered by genetic engineering techniques to contain the BAR gene (Christou et al. 1991). The BAR gene, which is a part of the bialaphos biosynthesis...
pathway, encodes PAT and is used as an assayable marker gene (D'Halluin et al. 1992). Genetically engineered rice plants produce phosphinothricin acetyl transferase, PAT, which makes the plant resistant to glufosinate. Phosphinothricin, which is the active portion of the glufosinate molecule, is an inhibitor of glutamine synthetase and thus prevents incorporation of ammonia into amino acids. Inhibition of glutamine synthetase (E.C. 6.3.1.2) by glufosinate results in the accumulation of toxic levels of ammonia in plant cells. Acetylation of glufosinate at its free amino group by PAT disrupts glufosinate's inhibitory activity of glutamine synthetase, thus making the plant resistant to glufosinate (D'Halluin et al. 1992).

Application of herbicides at 3- to 4- leaf or tillering stage of rice growth is a widely followed practice in rice weed management. However, there are no postemergence herbicides that control red rice. Control of red rice is further complicated in fields due to germination and emergence over a longer period of time than cultivated rice. Water management is an important tool in reducing red rice emergence. Drained fields may still have lower sections with standing water, or may collect water after a rain. Depending on red rice height and water depth, this may leave some red rice partially or totally
covered with water. The influence of water on the ability to control red rice with glufosinate is not known. Thus, the main objectives of this study were to evaluate glufosinate resistance of transgenic lines transformed with the BAR gene and red rice efficacy with glufosinate under flooded and non-flooded conditions.

**MATERIALS & METHODS**

**Transgenic rice studies.** Transgenic rice lines were evaluated in 1993, 1994, and 1995 on the main unit of the Rice Research Station, Crowley, La, on a Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualf) soil. Fifteen transgenic rice lines including six derived from the transformation of Gulfmont and nine derived from the transformation of Koshihikari rice and non-transgenic Gulfmont and Koshihikari were evaluated (Table 2.1). The transgenic lines possessed two vector plasmids, pWRG4517, and pWRG2426. Plasmid pWRG4517 contained BAR gene for glufosinate resistance and Hm gene, which confers resistance to hygromycin (Agracetus Inc. 1991). The pWRG2426 construct consisted of the GUS gene in addition to the above two genes (Agracetus Inc. 1991). In the transgenic rice studies, plot size was 3.7 by 1.4 meters.
Table 2.1. BAR-transformed and non-transformed commercial varieties evaluated for response to applications of the herbicide glufosinate.

<table>
<thead>
<tr>
<th>Entry number</th>
<th>Line number</th>
<th>Parent cultivar</th>
<th>Vector plasmid</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>517-1-R1</td>
<td>Gulfmont</td>
<td>pWRG4517</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>517-2-R1</td>
<td>Gulfmont</td>
<td>pWRG4517</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>517-3-R1</td>
<td>Gulfmont</td>
<td>pWRG4517</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>517-5-R1</td>
<td>Gulfmont</td>
<td>pWRG4517</td>
<td>1-2</td>
</tr>
<tr>
<td>5</td>
<td>517-7-R1</td>
<td>Gulfmont</td>
<td>pWRG4517</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>526-1</td>
<td>Gulfmont</td>
<td>pWRG2426</td>
<td>&lt;2</td>
</tr>
<tr>
<td>7</td>
<td>495-1-R1</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>495-1-R2</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>496-1-R1</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>&gt;10</td>
</tr>
<tr>
<td>10</td>
<td>496-1-R2</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>&gt;10</td>
</tr>
<tr>
<td>11</td>
<td>496-2-R1</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>1-2</td>
</tr>
<tr>
<td>12</td>
<td>496-3-R1</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>3-4</td>
</tr>
<tr>
<td>13</td>
<td>496-3-R2</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>4-6</td>
</tr>
<tr>
<td>14</td>
<td>496-4-R1</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>4-6</td>
</tr>
<tr>
<td>15</td>
<td>496-4-R2</td>
<td>Koshihikari</td>
<td>pWRG2426</td>
<td>4-6</td>
</tr>
<tr>
<td>16</td>
<td>Gulfmont</td>
<td>(Non-transgenic)</td>
<td>pWRG4517</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Koshihikari</td>
<td>(Non-transgenic)</td>
<td>pWRG2426</td>
<td></td>
</tr>
</tbody>
</table>
with seven rows. At the 3- to 4-leaf stage, rice lines were sprayed with glufosinate at 0, 1.1 and 2.2 kg ai/ha. The field was not flooded until 2 days after glufosinate application. The experimental design was a split-plot with herbicide rate as main plots and rice lines as sub-plots. The data were subjected to analysis of variance (ANOVA) to estimate main effects of year, rice line, and glufosinate rate and interactions between main effects. When interactions were not significant, data were pooled. Treatment means were separated using Fisher's protected LSD at the 0.05 level of probability.

Red rice experiments. Field and greenhouse experiments were conducted to evaluate the effect of glufosinate and flooding on control of 3- to 4-leaf awnless strawhull red rice. Field experiments were conducted in 1994 and 1995 on the South farm of the Rice Research Station, Crowley, La, on a soil naturally infested with red rice. Herbicide treatments were a factorial of either flood (approximately 1.25 cm) or no flood and nine glufosinate rates. Glufosinate was applied at 0, 0.3, 0.4, 0.6, 0.8 and 1.1 kg ai/ha as a single application or a sequential application of 0.3, 0.4 and 0.6 kg/ha applied one week apart into 6.2 m by 1.8 m plots.

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Greenhouse experiments were conducted in 1994 and 1995 to further evaluate how different depths of flood water affect red rice control with glufosinate. The design was a randomized block design with a complete factorial arrangement of flood depths (0, 25, 50, 75 and 100% of plant height) and glufosinate rates (0, 0.3, 0.4, and 0.6 kg/ha). Twenty seeds of awnless strawhull red rice were sown into 30 by 15 by 8 cm plastic tubs and were later thinned to 10 plants per tub. In all the experiments, herbicide treatments were applied in a 95 L/ha spray volume at 3- to 4- leaf stage of red rice (approx. 25 cm tall) with a CO₂ pressurized back pack sprayer with flat fan nozzles⁴ spaced at 0.38 m. Water was siphoned from the tubs and tubs were placed back in the greenhouse 24 hr after treatment. A shallow flood (2 cm) was maintained to promote plant growth in the greenhouse experiment.

For both rice and red rice experiments, phytotoxicity of glufosinate was visually evaluated 21 DAT⁵ on a scale of 0 (no injury) to 100% (plant death). Injury symptoms included chlorosis, necrosis, and stunting of plants. In addition to visual observations on injury, plant heights and dry weights of red rice plants were recorded in the

⁴Teejet FFVS 8002 tips, Spraying Sysems Co., Wheaton, IL 60187.

⁵DAT, days after treatment
greenhouse study. Height from the base of the plant to the
tip of the longest leaf was determined. Red rice plants (3
from each pot) were harvested at 21 DAT and were oven dried
at 60 C and weighed to determine dry weight. Weed-free
plots were maintained in each block in the transgenic rice
studies. Yield data from 1993 was not included since plots
were only one row, 2 m long. Greenhouse experiments were
repeated over time.

Data were subjected to analysis of variance to
determine the main effects of time, flooding depth and
glufosinate rate and interactions between main effects to
determine whether data could be pooled over years. Means
were separated by Fisher's protected LSD at 0.05 level of
significance.

RESULTS AND DISCUSSION

Transgenic rice experiments. The effect of glufosinate on
transgenic and commercial rice lines 21 DAT are presented
in Table 2.2. There was a significant interaction
between year, rice line and glufosinate rate; therefore
data on injury and yield were presented by year. Injury on
Gulfmont lines ranged between 0 to 8%, which was similar to
non-sprayed controls all three years. Although Gulfmont
line 526-1 was transformed with the same plasmid as the
Table 2.2. Effect of glufosinate on transgenic Gulfmont and Koshihikari rice lines and commercial Gulfmont and Koshihikari rice varieties 21 DAT in Crowley, LA.

<table>
<thead>
<tr>
<th>Rice injury</th>
<th>Glufosinate rate kg ai/ha</th>
<th>1993</th>
<th>1994</th>
<th>1995</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1.1 2.2</td>
<td>0</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>0 1.1 2.2</td>
<td>0</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Gulfmont lines</td>
<td></td>
<td>0</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>517-1-R1</td>
<td>0 3 5</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>517-2-R1</td>
<td>0 1 6</td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>517-3-R1</td>
<td>0 1 3</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>517-5-R1</td>
<td>0 0 1</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>517-7-R1</td>
<td>0 0 0</td>
<td>0</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>526-1</td>
<td>0 3 0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Koshihikari lines</td>
<td></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>495-1-R1</td>
<td>0 3 1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>495-1-R2</td>
<td>0 5 14</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>496-1-R1</td>
<td>0 24 29</td>
<td>0</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>496-1-R2</td>
<td>0 6 53</td>
<td>0</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>496-2-R1</td>
<td>0 0 6</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>496-3-R1</td>
<td>0 11 18</td>
<td>4</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>496-3-R2</td>
<td>0 4 8</td>
<td>1</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>496-4-R1</td>
<td>0 9 13</td>
<td>1</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>496-4-R2</td>
<td>0 9 19</td>
<td>3</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Gulfmont</td>
<td>0 95 100</td>
<td>1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>0 100 100</td>
<td>0</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
Koshihikari lines (pWRG2426), injury was 3% or less, indicating that the differences in plasmids were not responsible for differences in susceptibility to glufosinate. Koshihikari line 496-2-R1 was not injured with any rate of glufosinate all three years. Injury of non-treated control Koshihikari rice lines, 495-1-R1 and 495-1-R2 in 1994 and 1995 was due to propanil [N-(3,4-dichlorophenyl) propanamide], which was sprayed to control barnyardgrass, [Echinochloa crus-galli (L.) Beauv]. Though propanil is a selective herbicide on rice, these two BAR-transformed Koshihikari rice lines were apparently more susceptible to propanil. On Koshihikari lines, 496-1-R1, 496-3-R1 and 496-4-R2 rice injury increased with 1.1 and 2.2 kg/ha glufosinate in 1993 and 1994, but were not different from their respective non-sprayed rice lines in 1995. On line 495-1-R1 there was no injury in 1993 and 1994, but in 1995 injury increased to about 21% with 1.1 or 2.2 kg/ha glufosinate. Rice line 495-1-R2 was injured with 2.2 kg/ha glufosinate in 1993, but not in 1994 or 1995. Rice lines 496-1-R2 and 496-3-R2 were both injured by 1.1 and 2.2 kg/ha glufosinate in 1994, but not in 1995. Koshihikari line 496-4-R1 was injured with either 1.1 or 2.2 kg/ha glufosinate in 1993, but only with 1.1 kg in 1994, while it was not injured by 2.2 kg/ha glufosinate in
1995. Nontransgenic Gulfmont and Koshihikari rice were injured 90% or more. First injury symptoms on nontransgenic lines due to treatment with glufosinate were observed 3 DAT and by 10 to 14 days injury was 100%. Early symptoms included chlorotic spotting followed by total foliar chlorosis and necrosis. D'Halluin et al. (1992) also reported that nontransgenic sugarbeets (Beta vulgaris L.) treated with 0.2 kg/L glufosinate were injured 4 days after treatment and were killed at 12 days. The non-selective action of glufosinate reported by others agree with the sensitivity of nontransgenic plants to glufosinate observed in our experiments (D'Halluin et al. 1992). The resistance of transgenic rice lines to glufosinate application and death of non-transgenic plants as was found in this experiment was similar to Leemans et al. (1987) who reported that transgenic tobacco (Nicotiana tabacum L.) plants expressing BAR gene were resistant to glufosinate at 6.6 kg/ha.

Yield data of transgenic rice lines in 1994 and 1995 as a percent of their respective non-treated controls are presented in Table 2.3. There was no treatment by year interaction and hence, data were pooled. Yield of Gulfmont lines ranged from 83 to 96% and 93 to 106% of the yield of
Table 2.3. Effect of glufosinate on grain yield of transgenic Gulfmont and Koshihikari rice lines expressed as a percent of their non-treated control.

<table>
<thead>
<tr>
<th>Glufosinate rate (kg ai/ha)</th>
<th>Rice lines</th>
<th>1.1</th>
<th>2.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gulfmont lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>517-1-R1</td>
<td>517-2-R1</td>
<td>85</td>
<td>93</td>
</tr>
<tr>
<td>517-3-R1</td>
<td>517-5-R1</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>517-7-R1</td>
<td>96</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>526-1</td>
<td>83</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Koshihikari lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>495-1-R1</td>
<td>495-1-R2</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>496-1-R1</td>
<td>496-1-R2</td>
<td>102</td>
<td>93</td>
</tr>
<tr>
<td>496-2-R1</td>
<td>97</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>496-3-R1</td>
<td>93</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>496-3-R2</td>
<td>86</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>496-4-R1</td>
<td>85</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>496-4-R2</td>
<td>97</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

aData are pooled over 1994 and 1995. bRice yield of non-treated nontransformed Gulfmont and Koshihikari were 5947 and 5632 kg/ha, respectively.
non-transformed control respective controls with 1.1 and 2.2 kg glufosinate, respectively. Rice yield of Koshihikari lines was at least than 85% of the control with 1.1 kg/ha glufosinate, but ranged from 70 to 108% percent of the control with 2.2 kg/ha of glufosinate. Yield of Gulfmont lines 517-5-R1 and 526-1 was less than the untreated control; Yields of other lines were equivalent to the control. Except for 517-5-R1, the response of all the other Gulfmont lines was similar for the two rates of glufosinate.

Among Koshihikari lines, rice yield of 495-1-R2 treated with 1.1 kg/ha glufosinate was greater than when not treated, but yield from all the other lines treated with 1.1 kg/ha glufosinate was equivalent to the non-transformed control. Rice yields of Koshihikari lines 496-1-R2, 496-3-R2, 496-4-R2 treated with 2.2 kg/ha glufosinate were reduced to 70, 81, and 79% of their respective treated controls. Rice yields of all other Koshihikari lines were not negatively affected by 2.2 kg/ha glufosinate.

