IMAZETHAPYR: RED RICE CONTROL AND RESISTANCE, AND ENVIRONMENTAL FATE

A Dissertation

by

LUIS ANTONIO DE AVILA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2005

Major Subject: Agronomy

IMAZETHAPYR: RED RICE CONTROL AND RESISTANCE, AND ENVIRONMENTAL FATE

A Dissertation

by

LUIS ANTONIO DE AVILA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Scott A. Senseman
Committee Members,	Garry N. McCauley
	James M. Chandler
	Kirby C. Donnelly
Head of Department,	C. Wayne Smith

August 2005

Major Subject: Agronomy

ABSTRACT

Imazethapyr: Red Rice Control and Resistance, and Environmental Fate. (August 2005)
Luis Antonio de Avila, B.S., Universidade Federal de Santa Maria (UFSM - Brazil);
M.S., Universidade Federal de Santa Maria (UFSM - Brazil)
Chair of Advisory Committee: Dr. Scott Allen Senseman

Imazethapyr was recently approved for use in rice, but limited information is available regarding its efficacy, environmental fate or potential red rice resistance. Therefore, experiments were conducted to 1) determine the effect of flooding time, and stage of imazethapyr application in red rice control, 2) assess the acetolactate synthase resistance to imazethapyr on red rice ecotypes, 3) determine the relative photolysis of imazethapyr, and 4) determine the effect of soil and moisture on imazethapyr adsorption and availability.

When imazethapyr was applied in sequential application of PRE followed by a POST application, to achieve >95% red rice control, flood needed to be established within 14 DAT when imazethapyr was applied EPOST, and 7 DAT when imazethapyr was applied LPOST. Delaying the flood up to 21 DAT reduced rice grain yield for both EPOST and LPOST application timings.

Based on enzymatic activity, the mean I_{50} values were 1.5, 1.1, 1.5, 1.6, 20.8, and 590.6 μM of imazethapyr, respectively, for LA 5, MS 5, TX 4, 'Cypress', 'CL-121', and 'CL-161'. CL-161 was 32 times more resistant than CL-121, and at least 420 times more resistant than the average of the red rice ecotypes and 'Cypress'. Results from the ALS assay showed that red rice ecotypes and Cypress had high susceptibility to imazethapyr when compared with the tolerant CL-121 and the resistant CL-161. Measurable enzymatic tolerance to ALS-inhibiting herbicides has not yet developed in these red rice ecotypes.

Imazethapyr quantum yield (ϕI) was 0.023 ± 0.002 while the hydroxyl radical rate constant ($k_{\bullet OH}^{I}$) was 2.8 ± 0.44 x 10^{13} M^{-1} h^{-1}. These results show that imazethapyr is susceptible to both direct and indirect photolysis. The results also show that imazethapyr photolysis in paddy water will be affected by turbidity due to its impact on the availability of sunlight to drive direct and indirect photolysis reactions.

Imazethapyr was more available and more concentrated in sandy soil. With higher amounts of water in soil there was greater amount of imazethapyr in soil solution and a lower concentration of herbicide due to dilution. The double centrifuge method provided a better estimate of plant available herbicide.

DEDICATION

To my beloved wife Marcia and daughter Carolina.

To my Father (In memoriam) and my Mother (In memoriam).

To my family in Brazil.

To the Brazilian people.

ACKNOWLEDGMENTS

I would like to thank to Dr. Senseman for his guidance during my degree. I would like to thank Dr. McCauley for his advice and help in bringing me here for my degree and to Dr. Chandler and Dr. Donnelly for serving on my committee and for all their help. I am very grateful to Dr. Massey and Dr. Armbrust in their help in the photolysis part of this work, and to Dr. Lee for his help in the ALS and adsorption experiments.

I am also grateful to CAPES-BRAZIL for sponsoring my studies and to the Federal University of Santa Maria (Universidade Federal de Santa Maria - UFSM) for allowing me to leave for my degree. And I am especially thankful to Dr. Claudio Lovato, Dr. Enio Marchezan, and Dr. Luiz Marcelo Costa Dutra for teaching my classes during my absentia.