Red rice flooding experiments. Control of red rice 21 DAT was affected by flooding and glufosinate rates (Table 2.4). As there was no treatment by year interaction, data were
averaged over years for field studies. Flooding reduced the efficacy of single glufosinate applications against red rice. Under non-flooded conditions, in general, as the rate of glufosinate increased from 0.3 to 1.1 kg/ha red rice control increased. However, no significant

Table 2.4. Red rice control 21 DAT as affected by flooding and glufosinate rates in the field.

<table>
<thead>
<tr>
<th>Glufosinate rates</th>
<th>No flooded</th>
<th>Flooded</th>
</tr>
</thead>
<tbody>
<tr>
<td>kg ai/ha</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.3</td>
<td>62</td>
<td>44</td>
</tr>
<tr>
<td>0.4</td>
<td>67</td>
<td>48</td>
</tr>
<tr>
<td>0.6</td>
<td>78</td>
<td>42</td>
</tr>
<tr>
<td>0.8</td>
<td>77</td>
<td>63</td>
</tr>
<tr>
<td>1.1</td>
<td>86</td>
<td>57</td>
</tr>
<tr>
<td>0.3 + 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td>0.4 + 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>91</td>
</tr>
<tr>
<td>0.6 + 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are pooled over 1994 and 1995.

<sup>b</sup>Sequential application one week apart.
differences were observed in red rice control with single applications of 0.4 to 1.1 kg/ha glufosinate under flooded conditions except at 0.8 kg/ha and were 63% or less. Flood water reduced the efficacy of glufosinate by 18 to 34 percentage points compared to no flood. The increased performance of sequential applications even under flooded situations is due to the second application made again one week later when there was no standing water in the field. When there was no flood, a sequential application of 0.3 kg/ha was similar to a single application of 1.1 kg/ha glufosinate. However, under a flooded situation, sequential applications were superior to all single applications. The red rice control achieved with different rates of glufosinate (sequential) was similar in both situations. Smith (1989) reported that all sequential applications of paraquat and some glyphosate sequential applications 1 month apart consistently controlled >90% tall fescue (Festuca arundinacea Schreb.). In contrast, single applications of 0.14 kg ai/ha paraquat and 0.84 kg ai/ha glyphosate provided less control of tall fescue. Sequential applications of 0.14 kg ai/ha paraquat applied 1 and 3 wk after peanut (Arachis hypogaea L.) emergence provided 81% ragweed (Ambrosia artemisiifolia L.) control compared with only 51% with a single application (Wilcut
Our findings confirm that sequential applications outperform single applications in controlling red rice in flooded situations.

The effect of glufosinate on red rice subjected to different depths of flooding in the greenhouse is presented in Table 2.5. When not flooded, red rice control with glufosinate was 96 to 100%. Control of red rice flooded to 25% of its height was similar to the control (no flood) for all glufosinate rates. Red rice control was 88 to 100% with a 0 or 25% flood at all glufosinate rates, however, control was reduced to 48, 38, and 78% or less with 0.3, 0.4, and 0.6 kg/ha glufosinate, respectively, when flood depth was 50% or more. These data suggest that flood depth between 25% and 50% of red rice height is critical for red rice control with glufosinate. The minor injury on plants under 100% submergence was due to herbicide exposure of some leaf tips above the water surface. Glufosinate did not reduce plant height compared to non-treated plants with 75 and 100% red rice submergence. At all rates of glufosinate, as the depth of flood water increased from 0 to 100%, plant heights generally increased indicating that an increasing flood depth reduces the herbicide activity. Red rice dry weight decreased with 25% or less flooding at...
Table 2.5. Red rice control in the greenhouse as affected by glufosinate rate and flooding depth 21 DAT.

<table>
<thead>
<tr>
<th>Glufosinate rate (kg ai/ha)</th>
<th>Red rice injury*</th>
<th>Red rice height</th>
<th>Red rice dry. weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flooding depth*</td>
<td>0    0.3 0.4 0.6</td>
<td>0    0.3 0.4 0.6</td>
<td>0 0.3 0.4 0.6</td>
</tr>
<tr>
<td></td>
<td>%    % % %</td>
<td>%    % % %</td>
<td>% % % %</td>
</tr>
<tr>
<td>0</td>
<td>0 96 96 100</td>
<td>49   10 5 0</td>
<td>394 15 43 0</td>
</tr>
<tr>
<td>25</td>
<td>0 91 88 96</td>
<td>57   19 19 10</td>
<td>494 161 66 48</td>
</tr>
<tr>
<td>50</td>
<td>0 40 31 78</td>
<td>50   46 36 24</td>
<td>378 349 357 29</td>
</tr>
<tr>
<td>75</td>
<td>0 48 38 35</td>
<td>42   46 41 46</td>
<td>347 247 241 179</td>
</tr>
<tr>
<td>100</td>
<td>0 8 18 14</td>
<td>54   51 48 48</td>
<td>348 329 291 349</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>13   10</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

*Flooding depths represent the percent of the plant height submerged in water prior to glufosinate application.

b Data are pooled over experiments.
0.3 to 0.6 kg/ha glufosinate; however, with 0.6 kg/ha glufosinate red rice dry weight was reduced in comparison to its respective check when flooded to depth of 50%. In addition, with 0.6 kg/ha glufosinate, red rice dry weights were similar when flooded from 0 to 75%. With a majority of the rates and parameters evaluated, it appeared that red rice control decreased when flood water covered more than 25% of the plant. The decrease in glufosinate activity with flooding probably was due to decreased herbicide contact with the red rice foliage. Recommendations based on previous research suggested that fields should be completely drained before application of propanil (Anonymous 1987; Anonymous 1995). A second application of propanil may be required if flood water is not completely drained (Anonymous 1995). Weeds must be exposed above the water for good control of ducksalad (*Heteranthera mimosa* L.), dayflower (*Commelina bengalensis* L.), redstem (*Erodium cicutarium* L.) and certain other broadleaf weeds with bentazon, [3-(1-methylethyl)-(1H)-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide] (Anonymous 1987; Anonymous 1993; Anonymous 1995). This emphasized the importance of sequential applications, as was found from our experiment.

This research suggested that sequential applications of glufosinate improved consistency in red rice control in
both flooded and non-flooded conditions, which would allow more flexibility than timing a single herbicide application. Results of this research also indicate that selective control of red rice in BAR-transformed rice is an effective tool in a crop-weed system in which no herbicide based postemergence selectivity is possible for red rice.

LITERATURE CITED


D'Halluin, K., M. De Block, J. Janssens, J. Leemans, A. Reynaerts, and J. Botterman. 1992. The BAR gene as a


CHAPTER 3
RESPONSE OF BAR TRANSFORMED RICE (Oryza sativa L.) AND RED RICE (Oryza sativa L.) TO GLUFOSINATE APPLICATION TIMING

INTRODUCTION

Advances in the genetic transformation of plants have allowed incorporation of herbicide resistance into crop plants. One of the first applications of genetic engineering in rice has been the development of tolerance to glufosinate by incorporating the BAR gene (Christou et al. 1991). The BAR gene encodes for the enzyme phosphinothricin acetyl transferase (PAT) which is used as an assayable marker gene (D'Halluin et al. 1992) and makes plants resistant to glufosinate. Phosphinothricin [homoalanin-4-yl-(methyl)phosphinic acid], which is the active portion of the glufosinate molecule inhibits glutamine synthetase (E.C. 6.3.1.2.) resulting in rapid accumulation of ammonia, cessation of photorespiration and photosynthesis, and chloroplast disruption (DeVine et al. 1993; Tachibana et al. 1986; Wild and Ziegler 1989). The BAR gene promotes detoxification of glufosinate by acetylation of the amino group (Droge et al. 1992) by PAT disrupting glufosinate's inhibitory activity of glutamine synthetase, thus making the plant resistant to glufosinate (D'Halluin et al. 1992). Transformed rice plants, in
addition to BAR gene, contain Hm gene that confers resistance to the antibiotic hygromycin. Hygromycin was a selective agent that killed non-transformed plants. Engineering rice for tolerance to glufosinate offers the possibility of selective control of red rice and other weeds in a rice cropping system (Braverman and Linscombe 1993; Braverman and Linscombe 1994. Red rice is one of the worst weed problems in cultivated rice production (Smith 1983). In Louisiana, of the 233,000 ha of rice grown, 75% or more of the area is infested with red rice. Rice grain yield reductions from season-long interference with red rice at 215 plants/m² can be 82% (Diarra et al. 1985).

Application of herbicides at the 3- to 4- leaf stage of rice is a common practice in rice weed management. Pantone and Baker (1992) reported that the tolerance of rice to bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy] acetic acid] was dependent on growth stage. Rice is tolerant to 2,4-D [(2,4-dichlorophenoxy)acetic acid] during the late tillering to early jointing stages, but may be severely injured prior to tillering or in the boot stage (Smith et al. 1977). While previous research noted the tolerance of BAR transformed rice to glufosinate (Braverman and

'Sanders, D. E. 1996. Personal communication.'
Linscombe 1993; Braverman and Linscombe 1994), there has not been any information on how BAR-transformed rice and red rice would respond to application of glufosinate at different growth stages. The objectives of this study were to evaluate the effects of glufosinate alone on different growth stages of BAR transformed rice and red rice; glufosinate-benomyl combination on boot stage of BAR transformed rice, and to compare the efficacy of single and sequential glufosinate applications on control of red rice.

**MATERIALS AND METHODS**

**Transgenic rice.** Experiments were conducted to evaluate the effect of glufosinate at 2.2 kg/ha on different growth stages of BAR-transformed 'Gulfmont' rice in 1994 and 1995 at the Rice Research Station, near Crowley, LA. Previous experiments by Braverman et al. (1994) suggested that BAR-transformed rice can tolerate 2.2 kg/ha glufosinate and thus the rate 2.2 kg/ha was selected for use on transformed rice in this experiment. Soil was a Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualf). BAR-transformed 'Gulfmont' rice, line 517-7-R1 containing the vector plasmid pWRG4517 obtained from Dr. Paul Christou of the John Innes Institute (formerly with Agracetus Inc., Middleton, WI) was utilized. Plasmid pWRG4517 contained the BAR gene for glufosinate resistance and the Hm gene.
which confers resistance to hygromycin (Agracetus Inc. 1991).

BAR transformed rice was drill seeded in conventionally prepared seedbeds on May 16, 1994 and May 20, 1995. BAR transformed rice was intentionally seeded late to create a flowering differential between transformed rice and rice being grown in the vicinity at Rice Research Station. Plot size was 4.8 by 1.4 m. The experimental design was a randomized complete block with treatments replicated four times. Glufosinate was applied at 2.2 kg/ha to drill-seeded rice with 1- to 2- leaves, 3- to 4-leaves, at panicle initiation (PI7), and in the boot stage. Non-treated control plots of BAR transformed Gulfmont rice were also established for comparison at each growth stage for both drill and water seeded rice. Glufosinate at 0 and 2.2 kg/ha was also evaluated on 1- to 2- leaf water-seeded BAR transformed Gulfmont rice. Water seeded transformed rice was established by sowing pre-germinated rice seed into 60 cm diam PVC enclosure that contained about a 2 cm flood. Yield data for 1 to 2 leaf water seeded rice was not determined because of the small plot size. Benomyl [methyl 1-(butylcarbonyl)-2-benzimidazolcarbamate] is commonly used to control fungal diseases such as blast in

\(^7\text{PI, panicle initiation.}\)
rice (Anonymous 1987). To determine if a combination of glufosinate and benomyl would affect the resistance of BAR-transformed Gulfmont rice, benomyl was mixed at the rate of 1.1 kg ai/ha with glufosinate at 2.2 kg/ha. This mixture was applied at the boot stage only, according to the recommended application timing for benomyl (Anonymous 1993).

Visual estimates of transformed rice injury were recorded at 14 DAT on a scale of 0 to 100% where 0 = no injury and 100 = plant death. Injury symptoms included chlorosis, necrosis, and stunting. Transgenic rice heights were measured 14 DAT from the base of the plant to the tip of the longest leaf. Rice was mechanically harvested and grain yield data was converted to 12% moisture. The BAR transformed rice study was conducted under weed-free conditions. Propanil \([N-(3,4-dichlorophenyl)propanamide]\) and bensulfuron \([2-[[[[4,6-dimethoxy-2-pyrimidiny]l amino]carbonyl] amino]sulfonyl] methyl]benzoic acid]\) were applied as blanket treatments to all the plots in order to control other weeds so that yield reductions due to glufosinate injury could be determined without weed competition.

**Red rice.** Experiments on transgenic rice and red rice were conducted at different locations to conform to
environmental regulations imposed by Animal and Plant Health Inspection Service (APHIS). The effect of glufosinate on a natural infestation of awnless, straw hull red rice was evaluated in 1993, 1994, and 1995 on the South Farm of the Rice Research Station, Crowley, LA.

Treatments consisted of a factorial arrangement of 3 growth stages of red rice (3- to 4- leaf, PI, boot stage) and 9 rates of glufosinate. Plot size was 6.2 by 1.8 m. The rates of glufosinate were 0.3, 0.4, 0.6, 0.8, and 1.1 kg ai/ha as single application or sequentially at 0.3, 0.4 and 0.6 kg ai/ha per application, one week apart.

Herbicides were applied with a CO₂-pressurized backpack sprayer in a 95 L/ha spray volume with flat fan nozzles spaced 0.38 m apart in both the experiments. Visual estimates of red rice control were recorded at 14 DAT on a scale of 0 to 100% where 0 = no injury and 100 = plant death. Injury symptoms on red rice included chlorosis, necrosis, and stunting.

Data from rice and red rice studies were subjected to analysis of variance. In rice studies, data were pooled over years when treatment by year interactions were not significant. Means of significant main effects and interactions in red rice studies were separated using Fisher's Protected LSD Test at P=0.05.
RESULTS AND DISCUSSION

Transgenic rice. The effect of glufosinate on the visual injury at different growth stages of transgenic Gulfmont rice 14 DAT are presented in Table 3.1. Due to a significant year interaction, data are presented for individual years. Injury was less when glufosinate was

Table 3.1. Injury and plant heights of BAR transformed rice 14 DAT as affected by application timing of 2.2 kg ai/ha of glufosinate.

<table>
<thead>
<tr>
<th></th>
<th>Rice injury</th>
<th>Plant height(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1994</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>1995</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water seeded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 leaf</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Dry seeded</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2 leaf</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>3-4 leaf</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>PI(^b)</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>Boot(^c)</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Boot</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

LSD (0.05) 3 6

\(^a\)Rice heights expressed as percent of non-treated controls. Average plant heights were 20, 45, 76, and 98 cm at 1- to 2- leaf, 3- to 4- leaf, PI and boot stages, respectively.

\(^b\)Abbreviations: PI, panicle initiation.

\(^c\)Benomyl (1.1 kg/ha) + glufosinate (2.2 kg/ha) mixture applied at boot stage.
applied at the boot stage compared with applications made to rice in the 1- to 2- leaf or 3- to 4- leaf stages. Injury from glufosinate applied at PI was 28 and 8% in 1994 and 1995, respectively. In both years, no differences in injury were found between drill seeded and water seeded rice with 2.2 kg/ha glufosinate. Glufosinate applied at the 3- to 4- leaf stage injured rice 19% in 1994 and 13% in 1995. The reduction in injury from 1994 to 1995 may have been due to the advancement of the transgenic line an additional generation. In the 1994 study, segregation for susceptibility was observed which may have influenced injury ratings. By 1995, most of this segregation had been eliminated through the selection pressure of glufosinate application and this may be the reason for the interaction observed.

Although significant differences in plant height were noted between years and growth stages, glufosinate did not severely impact plant height. Plant heights expressed as a percent of their respective controls (Table 3.1) suggested that with the exception of glufosinate applied at the 3- to 4- leaf stage, the heights of rice treated with glufosinate at all the other stages was similar to the controls. In spite of greater injury sustained at some stages, plant heights were at least 93 or 96% or more of the controls in 1994 and 1995, respectively.
Rice yields were calculated as a percent of the respective control (Table 3.2). Yield of non-treated control rice plants at different growth stages varied from 4110 to 4750 kg/ha in 1994 and 5260 to 5500 kg/ha in 1995.

Except for boot stage applications, rice yields of other treatments expressed as a percent were similar to their respective controls. Rice yield from the plots

Table 3.2. Yield of BAR-transformed Gulfmont rice treated with glufosinate at 2.2 kg ai/ha as influenced by application timing.

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Rice yield</th>
<th>( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry seeded</td>
<td>1-2 leaf</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>3-4 leaf</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>PI(^b)</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Boot(^c)</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Boot</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>LSD (0.05)</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^a\)Percent rice yield with respect to non-treated controls. Average rice yield of non-treated control was 4430 kg/ha in 1994 and 5360 kg/ha in 1995.

\(^b\)Abbreviations: PI, panicle initiation.

\(^c\)Benomyl (1.1 kg/ha) + glufosinate (2.2 kg/ha) mixture applied at boot stage.
treated at the boot stage with glufosinate plus benomyl and without benomyl were reduced to 84% of their controls. Rice yield was also reduced to 88% of its control when treated with glufosinate at the 1- to 2- leaf stage but this was not different from the yield from control plots. Rice yield was 92% of its non-treated control at 3- to 4- leaf stage, which was the greatest of all the treatments. Poor correlation between injury on BAR transformed rice and yield ($R = 0.025$) suggests that though the injury was evident on plants at 14 DAT, transgenic plants were able to overcome the injury and yields were not affected. Although there was greater foliar injury at the 3- to 4- leaf stage, it also had a greater time between injury and grain production in which to recover, while plants injured from glufosinate at the boot stage had less time until grain production occurred. Similarly, Delannay et al. 1995 reported that there was no significant lasting injury on glyphosate [N-(phosphonomethyl)glycine] resistant soybean [Glycine max (L.) Merr.].

Red rice. Red rice control was affected by glufosinate rate and growth stages when evaluated 14 DAT (Table 3.3). Glufosinate at 1.1 kg/ha controlled red rice more effectively when applied to 3- to 4- leaf rice (91%) compared with applications made at PI (74%) or the boot
stage (77%). Sequential glufosinate applications at 0.4 or 0.6 kg/ha controlled red rice at least 87% at all growth stages. Sequential applications of 0.3 kg/ha glufosinate on red rice were equal to a single application of 1.1 kg/ha.

Table 3.3. Red rice control 14 DAT as influenced by application timing of glufosinate at various rates.

<table>
<thead>
<tr>
<th>Glufosinate rate (kg ai/ha)</th>
<th>3-4 leaf</th>
<th>PIb</th>
<th>Boot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.3</td>
<td>66</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>0.4</td>
<td>69</td>
<td>31</td>
<td>50</td>
</tr>
<tr>
<td>0.6</td>
<td>78</td>
<td>35</td>
<td>51</td>
</tr>
<tr>
<td>0.8</td>
<td>84</td>
<td>52</td>
<td>68</td>
</tr>
<tr>
<td>1.1</td>
<td>91</td>
<td>74</td>
<td>77</td>
</tr>
<tr>
<td>0.3c</td>
<td>95</td>
<td>72</td>
<td>83</td>
</tr>
<tr>
<td>0.4c</td>
<td>100</td>
<td>87</td>
<td>91</td>
</tr>
<tr>
<td>0.6c</td>
<td>100</td>
<td>94</td>
<td>88</td>
</tr>
<tr>
<td><strong>LSD (0.05)</strong></td>
<td></td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

aData are pooled over three years.

bAbbreviations: PI, panicle initiation.

cSequential treatment applied 1 week apart.
at all growth stages. At the 3- to 4- leaf stage, red rice control was 95% or more at all rates of glufosinate applied sequentially. Similar results have been found with sequential applications of 0.14 kg ai/ha paraquat (1,1'-dimethyl-4,4'-bipyridinium ion) applied 1 and 3 wk after peanut (Arachis hypogaea L.) emergence which provided 81% common ragweed (Ambrosia artemisiifolia L.) control compared to only 51% with single application 0.14 kg/ha of paraquat 1 wk after emergence (Wilcut and Swann 1990).

At PI and booting stages, at least 0.6 kg/ha of glufosinate applied sequentially was required for red rice control. However, 0.3 kg/ha glufosinate applied sequentially at PI was consistently less effective than sequential applications of 0.4 or 0.6 kg/ha. Data suggest that sequential application of 0.3 to 0.4 kg/ha of glufosinate would be more effective in controlling more mature red rice. Lee et al. 1982, Mathes et al. 1980 and McClelland et al. 1978 also reported that sequential applications of several other herbicides were more appropriate in controlling later stages of weeds in other crops as well, which agrees with our findings. At all the three growth stages, 1.1 kg/ha glufosinate was required to achieve red rice control equivalent to 0.3 kg/ha applied sequentially.
Pantone and Baker (1992) found that bromoxynil and triclopyr injured 'Lemont', 'Tebonnet' and 'Mars' varieties of rice less when applied at PI stage compared with earlier growth stages. Our results also suggest that response to glufosinate is dependent on growth stage. Ralph et al. (1992) concluded that glyphosate at 2.2 kg ai/ha was less effective in controlling larkspurs (Delphinium spp.) when applied in the flower stage compared to earlier growth stages.

This test was purposefully conducted with glufosinate at 2.2 kg/ha on transgenic rice which was previously estimated as 4 times the rate required to control red rice to determine how BAR transformed rice would respond at different growth stages. Since only 0.6 kg/ha glufosinate applied sequentially provided excellent control of red rice, the injury to BAR transformed rice should be less than the injury for the high rate. Also, this experiment was conducted with a monocultured red rice where competition was very high among red rice plants. While direct comparisons of the selectivity between BAR transformed rice and red rice were not possible, this research suggests that red rice was between 4 to 5 times more susceptible at 3- to 4- leaf, 2 to 4 times more susceptible at PI, and 5 to 11 times more susceptible at
boot stage than BAR transformed rice with single applications of glufosinate based on the selectivity index values calculated (data not shown). The relative injury to red rice compared with BAR transformed rice may be greater with decreased rates of glufosinate on BAR transformed rice due to reduced injury.

This research suggests that the sequential applications of glufosinate are better for consistent control of red rice. The use of a weed control system with BAR transformed rice would significantly reduce the amount of herbicide active ingredients compared with propanil and molinate (5-ethylhexahydro-1H-azepine-1-carbothioate)-based systems with the added advantage of being able to control red rice postemergence. The results of this research also indicate that use of glufosinate in BAR transformed rice increases the flexibility of red rice control in rice compared with herbicides and cultural practices presently available.

**LITERATURE CITED**


CHAPTER 4

GLUFOSINATE RESISTANT BAR TRANSFORMED RICE (Oryza sativa L.) AND RED RICE (Oryza sativa L.) RESPONSE TO GLUFOSINATE ALONE AND IN MIXTURES

INTRODUCTION

Newly developed herbicide-resistant crops may serve as effective tools to selectively control problem weeds in agronomic crops. Transforming rice with the bialaphos resistance (BAR) gene so that it is resistant to the nonselective herbicide glufosinate allows for the selective control of red rice and other problem weeds in rice cropping systems (Braverman and Linscombe 1994). The BAR gene in the resistant rice plant promotes detoxification of glufosinate by acetylation of its amino group (Droge et al. 1992).

Historically, glufosinate has been used to control annual and perennial weeds in non-crop land areas and as a non-selective contact type herbicide prior to crop emergence in minimum tillage systems (Haas and Muller 1987). The efficacy of glufosinate in controlling grasses and other weeds may be enhanced by combining it with other herbicides. Although glufosinate controls a wide spectrum of broadleaf weeds, it is less effective on grasses (Anonymous 1993). Thus, supplemental grass control may be necessary. Combinations of contact herbicides with other
herbicides having foliar and soil residual activity can enhance initial weed control, provide residual weed control, and reduce the number of trips across the field (Bruce and Kells 1990; Bruff and Shaw 1992a, 1992b, Lanie et al. 1994). Control of pitted morningglory (Ipomea lacunosa, L.) with glufosinate was 63%, but glufosinate combined with metribuzin (0.42 kg ai/ha), [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one], or imazaquin {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid} resulted in 99% and 98% control, respectively (Lanie et al. 1994). Combining herbicides also may be beneficial in delaying the appearance of resistant weed biotypes, which is an important threat in monocultures (Hatzios and Penner 1985).

Glufosinate has recently been evaluated for its potential use in rice. Deep water may reduce the effectiveness of glufosinate under flooded rice conditions since glufosinate must contact the weed foliage. Our earlier studies showed that glufosinate performs best when no more than 25 to 50% of red rice is under the flood. In drained rice fields when standing water is present in lower sections especially after a rain, other herbicides that have activity in the flood water may be needed for
effective weed control. This would be especially important for aquatic weeds such as ducksalad \textit{(Heteranthera limosa (Sw.) Willd.)} and alligatorweed \textit{(Alternanthera philoxeroides (Mart.) Griseb.)}, which are not controlled effectively with glufosinate in rice fields of Louisiana\(^{8}\).

While the need for combining glufosinate with other herbicides has been recognized, the phytotoxicity of these combinations to BAR transformed rice and red rice is not known. The objectives of this study were to evaluate red rice control and crop safety of BAR-transformed rice associated with combinations of glufosinate and contact and residual herbicides.

**MATERIALS AND METHODS**

**Transgenic rice.** Field studies were conducted in 1994 and 1995 at the Rice Research Station located near Crowley, LA on a Crowley silt loam soil (fine montmorillonitic, thermic Typic Albaqualf) with a pH of 5.6 and 1.2\% organic matter. Transformed Gulfmont rice (line 517-7-R1) containing the vector plasmid pWRG4517 (Agracetus Inc. 1991) was drill seeded in rows spaced 20 cm apart in conventionally tilled seedbeds on May 26, 1994 and May 28, 1995. Plasmid pWRG 4517 contained BAR gene for glufosinate resistance and Hm gene that acts as a selective agent by conferring

resistance to the antibiotic hygromycin. Glufosinate at 2.2 kg ai/ha was applied at the 3- to 4- leaf stage either alone or with pendimethalin (1.1 kg ai/ha), thiobencarb (3.4 kg ai/ha), quinclorac (0.3 kg ai/ha), propanil (3.4 kg ai/ha), bensulfuron methyl (0.07 kg ai/ha), bentazon (1.1 kg ai/ha), acifluorfen (0.6 kg ai/ha), or triclopyr (0.4 kg ai/ha). A non-treated control was also included. Plots were maintained weed free by a blanket treatment of 3.4 kg/ha propanil at the 2- leaf stage and hand removal throughout the season. Herbicide applications were made on June 20, 1994 and June 21, 1995. The experimental area was drained one day before herbicide applications. Plot size was 4.8 by 1.4 m. The experimental design was a randomized complete block with four replications.

Red rice. A natural infestation of awnless, straw hull red rice was evaluated at the Rice Research Station to compare the effect of glufosinate applied alone or with pendimethalin, thiobencarb, quinclorac, propanil, bensulfuron, bentazon, acifluorfen and triclopyr herbicide treatments. With the exception of glufosinate, which was applied at 0.6 kg/ha, other herbicides were applied at the rates mentioned previously to 3- to 4- leaf red rice on May 8, 1994 and April, 28, 1995. Previous studies showed that at 0.6 kg/ha, glufosinate controls nearly 80% of red rice.
In the present red rice studies, the 0.6 kg/ha rate of glufosinate was selected instead of 2.2 kg/ha which was used on BAR transformed rice (which would result in approximately 100% control) since the intent was to show antagonism or increased red rice control with the combinations. Plot size was 6.2 by 1.8 m. The experimental design was a randomized complete block with 4 replications. Herbicides were applied when red rice had 3- to 4- leaves.