I am very thankful to my wife Marcia Vizzotto who helped me during my career, doing an outstanding job as wife, as graduate student, and now as mother of our beloved Carolina Vizzotto de Avila. I am deeply grateful to my father Ataliba de Avila and my mother Almerinda Maria Pedrotti de Avila who are not among us, for their support during my studies, and for serving as a model for my life. I would like to thank my family, my brothers, and sisters, and my friends I left in Brazil and to new friends that I met in College Station.

I am thankful to my mentors during my academic studies, in chronologic order: Dr. José Fernando Schlosser, Prof. Isaías Salin Farrett, Dr. Afrânio Almir Righes, Dr. Reimar Carlesso, Dr. Nelson Diehl Kruse, Dr. Sérgio Luiz de Oliveira Machado, Dr. Enio Marchezan, Dr. Garry Nathan McCauley, Dr. James Mike Chandler, and Dr. Scott Allen Senseman, for their contribution to my professional, scientific and personal character as advisers during my B.S., M.S., and Ph.D. degrees.

My gratitude also goes to my colleagues at Texas A&M, Dr. Do Jin Lee, Dr. Rick Haney, Dr. Larry Jason Krutz, Dr. Audie Sciumbato, Dr. John Houston O'Barr, Dr. Greg Steele, Dr. Kathy Carson, Dr. Brian Ottis, Mr. Mark Matocha, Mr. Jeff Ullman, Mrs. Sarah Lancaster, Mrs. Judy Vader, and Mrs. Cecilia Gerngross.

TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

Red rice (*Oryza sativa* L.) is one of the most troublesome weeds in the United States rice production (Webster 2000). Red rice interference in rice causes reductions in grain yield, with the degree of loss depending on the infestation level and crop management (Diarra et al. 1985; Montealegre and Vargas 1989; Pulver 1986; Souza and Fischer 1986; Kwon et al. 1991). Competition data showed that each seedhead of red rice per square meter caused a rice yield reduction of 16 to 18 kg ha^{-1} (Montealegre and Vargas 1989; Souza and Fischer 1986).

Development of commercial rice tolerant to the imidazolinone herbicide family allows the use of imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} (Figure 1) in commercial rice for weed control (Croughan 1994). This herbicide has a broad weed control spectrum and is primarily used in soybean and peanut (Vencill 2002). Imazethapyr has been shown to control some important rice weeds in imidazolinone-tolerant rice, in both drill seeded (Webster and Baldwin 1998) and pre germinated water seeded systems (Masson and Webster 2001). Imazethapyr has shown better weed control when applied postemergence as compared to a preemergence (PRE) application (Masson and Webster 2001; Steele et al. 2002). Some problem weeds, such as red rice require sequential applications to obtain the desired control and to reduce crop injury (Dillon et al. 1999; Steele et al. 2002).

Imazethapyr applied pre-plant incorporated at 140 g ha^{-1} in Arkansas, provided 98% control of red rice (Dillon et al. 1999). The same rate applied preemergence provided 99% control and the delayed pre-emergence treatment provided 93% control (Dillon et al. 1999).

This dissertation follows the style of Weed Science.

In Texas, at least 96% red rice control was observed with single postemergence applications of imazethapyr at 70 g ha^{-1} or higher rates (Steele et al. 2002). Red rice control with sequential applications was better than any single application, regardless of rate or timing (Steele et al. 2002). However in the case of red rice, 100% control is desirable for the program to be effective due to potential outcrossing between commercial rice with red rice (Dillon et al. 1999).

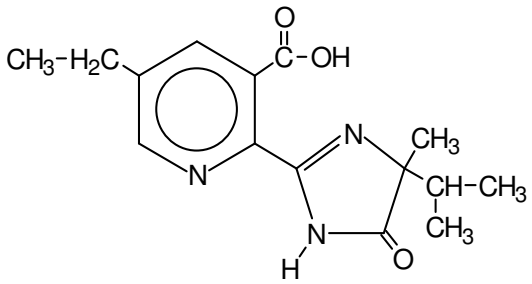

Figure 1. Imazethapyr chemical structure.