Herbicides in both transgenic and red rice studies were applied with a CO₂-pressurized backpack sprayer in a 95 L/ha spray volume at 140 kPa. Visual injury on transgenic and red rice was evaluated at 21 days after treatment (DAT) on a scale of 0 to 100 where 0 = no injury and 100 = plant death. Foliar injury symptoms included chlorosis, necrosis, and stunting. Plant heights were also recorded in both the experiments 21 DAT by measuring from the base of the plant to the tip of the longest leaf. Transgenic rice was machine harvested and grain yield was adjusted to 12% moisture. Panicle maturity of red rice was scored visually two weeks before harvest based on grain filling and grain color on a scale of 0 to 100 where 0 = immature and 100 = complete maturity of the seed as a percent of the non-treated control. Red rice was hand harvested from one square meter when more than 90% of seed
in non-treated controls plots was mature and one-hundred seed weight of oven dried seed was determined.

All data reported for each year and across years were subjected to analysis of variance. Means were separated using Fisher's protected LSD at the 5% level of significance. For transgenic rice, a year by treatment interaction was not significant for plant injury and height and data were pooled. This was not the case for yield. For red rice, data were pooled over years for all variables.

RESULTS AND DISCUSSION

Transgenic rice. Except for glufosinate applied with triclopyr and acifluorfen, transgenic rice injury 21 DAT from all other herbicide combinations was no more than 13% (Table 4.1). Combinations of glufosinate with triclopyr or acifluorfen injured rice 59 and 22%, respectively. Rice injury with glufosinate applied alone was 6% and equivalent to the non-treated control. Only propanil, acifluorfen, or triclopyr combined with glufosinate injured rice more than glufosinate alone. Rice height with all glufosinate-herbicide combinations was similar to the non-treated
Table 4.1. Effects of glufosinate alone and in combination with other herbicides on injury, plant height, and yield of BAR-transformed rice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate</th>
<th>Injury</th>
<th>Rice Height</th>
<th>Rice yield</th>
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<tbody>
<tr>
<td></td>
<td>kg/ha</td>
<td>%</td>
<td>cm</td>
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</tr>
<tr>
<td>Glufosinate + pendimethalin</td>
<td>2.2 + 1.1</td>
<td>9</td>
<td>53</td>
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<td></td>
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<td>4640</td>
</tr>
<tr>
<td>Glufosinate + thiobencarb</td>
<td>2.2 + 3.4</td>
<td>8</td>
<td>51</td>
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<td></td>
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<tr>
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<td>8</td>
<td>52</td>
<td>3820</td>
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<tr>
<td>Glufosinate + propanil</td>
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<td>53</td>
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<td>Glufosinate + bentazon</td>
<td>2.2 + 1.1</td>
<td>8</td>
<td>49</td>
<td>3600</td>
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<td>52</td>
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<td>4140</td>
</tr>
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<td>Glufosinate + triclopyr</td>
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<td>59</td>
<td>43</td>
<td>2195</td>
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<tr>
<td>Glufosinate</td>
<td>2.2</td>
<td>6</td>
<td>53</td>
<td>3620</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>4830</td>
</tr>
<tr>
<td>Non-treated control</td>
<td>0</td>
<td>48</td>
<td>4530</td>
<td>5580</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>7</td>
<td>5</td>
<td>1060</td>
<td>570</td>
</tr>
</tbody>
</table>

*Rice plant injury and height were determined 21 DAT and pooled over years.*
control. The foliar injury with the combination of glufosinate and triclopyr (59%) was reflected in a 19% height reduction when compared with glufosinate alone.

In 1994 pendimethalin, acifluorfen, or triclopyr applied with glufosinate reduced grain yield at least 26% compared with the non-treated control, but yield with all treatment combinations were equivalent to glufosinate alone (Table 4.1). In 1995, all herbicide treatments reduced yield when compared with the non-treated control. Rice yield from glufosinate application alone was reduced 13%. Rice yield following application of glufosinate plus pendimethalin or bensulfuron were equivalent to glufosinate alone, but yields for the other combination were 14 to 75% less than for glufosinate alone.

Rice yield was reduced both years with the combination of triclopyr and glufosinate (Table 4.1). Triclopyr plus glufosinate reduced rice yields by 39 and 75% in 1994 and 1995 respectively, compared with glufosinate alone and 52 and 79%, respectively, compared with the non-treated control. Pantone and Baker (1992) observed a 22% yield reduction of 'Mars' rice when 0.4 kg/ha triclopyr was applied at 2- to 3- leaf stage. Triclopyr, however, does not injure rice when applied from early tillering to internode elongation stages (Smith and Hill 1990). The increase in the free ammonia pool due to glufosinate
activity may have increased the response to triclopyr by promoting abnormal RNA and DNA based increases in cell division, or both herbicides may have contributed to cell wall plasticity and membrane destruction (Anonymous 1994). Surfactants in the glufosinate formulation may have also enhanced triclopyr absorption.

Red rice. Red rice control was 92% with propanil or acifluorfen plus glufosinate and was greater than for the other glufosinate-herbicide combinations and glufosinate alone (81 to 85% (Table 4.2). Red rice control with glufosinate alone was only 83% indicating that 0.6 kg/ha of glufosinate was not adequate. Earlier studies showed that a single application of 1.1 kg/ha glufosinate or sequential applications of 0.3 kg/ha glufosinate one week apart controlled 3- to 4- leaf red rice 91 and 95%, respectively.

Unlike that observed in transgenic rice (Table 4.1) increased injury was not apparent on red rice when the glufosinate- triclopyr combination was applied. Differences in the response of red rice and rice to the triclopyr-glufosinate combination may be related to differences in their growth and development. As previously mentioned, differences in sensitivity to triclopyr exist even among commercial rice cultivars. Improved weed control was observed with glufosinate combined with other
herbicides as compared to glufosinate alone (Lanie et al. 1994). Though, acifluorfen and propanil are predominantly broadleaf herbicides and are not very effective on red rice (Anonymous 1994), the activity of glufosinate on red rice may have been increased when combined with these herbicides. Increased efficacy of propanil in mixtures than when applied alone have been reported by earlier researchers. Three year studies by Street and Snipes (1989) showed that control of barnyardgrass \( \textit{Echinocloa crusgalli} \) (L.) Beauv.] increased two-fold when 3.4 kg/ha propanil was mixed with 2.2 kg/ha tridiphane \[2-(3,5-dichlorophenyl)-2-(2,2,2-trichloroethyl) oxirane\]. Increased barnyardgrass control was also reported when propanil was combined with quinclorac, thiobencarb, or pendimethalin over that of propanil alone (Baltazar and Smith 1994). The membrane damage that would have been caused due to the application of acifluorfen or propanil may have been increased due to the mixture of glufosinate to the above herbicides.

The visual assessments of foliar injury were reflected in red rice plant heights. Glufosinate combined with either propanil or acifluorfen reduced red rice plant heights more than when glufosinate was applied alone.
Table 4.2. Red rice control, plant heights, panicle maturity and 100-seed weight as affected by glufosinate alone and in combination with other herbicides*.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate</th>
<th>Red rice control</th>
<th>Red rice height</th>
<th>Panicle maturity</th>
<th>100-seed weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glufosinate + pendimethalin</td>
<td>0.6 + 1.1</td>
<td>83</td>
<td>23</td>
<td>85</td>
<td>1.8</td>
</tr>
<tr>
<td>Glufosinate + thiobencarb</td>
<td>0.6 + 3.4</td>
<td>84</td>
<td>25</td>
<td>73</td>
<td>1.5</td>
</tr>
<tr>
<td>Glufosinate + quinclorac</td>
<td>0.6 + 0.3</td>
<td>81</td>
<td>25</td>
<td>86</td>
<td>1.8</td>
</tr>
<tr>
<td>Glufosinate + propanil</td>
<td>0.6 + 3.4</td>
<td>92</td>
<td>21</td>
<td>52</td>
<td>1.3</td>
</tr>
<tr>
<td>Glufosinate + bensulfuron</td>
<td>0.6 + 0.07</td>
<td>81</td>
<td>26</td>
<td>77</td>
<td>1.7</td>
</tr>
<tr>
<td>Glufosinate + bentazon</td>
<td>0.6 + 1.1</td>
<td>81</td>
<td>25</td>
<td>74</td>
<td>1.7</td>
</tr>
<tr>
<td>Glufosinate + acifluorfen</td>
<td>0.6 + 0.6</td>
<td>92</td>
<td>21</td>
<td>52</td>
<td>1.3</td>
</tr>
<tr>
<td>Glufosinate + triclopyr</td>
<td>0.6 + 0.4</td>
<td>85</td>
<td>25</td>
<td>77</td>
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<tr>
<td>Glufosinate</td>
<td>0.6</td>
<td>83</td>
<td>25</td>
<td>75</td>
<td>1.7</td>
</tr>
<tr>
<td>Non-treated Control</td>
<td>0</td>
<td>30</td>
<td>100</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>6</td>
<td>3</td>
<td>17</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

*Red rice control and height were recorded 21 DAT. Data for all variables are pooled over years.
Mean plant height of non-treated controls was 30 cm, which was greater than for all herbicide treated plants. Height of red rice plants treated with glufosinate in combination with pendimethalin, thiobencarb, quinclorac, bensulfuron, bentazon, triclopyr were all equivalent to glufosinate alone.

The reduced plant height observed for the propanil and bentazon mixtures with glufosinate was accompanied by delayed panicle maturity (Table 4.2). Differences in panicle maturity between the red rice treated with a mixture of glufosinate and pendimethalin or quinclorac and the non-treated control was not observed. Propanil or acifluorfen mixed with glufosinate reduced panicle maturity and 100-seed weight more than any other treatments. Although differences were not noted among herbicide treatments, the glufosinate combinations and glufosinate alone all reduced 100-seed weight compared with the non-treated control. Red rice panicle maturity and 100-seed weight for the mixture of propanil or acifluorfen with glufosinate were only 52 and 44% of the non-treated control, respectively.

This study revealed that transformed rice injury with mixtures of glufosinate and pendimethalin, thiobencarb, quinclorac, bensulfuron, or bentazon was no more than for glufosinate alone. However, glufosinate applied with
propanil, acifluorfen, or triclopyr was injurious to rice. Considering previous research showing differences in varietal sensitivity to triclopyr (Pantone and Baker, 1992), the transformed rice lines may be more sensitive to triclopyr than non-transformed lines. Increased sensitivity to propanil by two BAR-transformed rice lines has been observed in preliminary evaluations with glufosinate. Although transformed rice injury was enhanced, red rice control was not increased by the triclopyr combination. Additionally, glufosinate activity on red rice was not antagonized by any of the herbicide combinations. As more glufosinate resistant rice seed becomes available, the interaction of glufosinate and triclopyr should be studied in further detail to explain the increased injury with this combination of herbicides.

LITERATURE CITED


Bruce, J. A. and J. J. Kells. 1990. Horseweed (Conyza canadensis) control in no tillage soybeans (Glycine max) with preplant and preemergence herbicides. Weed Technol. 4:642-647.

Bruff, S. A. and D. R. Shaw. 1992a. Early season herbicide applications for weed control in stale seedbed soybean (Glycine max). Weed Technol. 6:36-44.


INTRODUCTION

Herbicide resistance involves the selection and evolution of a mechanism that allows a herbicide in a population of plants to withstand repeated exposure to that herbicide (Harper 1956). Most commonly, resistance is used in the context of weeds resistant to herbicide. In this case, selection pressure over extended periods of time to herbicide with the same mechanism of action result in a shift to weed biotypes less susceptible to the specific herbicide. Introduction of herbicide resistance into cultivated species is an important application of biotechnological research (Chaleff and Bascomb 1987). One such practical application is the development of glufosinate resistant rice. Glufosinate resistant rice offers the possibility of direct application of glufosinate for selective control of red rice in rice. Red rice is the worst problem weed in rice production in the southern United States, South and Central America (Cohn and Hughes 1981). Control measures include summer fallow and crop rotation combined with herbicide treatment (Smith et al. 1977). However, no complete satisfactory means for the
control of red rice in cultivated rice currently exists. Glufosinate resistant rice would be an effective alternative.

The commercial varieties, Gulfmont and Koshihikari, were altered by genetic engineering to contain the bialaphos resistance (BAR)gene for glufosinate resistance (Christou et al. 1991). Genetically engineered plants produce phosphinothricin acetyl transferase (PAT) which makes the plant resistant to glufosinate. Glufosinate is an inhibitor of glutamine synthetase (E.C.6.3.1.2) and thus, prevents incorporation of ammonia into amino acids. Inhibition of glutamine synthetase by glufosinate results in toxic accumulation of ammonia in plant cells. Acetylation of glufosinate by PAT at its free amino group disrupts glufosinate's inhibitory activity of glutamine synthetase, making the plant herbicide resistant (D'Halluin et al. 1992).

Herbicide resistance is a heritable trait in the population, not a transient, phenotypic response to an environmental condition which might allow a plant to escape herbicide effects (LeBaron and Gressel 1982). Repeated use of the same herbicide or herbicides with the same mechanism of action will select for resistance in the population by

Abbreviations: BAR, Bialaphos resistance; PAT, Phosphinothricin acetyl transferase.
killing the susceptible biotype while the resistant biotype will survive, reproduce, and pass the herbicide resistance trait to the next generation (Gressel and Segel 1982). Rice is predominantly a self pollinated crop. However, chances of open pollination and hybridization with red rice still exist and can range from 1% in Lemont to over 50% in the Nortai variety (Langevin 1988). Therefore, the possibility for the movement of glufosinate resistance to red rice exists. Major concern is the long term effectiveness of glufosinate on red rice if outcrossing occurs. Furthermore, glufosinate resistant rice as a volunteer crop could be a weed in another variety. Cross resistance of BAR-transformed rice to other herbicides has not been investigated. If cross resistance exists, the magnitude of the problem of controlling glufosinate resistant red rice in rotational crops would escalate if only glufosinate is used.

Cross resistance is the increased resistance to one herbicide as a result of a selection pressure from another, and is more likely to occur if herbicides possess a similar mode of action (Gressel 1979). However, there have been cases of cross resistance to herbicides of unrelated modes of action. Heap and Knight (1986) showed that diclofop methyl (\(\pm 2-[4(2,4\text{-dichlorophenoxy})\text{phenoxy}]\text{propanoic acid}\))
resistant annual ryegrass \((\text{Lolium rigidum Lam.})\) was cross resistant to fluazifop butyl \((\text{(R)-2-[4-[(trifluoromethyl)-2-pyridinyl] oxyl} \text{ phenoxy} \text{ propanoic acid}),(\text{chlorsulfuron [2-chloro-N-[[4-methoxy-6-methyl-1,3,5-triazin-2-yl} \text{amino} \text{carbonyl} \text{benzenesulfonamide}] and DPX-T6376 (methyl 2-[[[4-methoxy-6-methyl-1,3,5-triazin-2-yl} \text{amino} \text{carbonyl} \text{amino} \text{sulfonyl} \text{benzoate}]. \) This was the first report on cross resistance and these results were confirmed by Matthews et al. (1990). Smeda et al. (1992) reported that trifluralin resistant green foxtail \((\text{Setaria viridis (L.) Beauv})\) was resistant to the structurally unrelated herbicides, DCPA \((\text{dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate})\) and terbutol \((2,6-di-tert-butyl-p-tolyl methylcarbamate)\). In a soybean-rice rotation, which is the predominant cropping system in southwest Louisiana \((\text{Griffin and Robinson 1989})\), metolachlor and alachlor \((2-chloro-2'-6'diethyl-N-methoxymethyl) acetamide)\) are commonly used to control red rice in soybean \((\text{Griffin and Harger 1986; Khodayari et al. 1987})\). Control of red rice often depends upon the herbicides used on rotational soybean crop \((\text{Huey and Baldwin 1980})\). Acetanilides, such as metolachlor \((\text{Griffin and Robinson 1989})\), dinitroanalines such as trifluralin, and imidazolinones, such as imazaquin \((2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-}
imidazol-2-yl]-3-quinolinecarboxylicacid), are commonly used in the control of red rice in soybeans (Anonymous 1994; Khodayari et al. 1987). Therefore, it is important to determine if BAR- transformed rice is cross resistant to any herbicides used in a rotational soybean crop. In addition, no-till rice production involving burndown herbicides is becoming increasingly popular in Louisiana (Bollich and Sanders 1993). Glyphosate, paraquat, and glufosinate are non-selective herbicides that control vegetation preplant in rice (Mendt and Braverman 1995). Therefore, cross resistance to these burndown herbicides that have a similar use pattern is of major concern.

Another important aspect of cross resistance is negative cross resistance. Negative cross resistance occurs when herbicides, other than the one to which a plant biotype has developed resistance, become more toxic to the resistant biotype than to the susceptible biotype (Hall et al. 1996). Our earlier studies showed that negative cross resistance has been observed in the BAR-transformed Gulfmont rice line, 517-1-R1, and transformed Koshihikari lines, 495-1-R1 and 495-1-R2, which were severely injured with triclopyr [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid] and propanil [N-(3,4-dichlorophenyl)propanamide], respectively.
Earlier field results on the evaluation of glufosinate on BAR-transformed Gulfmont and Koshihikari rice suggested that transformed Gulfmont was more resistant to glufosinate (2.2 kg/ha) than transformed Koshihikari (Braverman and Linscombe 1994). Studies by Baker et al. (1988) and Griffin and Baker (1990), suggested variable tolerance of rice cultivars (Lemont, Tebonnet and Mars) to fenaxoprop, \([\pm 2-\{(6-chloro-2-benzoxazolyl)oxy\}propanoic acid\] and that Mars variety was particularly susceptible to fenaxoprop. Further, Pantone and Baker (1992) reported that Lemont rice was more susceptible to triclopyr than either Mars or Tebonnet. Thus, baseline (inherent) differences in resistance of non-transformed rice cultivars may also be reflected in the differential response of BAR-transformed Gulfmont and Koshihikari rice to glufosinate.

With these considerations, studies were conducted with two objectives. The first was to determine if BAR-transformed rice is cross resistant to other preplant burndown herbicides or herbicides that are routinely used in soybean. The second objective was to evaluate resistance level of non-transformed cultivars to determine if the greater resistance to glufosinate observed in BAR-transformed Gulfmont rice in comparison with Koshihikari rice is due to greater inherent resistance in non-transformed Gulfmont.
MATERIALS AND METHODS

Cross resistance studies. This study was conducted with BAR-transformed Gulfmont rice, line 517-1-R1. Line 517-1-R1 has plasmid pWRG4517 containing the BAR gene for glufosinate resistance and the Hm gene which confers resistance to the antibiotic hygromycin. Non-transformed Gulfmont rice and awnless, strawhull red rice also were evaluated for comparison. This experiment was conducted in a greenhouse located on the campus of Louisiana State University, Baton Rouge, LA. Six to 10 seeds were sown 1 cm deep in 9-cm diam by 12-cm deep plastic cups containing Crowley silt loam (fine montmorillonitic, thermic Typic Albaqualf). Stock concentrates of trifluralin and metolachlor were prepared by dissolving the formulated products in ethanol. Final treatment solutions were prepared from stock solutions by diluting aliquots of this initial concentrate in acetone. The preplant incorporated, PPI\textsuperscript{10}, herbicides were applied on 290 g of soil which comprised the upper 4-cm of soil in the plastic cup. Plastic bags containing herbicide treated soil were left undisturbed for at least 5 h under a hood for solvent evaporation. Once the acetone solution evaporated, the bags were closed and shaken thoroughly, so that the

\textsuperscript{10}PPI, preplant incorporated; POST, postemergence

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herbicide-treated soil particles were evenly distributed. This treated soil was placed back into the plastic cups and seeds were sown into the treated soil. Plants were later thinned to three per cup.

The postemergence, POST, herbicides used in this study were glyphosate (0.3 and 0.6 kg ai/ha), sulfosate (1.8 and 3.6 kg ai/ha), paraquat (0.3 and 0.6 kg ai/ha), and imazethapyr (0.1 and 0.3 kg ai/ha). Rice plants that received POST applications were sown on the same day and manner as for PPI treatments. Non-treated control plants were maintained with all the three rice types for comparison. POST applications were made at 3- to 4- leaf stage of plant growth in a 95 L/ha spray volume with a CO₂ pressurized, backpack sprayer with flat fan nozzles spaced at 0.38 m. A completely randomized experimental design with four replications was used and the experiments were conducted in September, 1994 and March, 1996. Visual estimates of the percent injury on all rice lines were recorded 8 WAT for PPI treatments and 4 WAT for POST treatments on a scale of 0 to 100%, where 0 = no injury and 100 = plant death. Rice injury rating involved a combined assessment of foliar chlorosis, necrosis, and stunting. Plant heights were measured from base of the plant to the

11Teejet FFVS 8002 tips, Spraying Systems Co., Wheaton, IL 60187.
tip of the longest leaf 7 WAT for the plants that received PPI treatments.

**Baseline Resistance Studies.** In order to determine the basis for differential response of BAR-transformed rice cultivars to glufosinate, a baseline resistance study was conducted in the greenhouse in January and March of 1994. The experimental design was a randomized complete block with factorial arrangement of 28 rice genotypes and 5 glufosinate rates. Rice cultivars evaluated were commercial Gulfmont and Koshihikari in addition to 21 U.S. genotypes (Alan, AS 3510, Bengal, Cypress, Jasmine, Katy, LA 2115, Lacassine, Lemont, Mars, Maybelle, Mercury, Millie, Orion, (an imazethapyr resistant rice line12), Rico 1, RT 7015, Rosemont, Skybonnet, Torida, V 4716 and two Japanese cultivars (Nipponbare and Sasanishiki). Three biotypes of red rice (awnless strawhull, long awn strawhull, and long awn blackhull) also were evaluated for comparison. Glufosinate was applied at 0, 0.04, 0.08, 0.15, and 0.3 kg/ha. Earlier studies showed that the lower rate of glufosinate used to control red rice is 0.3 kg/ha and thus, these rates were sub-lethal to allow development

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of a dose-response curve. Visual injury symptoms were recorded 21 DAT on a scale of 0 to 100%, where 0 = no injury and 100 = plant death. Symptoms of glufosinate injury to rice included foliar chlorosis, stunting, and necrosis. The rate of glufosinate that causes 50% visual injury (I₅₀ value) was calculated for all the cultivars based on the regression equation fitted for each cultivar.

To further evaluate and confirm the visual differences in baseline resistance of Gulfmont and Koshihikari rice at a biochemical level, an ammonia assay was performed as an indicator of glufosinate sensitivity. This study was conducted in April and May, 1996, as a randomized complete block design with a factorial arrangement of five rice genotypes (Gulfmont, Koshihikari, Rico 1, Maybelle, and red rice) and 5 glufosinate rates (0, 0.04, 0.08, 0.15, and 0.3 kg/ha). Rico 1 and Maybelle were selected because they had the greatest and least I₅₀ values in the visual ratings, respectively and awnless straw hull red rice was included for comparison. Glufosinate applications were made as described previously. Plant leaf material was collected for ammonia assay 5 DAT and processed immediately using procedures described by D'Halluin et al. (1992). Rice leaf material (250 mg) was extracted in 1 ml water containing 50 mg PVPP (poly vinyl polypyrrolidine) and centrifuged for 5 min in an eppendorf centrifuge. The upper 200 µl
supernatant was diluted with 800 µl water. To 20 µl of the diluted plant extract, 1.5 ml reagent A (5 g phenol, 25 mg sodium nitroprusside, 500 ml water), followed by 1.5 ml reagent B (2.5 g NaOH, 1.6 ml NaOCl, 500 ml water) was added. The reaction mixture was incubated for 15 min at 37 C and the absorbance was measured at 625 nm. The ammoniacal nitrogen was determined on a standard curve (µg ammonical nitrogen/g fresh weight = g determined ammonical nitrogen x 450). [The standard curve was made using NH₄Cl in concentrations ranging from 0.1 to 2 g ammonical nitrogen (3.82 g NH₄Cl = 1 g NH₄ N)].

Data from both studies were subjected to analysis of variance. Means of significant main effects and interactions were separated using Fisher's protected LSD at P = 0.05.

RESULTS AND DISCUSSION

Cross resistance studies. Ratings of visual injury with PPI and POST treatments on BAR transformed rice in comparison to non-transformed rice and red rice are presented in Table 5.1 and 5.2. Injury symptoms included chlorosis and necrosis. As there was no interaction, data were pooled over years. There were significant differences in injury between trifluralin rates for all the rice types. There were no differences in injury between the 3 rice types with
0.6 kg/ha trifluralin. Trifluralin at 1.2 kg/ha caused similar injury (42 to 46%) on all rice lines. None of the rice seeds emerged in the soil treated with metolachlor PPI (Data not shown). This inhibition may be due to the absorption of metolachlor by the coleoptile and, as a result, none of the seedlings emerged from the soil (100% injury). Previously, metolachlor-treated soil was reported to inhibit emergence of rice at concentrations from 0.2 to 0.4 ppm (Braverman et al. 1985).

Postemergence applications of both rates of glyphosate, sulfosate, paraquat, and imazethapyr injured all rice lines, at least 78%, which was significantly greater than non-treated control plants. Injury on BAR-transformed rice due to applications of glyphosate, sulfosate, and paraquat was 100% and was similar to non-transformed rice and red rice. Injury on BAR-transformed rice due to imazethapyr (0.1 kg/ha) was 96% which was similar to injury at the same rate on red rice (83%) but was greater than that of injury on non-transformed rice (78%). However, no differences in the response of rice lines were observed with 0.3 kg/ha imazethapyr. With all
Table 5.1. Effect of trifluralin and metolachlor applied preplant incorporated on injury to BAR-transformed Gulfmont rice, non-transformed Gulfmont rice, and red rice 8 WAT.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate</th>
<th>BAR transformed</th>
<th>Non-transformed</th>
<th>Red rice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg ai/ha</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trifluralin</td>
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<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>1.2</td>
<td>45</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Rice injury data are averages pooled over experiments.*
Table 5.2. Effect of glyphosate, sulfoate, paraquat, and imazethapyr applied post-emergence on injury of BAR-transformed Gulfmont rice, non-transformed Gulfmont rice, and red rice 4 WAT.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate</th>
<th>BAR transformed rice</th>
<th>Non-transformed rice</th>
<th>Red rice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg/ha</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sulfoate</td>
<td>1.8</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sulfoate</td>
<td>3.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Paraquat</td>
<td>0.3</td>
<td>100</td>
<td>89</td>
<td>100</td>
</tr>
<tr>
<td>Paraquat</td>
<td>0.6</td>
<td>100</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>Imazethapyr</td>
<td>0.1</td>
<td>96</td>
<td>78</td>
<td>83</td>
</tr>
<tr>
<td>Imazethapyr</td>
<td>0.3</td>
<td>100</td>
<td>88</td>
<td>99</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

*Rice injury data are averages pooled over two experiments with four replications.
herbicides tested, BAR-transformed rice sustained injury similar to non-transformed rice and red rice with the single exception of imazethapyr at 0.1 kg/ha and was similar in all cases for red rice.

There was an experiment by treatment interaction for plant heights and thus data were presented individually for experiments conducted in 1994 and 1996 (Table 5.3). The interaction might have occurred due to the reduced plant height of BAR transformed rice and red rice in 1994 and non-transformed rice 1996 with 1.2 kg/ha trifluralin. No differences were found in plant heights among non-treated BAR-transformed rice, non-transformed rice and red rice in 1994 and 1996 except for non-transformed rice in 1994. Except for red rice treated with trifluralin at 0.6 kg/ha in 1996, plant heights of all the other treatments that received trifluralin (0.6 kg/ha) were not different from their respective non-treated controls.