Cross pollination between red rice and commercial rice is affected by several factors including; 1) variety selection, 2) distance between red rice and commercial rice plants in the field, 3) density of red rice in the field, and 4) environmental conditions such as moisture, humidity, temperature, etc. (Beachell et al. 1936). These authors showed that cross pollination in commercial rice vary between zero and 3.4%. This means that for every 1000 seeds, up to 34 seeds can contain genetic material that potentially originated from another rice plant. Langevin et al. (1990) observed that natural crossing between red rice and 'Lemont' variety was 1% but, for the 'Nortai' variety was 52%. It is possible that the next generation of the hybridization between rice and red rice can be more prolific than their parents, normally exhibiting more dry weight, more tillers, and bigger flag leaf (Langevin et al. 1990). In some cases, the hybrid can increase the number of spikelets compared to their parents (Oard et al. 2000).

The use of cultural practices may reduce the rates of crossing, thereby increasing the utilization lifetime of the Clearfield™ system in commercial rice. Water management is an important aspect in commercial rice weed control, particularly for red rice control. Varying water management alters the timing and total number of weeds emerging. Machado et al. (1998) surveyed the red rice seed bank dynamics in 27 commercial rice fields and found that the time of water introduction is one of the most important aspects in red rice management. Delaying water introduction to a field increased red rice emergence and reduced commercial rice grain yield. Early flooding after rice emergence significantly increased red rice control in drill-seeded rice (Machado et al. 1998; Noldin 1988).

Effective water management in imidazolinone-resistant rice can enhance red rice control and minimize outcrossing, ultimately, increasing the useful lifetime of the technology. For this reason it is necessary to study the effect of water management and timing of imazethapyr application on red rice control.

Although good red rice control by imazethapyr can be obtained, some red rice ecotypes have demonstrated tolerance to imazethapyr, including the blackhull TX 4 which has been controlled only up to 85% with imazethapyr at full field rate (Gealy and Black 1999). Red rice ecotypes differ in morphological characteristics (Diarra et al. 1985; Noldin et al. 1999b), growth pattern (Diarra et al. 1985; Noldin et al. 1999b), herbicide sensitivity (Noldin et al. 1999a, Gealy et al. 2000a), emergence (Gealy et al. 2000b) and genetic characteristics (Vaughan et al. 2001). Tolerant red rice ecotypes could diminish the benefit of the CLEARFIELD* system because the plants that are left uncontrolled in the field can outcross with the commercial rice variety variety (Langevin et al. 1990; Oka et al. 1961) resulting in tolerant offspring.

Imidazolinone tolerance or resistance is species dependent and has been attributed to many factors including differences in metabolism (Cole et al. 1989; Little and Shaner 1991; Masson and Webster 2001), foliar absorption, translocation (Ballard et al. 1995; Little and Shaner 1991; Shaner and Robson 1985), and an altered acetolactate synthase

(ALS) (Al-Khatib et al. 1998; Subramanian et al. 1994). It is not known which mechanism is responsible for imazethapyr tolerance in red rice.

The sensitivity of plants to the imidazolinones is species dependent. Selectivity has been attributed to differential metabolism (Cole et al. 1989; Little and Shaner 1991; Masson and Webster 2001), reductions in foliar absorption, translocation and metabolism (Ballard et al. 1995; Little and Shaner 1991; Shaner and Robson 1985). However, these aspects are not always sufficient to explain imazethapyr tolerance. In many cases, the resistance is due to altered acetolactate synthase (ALS) (Al-Khatib et al. 1998).

Acetolactate synthase or acetohydroxyacid synthase (AHAS) is the target site of the imidazolinone herbicides (Devine et al. 1993). ALS is a key enzyme in the biosynthesis of the branched-chain amino acids leucine, valine, and isoleucine. Inhibition of ALS leads to rapid growth cessation in susceptible species.