The plant height data agrees with the visual ratings, where trifluralin at 0.6 kg/ha caused only 6 to 10% plant injury (Table 5.1). However, plant heights of BAR-transformed rice, non-transformed rice and red rice in 1994 were different from 1996. In general, the heights of all the three rice types were significantly reduced when treated with 1.2 kg/ha trifluralin compared to their
Table 5.3. Effect of trifluralin and metolachlor applied preplant incorporated on plant heights* of BAR-transformed Gulfmont rice, non-transformed Gulfmont rice and red rice 7 WAT.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rate</th>
<th>BAR transformed</th>
<th>Non-transformed</th>
<th>Red rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluralin</td>
<td>0.6</td>
<td>38 38</td>
<td>38 36</td>
<td>44 36</td>
</tr>
<tr>
<td>Trifluralin</td>
<td>1.2</td>
<td>27 36</td>
<td>41 31</td>
<td>30 37</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>1.8</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>3.6</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>40 43</td>
<td>37 41</td>
<td>44 47</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

*Plant height data are averages of four replications. Experiments were conducted in 1994 and 1996.
respective untreated controls. Moreover, plant heights of BAR-transformed rice and red rice treated with 1.2 kg/ha trifluralin were significantly less compared to the plant height of non-transformed rice treated with the same rate of trifluralin in 1994. At all herbicide rates, the height of BAR-transformed rice was shorter or equal to non-transformed rice or red rice. Therefore, both visual ratings and plant height data indicate that BAR transformed rice is not cross resistant to these herbicides.

Baseline Resistance Studies. Glufosinate rates required to cause 50% injury ($I_{50}$) in Gulfmont and Koshihikari rice and other U.S., Japanese, and red rice lines are listed in Table 5.4. There were significant differences in $I_{50}$ values between Gulfmont and Koshihikari and between other U.S. and Japanese rice lines. The $I_{50}$ value for Gulfmont rice (0.13 kg/ha glufosinate) was over two times the rate required to cause 50% injury on Koshihikari rice (0.06 kg/ha glufosinate). In previous field research (Braverman and Linscombe 1994), BAR-transformed Gulfmont rice was more resistant to glufosinate than BAR-transformed Koshihikari rice. In the present study, the greater tolerance of glufosinate by non-transformed Gulfmont than non-transformed Koshihikari agrees with field observations.
Table 5.4. Regression equations*, correlation coefficients, and calculated I<sub>50</sub>* values (50% visual injury 21 DAT) for U.S., Japanese and red rice genotypes.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Rice type&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Equation</th>
<th>Correlation coefficient</th>
<th>I&lt;sub&gt;50&lt;/sub&gt; values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gulfmont</td>
<td>U.S.</td>
<td>( y = 12 + 301b )</td>
<td>0.94</td>
<td>0.13 kg/ha</td>
</tr>
<tr>
<td>Koshihikari</td>
<td>Japan</td>
<td>( y = 19 + 517b )</td>
<td>0.99</td>
<td>0.06</td>
</tr>
<tr>
<td>Rico 1</td>
<td>U.S.</td>
<td>( y = 2 + 279b )</td>
<td>0.97</td>
<td>0.17</td>
</tr>
<tr>
<td>RT 7015</td>
<td>U.S.</td>
<td>( y = 3 + 287b )</td>
<td>0.99</td>
<td>0.17</td>
</tr>
<tr>
<td>Katy</td>
<td>U.S.</td>
<td>( y = 3 + 305b )</td>
<td>1.00</td>
<td>0.16</td>
</tr>
<tr>
<td>Rosemont</td>
<td>U.S.</td>
<td>( y = 7 + 269b )</td>
<td>0.98</td>
<td>0.16</td>
</tr>
<tr>
<td>Jasmine</td>
<td>U.S.</td>
<td>( y = 5 + 311b )</td>
<td>0.98</td>
<td>0.15</td>
</tr>
<tr>
<td>Mercury</td>
<td>U.S.</td>
<td>( y = 5 + 311b )</td>
<td>0.98</td>
<td>0.15</td>
</tr>
<tr>
<td>Millie</td>
<td>U.S.</td>
<td>( y = 2 + 324b )</td>
<td>0.98</td>
<td>0.15</td>
</tr>
<tr>
<td>Orion</td>
<td>U.S.</td>
<td>( y = 7 + 283b )</td>
<td>0.94</td>
<td>0.15</td>
</tr>
<tr>
<td>Alan</td>
<td>U.S.</td>
<td>( y = 6 + 320b )</td>
<td>0.96</td>
<td>0.14</td>
</tr>
<tr>
<td>LA2115</td>
<td>U.S.</td>
<td>( y = 5 + 332b )</td>
<td>0.91</td>
<td>0.14</td>
</tr>
<tr>
<td>AS3510</td>
<td>U.S.</td>
<td>( y = 8 + 317b )</td>
<td>0.95</td>
<td>0.13</td>
</tr>
</tbody>
</table>

(Table 5.4. con'd.)
<table>
<thead>
<tr>
<th>Variety</th>
<th>Origin</th>
<th>Equation</th>
<th>r</th>
<th>s.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mars</td>
<td>U.S.</td>
<td>( y = 3 + 365b )</td>
<td>0.99</td>
<td>0.13</td>
</tr>
<tr>
<td>Lacassine</td>
<td>U.S.</td>
<td>( y = 14 + 290b )</td>
<td>0.88</td>
<td>0.12</td>
</tr>
<tr>
<td>Lemont</td>
<td>U.S.</td>
<td>( y = 15 + 295b )</td>
<td>0.85</td>
<td>0.12</td>
</tr>
<tr>
<td>Skybonnet</td>
<td>U.S.</td>
<td>( y = 4 + 375b )</td>
<td>0.98</td>
<td>0.12</td>
</tr>
<tr>
<td>Bengal</td>
<td>U.S.</td>
<td>( y = 19 + 303b )</td>
<td>0.89</td>
<td>0.10</td>
</tr>
<tr>
<td>Cypress</td>
<td>U.S.</td>
<td>( y = 15 + 352b )</td>
<td>0.92</td>
<td>0.10</td>
</tr>
<tr>
<td>V 4716</td>
<td>U.S.</td>
<td>( y = 17 + 334b )</td>
<td>0.90</td>
<td>0.10</td>
</tr>
<tr>
<td>Sasanishiki</td>
<td>Japan</td>
<td>( y = 15 + 389b )</td>
<td>0.90</td>
<td>0.09</td>
</tr>
<tr>
<td>Imazethapyr R line</td>
<td>U.S.</td>
<td>( y = 18 + 400b )</td>
<td>0.95</td>
<td>0.08</td>
</tr>
<tr>
<td>Torida</td>
<td>U.S.</td>
<td>( y = 19 + 389b )</td>
<td>0.93</td>
<td>0.08</td>
</tr>
<tr>
<td>Nipponbare</td>
<td>Japan</td>
<td>( y = 17 + 413b )</td>
<td>0.96</td>
<td>0.08</td>
</tr>
<tr>
<td>Maybelle</td>
<td>U.S.</td>
<td>( y = 29 + 525b )</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Awnless strawhull</td>
<td>RR</td>
<td>( y = 28 + 550b )</td>
<td>0.96</td>
<td>0.04</td>
</tr>
<tr>
<td>Long awn blackhull</td>
<td>RR</td>
<td>( y = 30 + 500b )</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>Long awn strawhull</td>
<td>RR</td>
<td>( y = 28 + 550b )</td>
<td>0.99</td>
<td>0.04</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td></td>
<td></td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

*\( y \) = injury 21 DAT; \( I_{50} \) values were calculated from regression equation for each rice line. *\( b \) Rice types included cultivars from the U.S., Japan, and red rice (RR).
Therefore, the greater resistance of BAR-transformed Gulfmont rice was related to its inherent resistance level. These studies suggest that expression of the BAR gene is amplified proportionately to the baseline resistance level. Studies by Nyffeler et al. (1980) suggested that the equi-effective dose (ED$_{50}$) for 50% growth inhibition by metolachlor of different cultivars of sorghum (*Sorghum bicolor* L.) with safener CGA-43089 [α-(cyanomethoximino)-benzacetonitrile] increased proportionately to that of the ED$_{50}$ without safener.

Among U.S. rice cultivars, I$_{50}$ values ranged from 0.08 to 0.17 kg/ha glufosinate except for Maybelle (0.04 kg/ha). The I$_{50}$ values for Japanese cultivars, Nipponbare (0.08 kg/ha glufosinate) and Sasanishiki (0.09 kg/ha glufosinate) were similar with an average value of 0.08 kg/ha for Japanese cultivars. However, the average I$_{50}$ value for U.S. rices was 0.13 kg/ha glufosinate which was almost twice that of Japanese cultivars. The I$_{50}$ values among the red rice biotypes did not differ and were similar to Koshihikari. However, significant differences existed in I$_{50}$ values among U.S. cultivars. Cultivars, Torida, Imazethapyr resistant line, V 4716, Bengal, and Cypress had I$_{50}$ values of 0.08 to 0.10. The I$_{50}$ value of the imazethapyr resistant rice line was only 0.08 kg/ha even
though its parent lines, Lemont and Mercury had $I_{50}$ values of 0.12 and 0.15 kg/ha, respectively. Rico 1 and RT 7015 had the greatest $I_{50}$ values (0.17 kg/ha), but were not different from Alan (0.14 kg/ha), LA 2115 (0.14 kg/ha), Jasmine (0.15 kg/ha), Mercury (0.15 kg/ha), Millie (0.15 kg/ha), Orion (0.15 kg/ha), Katy (0.16 kg/ha), and Rosemont (0.16 kg/ha). Other U.S. rice cultivars had similar $I_{50}$ values.

Differences in sensitivity to glufosinate, as measured by $I_{50}$ values, existed between Gulfmont and Koshihikari, between U.S. and Japanese cultivars, and among U.S. cultivars. Pantone and Baker (1992) reported differential response of U.S. rice cultivars, Mars, Lemont and Tebonnet to triclopyr.

Differences in resistance of Gulfmont and Koshihikari to glufosinate were confirmed by assaying ammonia accumulation 5 DAT (Table 5.5). U.S. cultivars, Rico 1 and Maybelle which had the greatest and least $I_{50}$ values, respectively, from the baseline resistance studies also were evaluated for ammonia accumulation for comparison, in addition to awnless, strawhull red rice. Ammonia accumulation was significantly different among rice cultivars and glufosinate rates 5 DAT. There were no differences in repetitions of the experiment, therefore data were pooled. Ammonia levels in rice cultivars treated
with 0.04 kg/ha glufosinate and Gulfmont and Rico 1 treated with 0.08 kg/ha were similar to non-treated controls and were not detectable (limit of detection was 73 µg/g fresh weight). Ammonia concentration with 0.08 kg/ha glufosinate was 436 µg/g fresh weight in Koshihikari.

Ammonia accumulation in Koshihikari treated with 0.08 kg/ha glufosinate but not in Gulfmont supports the 0.06 kg/ha I_{50} value for Koshihikari from the baseline resistance studies. At all rates tested, ammonia accumulation was greater in Koshihikari than in Gulfmont. Ammonia accumulation was approximately six and two times greater in Koshihikari than in Gulfmont with glufosinate at 0.15 and 0.3 kg/ha, respectively. With 0.15 kg/ha glufosinate, visual injury on Koshihikari (64%) was greater than Gulfmont (45%) at 21 DAT (data not shown) which agrees with increased ammonia accumulation in Koshihikari. Results demonstrate that differences in glufosinate tolerance of Gulfmont and Koshihikari at the physiological level that agree with those observed at the whole plant level (in terms of visual injury-I_{50} values). Results of the ammonia assay are in agreement with visual baseline resistance studies confirming that the differences in BAR-transformed Gulfmont and Koshihikari rice which were

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Table 5.5. Ammonia accumulation of commercial Gulfmont and Koshihikari rice varieties 5 DAT as a measure of baseline resistance compared to other rice cultivars.

<table>
<thead>
<tr>
<th>Glufosinate rate (kg/ha)</th>
<th>Rice cultivars</th>
<th>0</th>
<th>0.04</th>
<th>0.08</th>
<th>0.15</th>
<th>0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>0.04</td>
<td>0.08</td>
<td>0.15</td>
<td>0.3</td>
</tr>
<tr>
<td>Ammonia accumulation (g/g fresh weight)</td>
<td>Gulfmont</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>94</td>
<td>636</td>
</tr>
<tr>
<td></td>
<td>Koshihikari</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>436</td>
<td>604</td>
</tr>
<tr>
<td></td>
<td>Maybelle</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>545</td>
<td>599</td>
</tr>
<tr>
<td></td>
<td>Rico 1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>83</td>
<td>722</td>
</tr>
<tr>
<td></td>
<td>Awnless strawhull-red rice</td>
<td>ND</td>
<td>ND</td>
<td>416</td>
<td>620</td>
<td>1629</td>
</tr>
<tr>
<td></td>
<td>LSD(0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>169</td>
</tr>
</tbody>
</table>

*Abbreviations: ND, Not detected. Lower limit of detection was 73 μg/g fresh weight.
previously observed are related to their inherent resistance level.

With 0.08, 0.15, and 0.3 kg/ha of glufosinate, there were no differences between Maybelle, Koshihikari, and red rice in ammonia accumulation. Although there was an increase in ammonia accumulation in most rice lines when glufosinate rate was increased from 0.08 to 0.15 kg/ha, ammonia content was similar with either 0.08 or 0.15 kg/ha glufosinate in Maybelle and Koshihikari. Ammonia levels in Gulfmont and Rico 1, when glufosinate was applied at 0.15 kg/ha, were 94 and 83 μg/g fresh weight, respectively, which is approximately one seventh of the other cultivars evaluated at that rate. Ammonia accumulation increased seven and nine times for Gulfmont and Rico 1, respectively, when glufosinate rate was increased from 0.15 to 0.3 kg/ha. Even though there were significant differences in I50 values between Gulfmont and Rico 1, ammonia accumulation was similar in both cultivars at all rates of glufosinate. In general, differences in I50 values of Maybelle, Rico 1, and red rice in addition to Gulfmont and Koshihikari also agreed with the differences in ammonia accumulation. Some variation between visual ratings and ammonia accumulation may have been due to differential sensitivity to ammonia. In addition, the physiological response of ammonia
accumulation was much more rapid than the visual injury symptoms.

Based on greenhouse studies, there was no cross resistance in BAR-transformed rice to the herbicides trifluralin, metolachlor, glyphosate, sulfosate, paraquat, and imazethapyr. Therefore, these herbicides can control red rice and avoid development of populations resistant to glufosinate. Baseline resistance studies and results from the ammonia assay demonstrated that greater resistance of BAR-transformed Gulfmont rice to glufosinate compared with BAR-transformed Koshihikari is due to the greater intrinsic resistance of Gulfmont to glufosinate. If BAR-transformed Koshihikari rice is commercialized, lower rates of glufosinate may be needed to avoid the potential yield losses due to crop injury.

LITERATURE CITED


Griffin, J. L. and T. R. Harger. 1986. Red rice (Oryza sativa) and jungle rice (Echinocloa colonum) control in solid seeded soybeans (Glycine max). Weed Sci. 34:582-586.


Hall, J. C., M. J. Donnelly-Vanderloo, and D. J. Hume. 1996. Triazine resistant crops: The agronomic and


CHAPTER 6

GENETIC ANALYSIS OF GLUFOSINATE RESISTANCE IN
CONTROLLED CROSSES BETWEEN TRANSFORMED RICE
(Oryza sativa L.) AND THE WEED RED RICE (Oryza sativa L.)

INTRODUCTION

Red rice is one of the worst weed problems for commercial rice production in the US and other countries (Goss and Brown 1939; Smith 1983). It was recognized as a weed of rice in the U.S. as early as 1846 (Craigmiles 1978). In Louisiana, 75% or more of 230,000 ha of rice grown is infested with red rice\textsuperscript{13}. In addition to contaminating the rice seeds, red rice has poor milling quality, shatters, and lodges, making commercial rice harvest difficult (Diarra et al. 1985). Control measures for red rice include summer fallow and crop rotation combined with herbicide treatment (Smith et al. 1977). Several herbicides and application timings have been tried (Smith 1981; Parker and Dean 1976). No complete satisfactory means for the control of red rice in cultivated rice is available so far. The use of transgenic rice has been the latest approach to try to overcome the red rice problem (Braverman and Linscombe 1994).

\textsuperscript{13}Paul Seilhan, personal communication.

100
Over the past decade, recombinant DNA technology has been used where specific genes can be introduced into a plant in a relatively straightforward manner, provided the genes coding for the character have been identified (Brown et al. 1995). Genetic modification is only one technique that may help to meet the objectives of breeding future varieties (Jorgensen et al. 1996). Genetic manipulation for herbicide resistance in cultivated species is an important application of biotechnological research (Chaleff and Bascomb 1987). One application of biotechnology was engineering resistance to the herbicide glufosinate by the expression of the detoxifying enzyme, phosphinothricin acetyl transferase (PAT) (Vasil 1996).

The commercial rice varieties, Gulfmont and Koshihikari were altered by genetic engineering to contain the BAR gene for glufosinate resistance (Agracetus Inc. 1991). The BAR gene from *Streptomyces hygroscopicus* encodes for phosphinothricin acetyl transferase that catalyzes the transfer of an acetyl moiety from acetyl-coenzyme A to the amino group of the molecule (De Block et al. 1987). Glufosinate is an inhibitor of glutamine synthetase (E.C. 6.3.1.2) and thus prevents incorporation of ammonia into amino acids. Inhibition of glutamine synthetase by glufosinate results in toxic accumulation of
ammonia in plant cells. Acetylation of glufosinate by PAT at its free amino group disrupts the inhibition of glutamine synthetase thus making the plant herbicide resistant (D'Halluin et al. 1992).

A major benefit from herbicide resistant crops is the opportunity for new strategies and or increased flexibility in the management of problem weeds (Wilcut et al. 1996). Herbicide resistant crops also facilitate the addition of conservation tillage crop production practices because of more effective post-emergence treatments (Wilcut et al. 1996). Development of crop cultivars with resistance to post-emergence herbicides will encourage crop producers to use economic weed threshold predictions in making their weed management decisions (Coble and Mortensen 1992). In addition, herbicide resistant crops will potentially allow the use of more environmentally benign herbicides and lower use rates of herbicides than many soil applied herbicides (Burnside 1992; Knake 1992).

In contrast to these advantages, the main concern of introducing transgenic herbicide resistant crops into agriculture is the spread of the engineered gene(s), particularly by pollen, to related weed species (Keeler 1989; Williamson 1991). A possible negative environmental impact of this sexual transfer of engineered genes to related wild plants by natural hybridization is the
evolution of more aggressive weed genotypes (Keeler 1989; Hoffman 1990). Moreover, if crop-weed hybrid seeds were formed, and seeds were dormant (a trait often found in weeds), some hybrids would establish with the weed at a similar time and hybrids may continue to cross leading to a stable introgression (Jorgensen et al. 1996). Furthermore, gene exchange between a crop and a weedy relative may increase the adaptability of the weed, making it even more competitive. Added adaptability was noticed in weeds like wild beets, *Beta vulgaris* (Boudry et al. 1993), red rice, *Oryza sativa* (Arnold and Hodges 1995) and wild lettuce, *Lactuca sativa* (Williamson 1993). Rice is predominantly a self pollinated crop. But, chances of cross pollination and hybridization between rice and red rice still exist and can range from 1% in Lemont to over 50% in the Nortai variety (Langevin 1988). There are several reports of introgressive hybridization between rice and its weedy relatives (Oka and Chang 1959; Morishima et al. 1961). Herbicide resistance in many cases can be achieved by the transfer of a single gene (Schulz et al. 1990). Gene expression levels vary with genetic background, epistasis, linkage, and pleiotropy. Therefore, it can be difficult to predict how the genetically engineered gene will be expressed in a
related weed species (Colwell et al. 1985; Tiedje et al. 1989).

The objective of this study was to determine genetic control of glufosinate resistance in controlled crosses between cultivated rice and weedy red rice biotypes. To accomplish this, controlled crosses were made in the greenhouse to generate data that can be used as a model for possible genetic transfer that may occur under field conditions.

MATERIALS AND METHODS

Greenhouse studies were conducted in 1994 to 1996 at Louisiana State University, Baton Rouge, LA. Two transgenic lines derived from the cultivars Gulfmont and Koshihikari were shown in previous studies (Oard et al. 1996) to contain the BAR gene that conferred high levels of resistance to glufosinate in two years of field studies. Southern analysis revealed that transgenic line 517-7-R1 derived from Gulfmont contained stably integrated 2 copies of the BAR and Hm (hygromycin resistance) genes, and transgenic line 496-2-R1 derived from Koshihikari contained the BAR (1 to 2 copies), Hm, and GUS genes. The lines 517-7-R1 and 496-2-R1 were used as parents in reciprocal crosses with an awnless strawhull red rice biotype.
Six to ten seeds of each transgenic and red rice line were sown 1 cm deep in 9 cm diameter (400 ml) plastic cups containing Crowley silt loam soil (fine montmorillonitic, thermic Typic Albaqualf) on 17 August, 1994. In order to ensure synchronous flowering between rice lines and red rice, red rice was sown in one week intervals starting one month before and after sowing rice seed. At the 3- to 4-leaf stage, all lines were transplanted into 11.4 L plastic pots. Pots were lined with plastic and a continuous flood was maintained. Reciprocal controlled crosses were made between BAR transformed Gulfmont and red rice and BAR transformed Koshihikari rice and red rice. Emasculated panicles were enclosed in glycine bags soon after hand pollination and the F₁ seeds were harvested at maturity, air dried and placed in cold storage (0 C) for several months until use.

The F₁ seeds from the reciprocal crosses were germinated on September 20, 1995. Seeds did not have a full seed coat (the lemma and palea which form the seed coat were cut at the time of making crosses in order to remove stamens), and utmost care was taken in handling prior to germination. Seed coats were pinched off and the dehulled seeds were surface sterilized by soaking in 3% sodium hypochlorite solution for 12 h. Seeds were double rinsed with distilled water after surface sterilization and
then dipped in 800 ppm of dithane solution and removed immediately. Treated seeds were placed on moistened filter paper in a petri dish and placed in an incubator at 32 C. After germination, seedlings were transferred into 400 ml plastic cups containing sterilized peat. Seedlings were grown under fluorescent lighting using a light table that produced 75 µM/sq.m/sec of photosynthetically active radiation for one week before being transferred to the greenhouse. At the 3- to 4- leaf stage, rice was transplanted from plastic cups to 11.4 L pots. At the late tillering stage, a tiller from each plant was separated and planted in separate pots so that response to glufosinate application could be evaluated without killing susceptible individuals. Once the tiller was established, 2 weeks after transplant, glufosinate was sprayed at 2.2 kg ai/ha on 15 December, 1995 to evaluate if glufosinate resistance is expressed in F\textsubscript{1} plants. Parental transformed and red rice lines, in addition to non-transformed parental lines were grown and sprayed along with the F\textsubscript{1} plants. Glufosinate was applied in a 95 L/ha spray volume with a CO\textsubscript{2} pressurized backpack sprayer with flat fan nozzles\textsuperscript{14} spaced at 0.38 m.

\textsuperscript{14}Teejet FFVS 8002 tips, Spraying Systems Co., Wheaton, IL 60187.
Visual estimates of the percent injury were recorded 3 WAT on a scale of 0 to 100% where 0 = no injury and 100 = plant death. Plant heights were also measured 3 WAT in cm from base of the plant to the tip of the longest leaf. All the non-treated control plants and treated plants were assayed for ammonia concentration 5 DAT as a sensitive indicator of glufosinate resistance according to the procedures described by D'Halluin et al. (1992). Briefly, rice leaf material (250 mg) was extracted in 1 ml water containing 50 mg PVPP (poly vinyl polypyrrolidine) and centrifuged for 5 min in an eppendorf centrifuge. The upper 200 μl supernatant was diluted with 800 μl water. To 20 μl of the diluted plant extract, 1.5 ml reagent A (5 g phenol, 25 mg sodium nitroprusside, 500 ml water), followed by 1.5 ml reagent B (2.5 g NaOH, 1.6 ml NaOCl, 500ml water) was added. The reaction mixture was incubated for 15 min at 37 C and the absorbance was measured at 625 nm. The ammonical nitrogen was determined on a standard curve (g ammonical nitrogen/g fresh weight = g determined ammonical nitrogen x 450). [The standard curve was made using NH₄Cl in concentrations ranging from 0.1 to 2 g ammonical nitrogen (3.82 g NH₄Cl = 1 g NH₄'N)]. F₁ plants that were saved at the tillering stage were allowed to self pollinate. The panicles were bagged at early grain filling stage with glycine bags to prevent seed loss due to
shattering. Seeds from each plant were harvested at physiological maturity.

A total of at least 100 seeds were planted per cross to raise the F₂ generation. Three F₁ seeds per plant were sown in 400 ml plastic cups on 11 July, 1996. Transformed and commercial parents were also planted in addition to red rice on the same date. At the late 3- to 4- leaf stage, one basal leaf of each plant was dipped in 300 ppm of technical grade glufosinate solution¹⁵ for approximately 2 seconds. The dipped leaves were scored for resistance 7 DAT on a scale of 0 to 100%, where 0 is no leaf injury and 100 is plant death. Injury symptoms included chlorosis, necrosis, and plant stunting.

Glufosinate was sprayed at 1.1 kg/ha at the early tillering stage. Our previous research suggested that red rice control was greater than 90% with application of 1.1 kg/ha glufosinate and thus this rate was selected to use on F₂ plants to differentiate plants resistant or susceptible to glufosinate. Treated plants were visually evaluated as previously described for resistance or susceptibility to glufosinate. The ammonia assay was performed on all the sprayed plants 4 DAT as described by D'Halluin et al. (1992). The resistant plants that survived glufosinate

¹⁵AgrEvo USA Company. Little Falls Center One, 2711 Centerville Road, Wilmington, DE 19808.
application were grown till seed set. Based on the data from dip test, spray test, and ammonia assay frequency distribution curves were drawn for each cross to determine how the observed data would fit in different classes. Chi-square analysis was done with the assumptions based on frequency distribution curves for each cross.

RESULTS AND DISCUSSION

Injury on the different F₁ plants from the two reciprocal crosses due to the application of 2.2 kg/ha glufosinate 3 WAT ranged from 10 to 30 percent (data not shown). Even though there was minor injury on F₁ hybrids, the plants recovered from the herbicide effect within 3 WAT. Ammonia concentration as a measure of resistance to glufosinate application in the F₁ plants in comparison to their respective transformed and non-transformed parents is shown in Figure 6.1. Ammonia concentration in non-treated controls was not detectable (data not shown). Ammonia concentration in all the plants tested was greater at 4 DAT than at 8 DAT. These results suggest that the inhibitory activity of glufosinate on glutamine synthetase was substantially greater in the first few days of herbicide application and then returned to a basal level by 8 DAT. Ammonia concentration of F₁ plants from all crosses equaled 160 μg/g fresh weight and were not different from the
Figure 6.1. Ammonia accumulation 4 and 8 days after treatment (DAT) in glufosinate tolerant F$_1$ plants in comparison to transformed and non-transformed parents and red rice. Types of parental lines were red rice (R), Gulfmont (G), Koshihikari (K), transformed Gulfmont (TG), and transformed Koshihikari (TK). Rice types of F$_1$ crosses (maternal parent listed first) were transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RXTK).
than at 8 DAT. These results suggest that the inhibitory activity of glufosinate on glutamine synthetase was substantially greater in the first few days of herbicide application and then returned to a basal level by 8 DAT. Ammonia concentration of F₁ plants from all crosses equaled 160 µg/g fresh weight and were not different from the transformed parents. However, ammonia concentration in the non-transgenic Gulfmont, Koshihikari, and red rice were 14, 20, and 23 times greater, respectively, compared to the transformed parents and the F₁ hybrids (Figure 6.1.) Significant variation was observed in ammonia concentration among non-transformed Gulfmont, Koshihikari, and red rice. Ammonia concentration in Gulfmont was less than Koshihikari and red rice, as was found in our previous experiments indicating varietal differences in glufosinate resistance. Parental and F₁ data on visual injury and ammonia concentration together suggest that glufosinate resistance is controlled by a dominant gene(s) in the transformed lines because all the F₁ hybrids were tolerant to herbicide treatments. Research by Brown et al. (1995) also showed that glufosinate resistance is expressed in hybrid plants formed as a result of crosses between transgenic canola (Brassica napus L.) and its related weeds. For F₁ plants of reciprocal crosses, each comparison for ammonia
concentration and visual injury suggested that cytoplasmic factors were not involved in glufosinate resistance with the reciprocal crosses of 517-7-R1 and 496-2-R1 with red rice.

Based on the data from dip test, spray test, and ammonia assay frequency distribution curves were drawn. The data observed fit into two different classes (graphic presentation of data shown for spray test in Figure 6.2). Segregation of observed resistant and susceptible plants based on glufosinate dip test in the F₂ generation and mean percent injury are presented in Table 6.1. Because the data was in distinct classes, and herbicide resistance is primarily controlled by single dominant genes (Schulz et al. 1990), plants from all the crosses were tested based on the assumption that they should segregate in 3:1 ratio in the F₂ generation. In addition, 15:1, 9:7, and 13:3 gene ratios were evaluated for goodness to fit. In the single leaf dip test, average injury value of resistant and susceptible F₂ plants ranged between 5 to 7% and 96 to 99%, respectively. Among individuals from crosses involving transformed Gulfmont with red rice (94 plants) and transformed Koshihikari with red rice (95 plants), 71 and 67 plants were found to be resistant to glufosinate, respectively. The chi-square value was significant for all the crosses at the 95% level. The chi-square value was also
Figure 6.2. Frequency distribution of percent rice injury from glufosinate spray test of $F_2$ population resulted from crosses between transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RxTK).
Table 6.1. Segregation of glufosinate resistance in four F₁ populations based on glufosinate dip test* and chi-squared probabilities for fit to single-gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Distribution analysis of rice injury</th>
<th>Average rice Injury</th>
<th>chi-square value</th>
<th>Dip test consisted of placing a basal leaf at 3- to 4- leaf stage in 300 ppm glufosinate for 2 sec.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>observed</td>
<td>expected</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>GXR</td>
<td>71</td>
<td>23</td>
<td>71</td>
<td>24</td>
</tr>
<tr>
<td>RXG</td>
<td>97</td>
<td>43</td>
<td>105</td>
<td>35</td>
</tr>
<tr>
<td>KXR</td>
<td>67</td>
<td>28</td>
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<tr>
<td>RXK</td>
<td>64</td>
<td>22</td>
<td>65</td>
<td>21</td>
</tr>
</tbody>
</table>

*R, S = number of resistant and susceptible plants based on visual scoring of dip test.

All chi-square values are significant at p = 0.05

Rice types of F₁ crosses (maternal parent listed first) were BAR transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RxTK).
Table 6.2. Segregation of glufosinate resistance in four F₂ populations based on glufosinate spray test* and chi-squared probabilities for fit to single-gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Distribution analysis of rice injury</th>
<th>Average rice Injury</th>
<th>chi-square value(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>observed(^b)</td>
<td>expected(^b)</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>S</td>
<td>R</td>
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<td>GXR</td>
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<td>29</td>
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<tr>
<td>RXG</td>
<td>90</td>
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<td>22</td>
<td>73</td>
</tr>
<tr>
<td>RXK</td>
<td>69</td>
<td>21</td>
<td>68</td>
</tr>
</tbody>
</table>

*Spray test consisted of spraying plants at early tillering stage with 1.1 kg/ha glufosinate.

\(^b\)R, S = number of resistant and susceptible plants based on visual scoring of dip test.

\(^c\)All chi-square values are significant at p = 0.05

\(^d\)Rice types of F₁ crosses (maternal parent listed first) were BAR transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RxTK).
significant with crosses involving red rice as a female parent. The data obtained from spraying whole plants are better indicators of glufosinate resistance or susceptibility than dipping just a leaf which may not be a true representation. However, results of both the tests were similar.

Data on segregation of the glufosinate resistance based on glufosinate spray test is presented in Table 6.2. Plants were either killed (100% injury) or normal (0%) with the whole plant spray of glufosinate. Injury symptoms included severe yellowing and necrosis of the leaf tissue 3 DAT. For plants evaluated from crosses involving transformed Gulfmont as either maternal (102 plants) or paternal parent (120 plants), 73 and 90 were resistant while 29 and 30 plants were susceptible, respectively. Chi-square values for all the crosses were significant at the 95% level as in the dip test confirming the assumption that glufosinate resistance is under the influence of a single dominant gene. Therefore, glufosinate resistance in the F2 populations evaluated will segregate in 3 (resistant) : 1 (susceptible) ratio as was observed in our study.

Results for ammonia accumulation of observed resistant and susceptible individuals are presented in Table 6.3.
Table 6.3. Segregation of glufosinate resistance in four $F_2$ populations based on ammonia assay (4 DAT)* and chi-squared probabilities for fit to single-gene ratio in crosses between two transgenic rice varieties and weed red rice, 1996, greenhouse, Baton Rouge, LA.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Observed R</th>
<th>Observed S</th>
<th>Expected R</th>
<th>Expected S</th>
<th>Average ammonia concentration R</th>
<th>Average ammonia concentration S</th>
<th>Chi-square value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GXR</td>
<td>73</td>
<td>29</td>
<td>77</td>
<td>26</td>
<td>110</td>
<td>1761</td>
<td>0.47</td>
</tr>
<tr>
<td>RXG</td>
<td>90</td>
<td>30</td>
<td>90</td>
<td>30</td>
<td>180</td>
<td>2545</td>
<td>0.01</td>
</tr>
<tr>
<td>KXR</td>
<td>75</td>
<td>22</td>
<td>73</td>
<td>24</td>
<td>173</td>
<td>2432</td>
<td>0.17</td>
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<tr>
<td>RXK</td>
<td>69</td>
<td>21</td>
<td>68</td>
<td>23</td>
<td>126</td>
<td>1935</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*R, S = number of resistant and susceptible plants based on ammonia assay.

*All chi-square values are significant at $p = 0.05$

*Rice types of $F_1$ crosses (maternal parent listed first) were transformed Gulfmont x red rice (TGxR), red rice x transformed Gulfmont (RxTG), transformed Koshihikari x red rice (TKxR), and red rice x transformed Koshihikari (RxTK).
Ammonia accumulation at 4 DAT in glufosinate treated resistant plants was very low (160 ug/g fresh weight) whereas in susceptible plants it ranged between 2306 to 3594 ug/g fresh weight. This amount is approximately 14 to 23 times greater ammonia accumulated than in the glufosinate treated resistant plants. Ammonia concentration in non-treated plants was similar to that of the treated glufosinate resistant plants. All the data generated from the glufosinate dip test, spray test, and ammonia assay fit a 3:1 gene ratio but not the 15:1, 9:7, or 13:3 ratios. Results from the spray test and ammonia accumulation test suggested that a single dominant nuclear-encoded gene confers resistance to glufosinate at 1.1 kg/ha rate in the populations tested.

Based on these results from controlled crosses, it can be concluded that hybridization between rice and red rice occurs with either one of the rice types as a maternal parent. During large scale cultivation of glufosinate resistant rice, there is a distinct possibility for transfer and expression of resistance in F1 hybrids with the pollen movement in either direction. Thus far, studies on the influence of the glufosinate resistance on the competitive ability of rice indicates that the resistance does not impart a competitive advantage (Braverman 1997), but the competitive ability of the glufosinate resistant
red rice hybrids is not known. Natural introgression has still not been demonstrated with an engineered gene in rice due to environmental regulations. Future studies will investigate this risk analysis in field conditions as field size, proximity to wild relatives, and environmental conditions are all likely to affect risk.

LITERATURE CITED


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CHAPTER 7

SUMMARY

Preliminary studies that evaluated glufosinate at 1.1 and 2.2 kg ai/ha on BAR transformed Gulfmont and Koshihikari showed that Koshihikari lines were generally more sensitive than Gulfmont. Rice yields of all Gulfmont lines and six of nine Koshihikari lines were not affected by 2.2 kg/ha glufosinate. Efficacy of glufosinate on 3- to 4- leaf red rice was reduced when flood water was present at application. A sequential application of 0.25 kg/ha glufosinate one week after the first application controlled red rice as were a single application of 1.1 kg/ha. In greenhouse studies, efficacy of glufosinate was reduced when 25 to 50% of red rice was submerged under flood. Plant heights and dry weights of red rice increased as flood water depth increased at all rates of glufosinate. This research indicated that sequential applications are required for consistent red rice control in both flooded and non-flooded conditions.

Field studies evaluated single applications of glufosinate to BAR transformed Gulfmont rice and single and sequential applications of glufosinate on red rice at different growth stages. Injury on transformed Gulfmont was in the order of 1- to 2- leaf > 3- to 4- leaf > PI >
Though foliar injury was reduced due to applications at boot stage, grain yield was reduced by 16%. Single applications of glufosinate controlled red rice more effectively at 3- to 4-leaf followed by PI and boot. Injury to red rice was 2 to 11 times greater than the injury to BAR transformed rice depending on glufosinate rate and application timing. Sequential applications of glufosinate were more efficacious than single applications regardless of growth stage.

In field studies, post-emergence application of glufosinate alone and in combination with pendimethalin, thiobencarb, quinclorac, propanil, bensulfuron, bentazon, acifluorfen, or triclopyr were evaluated on BAR transformed Gulfmont rice and red rice. Combinations of triclopyr or acifluorfen with glufosinate were injurious to BAR transformed rice compared with glufosinate application alone. Though rice yields were not consistent between years with glufosinate applied alone, glufosinate-triclopyr mixture reduced rice yields in both years the experiment was conducted. Greater phytotoxicity of glufosinate-triclopyr observed on BAR transformed rice was not apparent with red rice. However, glufosinate applied with propanil or acifluorfen were more effective in controlling red rice than glufosinate alone and combination of glufosinate with other herbicides such as pendimethalin, thiobencarb,
quinclorac, bensulfuron, bentazon, and triclopyr. No antagonism on red rice control was observed due to other herbicide combinations with glufosinate.

BAR transformed Gulfmont rice in comparison with non-transformed Gulfmont and red rice were evaluated in greenhouse for cross-resistance to other herbicides that are commonly used in soybeans. With all the herbicides tested, injury on BAR transformed rice was either equal or lower than non-transformed rice and red rice. Since BAR transformed rice exhibited no cross-resistance to any of the herbicides, farmers can control red rice in soybeans without concern if a glufosinate resistant red rice develops. Studies that evaluated the baseline resistance level of non-transformed Gulfmont and Koshihikari in addition to red rice suggested that greater resistance of BAR transformed Gulfmont rice to glufosinate previously observed in the field can be related to its greater level of inherent resistance to glufosinate in non-transformed Gulfmont. If BAR transformed Koshihikari is commercialized, it is advisable that farmers use lower rates of glufosinate on Koshihikari than on Gulfmont.

Reciprocal controlled crosses were made in greenhouse to study the inheritance of glufosinate resistance from rice to red rice. Results from single leaf dip, whole plant spray and ammonia accumulation suggested that
glufosinate resistance segregates in 3:1 ratio in F$_2$
generation in all the crosses. Results suggest that there
is a possibility for the development of glufosinate
resistant red rice with the pollen movement in either
direction, i.e., from BAR transformed rice to red rice or
from red rice to BAR transformed rice. Further studies
should be conducted to investigate the risk involved in the
transfer of BAR gene under field conditions as influenced
by field size, proximity to wild relatives, and
environmental conditions may affect risk.
VITA

Sujatha Sankula was born on May 18, 1968, to Padmanabham and Rama Devi Sankula in Gudivada, Andhra Pradesh, India. During her schooling, Ms. Sankula actively participated in several district and national competitions in essay writing, elocution, poetry, etc. Having graduated with distinction from St. Theresa's college, Eluru, Andhra Pradesh, in 1985, Ms. Sankula found her aptitude for agriculture for her undergraduate degree. She enrolled in Andhra Pradesh Agricultural University, Bapatla, India and earned Bachelor of Science degree in 1989 during when she won a gold medal for securing highest Overall Grade Point Average (OGPA) in entomology in Bachelor of Science in the state of Andhra Pradesh. She was also an awardee of ICAR (Indian Council of Agricultural Research) 's 'Merit-cum-Means' scholarship and Chief Minister's Andhra Lalitha Kala Saarasvatonnata Scholarship during her undergraduate study. She specialized in Agronomy in her Master of Science program and upon graduating (1991) she taught biology and chemistry at junior and senior high school level. Ms. Sankula embarked on a doctoral degree program under the direction of Dr. Michael P. Braverman in the Department of Plant Pathology and Crop Physiology at Louisiana State University in the area of weed science in August, 1993.
During this time, she served as the president of Graduate Student Association of Plant Pathology and Crop Physiology. She received "Watamull Grant" awarded to the outstanding Indian student of L.S.U. in 1994. She presented three oral papers and two posters in the annual meetings of Southern Weed Science Society, one oral paper each at the annual meetings of Weed Science Society of America, Rice Technical Working Group, and Louisiana Plant Protection Association. During her stay at L.S.U. she headed the Programming Council of International Cultural Center, L.S.U and served as treasurer of Indian Student Association. Ms. Sankula has authored two refereed journal articles, 8 abstracts, and one Rice Research Station publication. Ms. Sankula was married to Murali Thota in February, 1996.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Sujatha Sankula

Major Field: Plant Health

Title of Dissertation: Potential for Glufosinate as a Selective Herbicide for Red Rice Control in BAR-Transformed Rice

Approved:

Michael Braverman
Major Professor and Chairman

John M. Landri
Dean of the Graduate School

EXAMINING COMMITTEE:

W. C. Rush

Date of Examination:
February 20, 1997