Therefore, it is necessary to screen red rice ecotypes that showed tolerance in the field have ALS enzyme tolerance/resistance to imazethapyr. By screening these ecotypes and determining the level of ALS tolerance/resistance, we may be able to more effectively use this technology.

The behavior of the herbicide in the environment has a great influence on its soil activity and weed control. Herbicide in the soil can be lost by volatilization, photolysis, microbial degradation, chemical degradation, or plant uptake (Goetz et al. 1990). The rates of degradation and the persistence of these herbicides are affected by temperature, moisture, organic matter, and soil adsorption (Goetz et al. 1990).

One of the most important factors related to imazethapyr degradation in soil is photolysis (Mangel 1991). Substantial losses by photodegradation of imazethapyr occur within 65 hours (Vencill 2002). Ultraviolet light caused 100% degradation of imazethapyr in aqueous solution after 48 h (Curran et al. 1992b).

Imazethapyr undergoes aqueous photolysis with half-lives ranging from 44 h at pH 5 to 57 h at pH 9 (Shaner and O'Conner 1991). Because imazethapyr is weakly absorbed to soil (Vencil 2002) and is hydrolytically stable in water and under anaerobic aquatic

conditions (Shaner and O'Conner 1991), photolytic mechanisms could play an important role in the dissipation of imazethapyr. Photolysis is an important dissipation mechanism for certain other pesticides used in commercial rice production (Armbrust 1999).

The rate of direct photolysis is determined by light intensity, and the extent of light absorption and quantum yield of the molecule (Zepp 1978; Zepp and Cline 1977). Quantum yield (ϕ) is used to estimate direct photolysis rates under different use scenarios (Mill 1999) and, therefore, is an important environmental parameter for photo-labile compounds (Wan et al. 1994).

Indirect photolysis is another important contributor to pesticide degradation in rice paddies (Armbrust 2000; Mabury and Crosby 1996). Singlet oxygen, alkylperoxy radicals, triplet states, and hydroxyl radicals are highly reactive chemical species present in natural waters that acts as intermediates in indirect photolytic reactions (Mabury and Crosby 1996). The hydroxyl radical ($\cdot OH$) is the most reactive species toward a wide variety of organic compounds (Buxton et al. 1988), and can be formed in surface water by several mechanisms, including the photolysis of nitrate or dissolved organic carbon, and by reactions between H_2O_2 with Fe(II) (Schwarzenbach et al. 1992). Inclusion of hydroxyl radical reactions in environmental models such as EXAMS often improves correlations between the observed and predicted behaviors of pesticides in natural waters (Armbrust 1999; Armbrust 2000).

The quantum yield and hydroxyl rate constant for imazethapyr have not been reported in the literature but are necessary to fully evaluate its environmental fate in aquatic systems such as rice paddies.

Imazethapyr is relatively safe to the environment due to its low mammalian toxicity (Vencill 2002). Imazethapyr dissipates in the environment mainly by biodegradation (Flint and Witt 1997). Studies have demonstrated little downward movement of imazethapyr in the field under normal application conditions (Gan et al. 1994). It has been postulated that net water flow in soil during the growing season is upward, and hence limits the downward movement of the weakly adsorbed imazethapyr residues (Gan et al. 1994). Upward movement of imazethapyr has been detected in course soil

(Wyk and Reinhardt 2001). Furthermore, the soil surface becomes more acidic as moisture levels decrease, thus further immobilizing the residues due to increased sorption at the lower soil pH values (Gan et al. 1994). Contrasting with those results, imazethapyr was the most frequent herbicide detected in rivers and ground water in the Midwest US (Battaglin et al. 2000) and when studied with undisturbed soils, imazethapyr moved in a 30-cm soil column (O'Dell et al. 1992). Results have indicated that imazethapyr could leach in course soil up a 30-cm depth, depending on rainfall amounts (Wyk and Reinhardt 2001). This difference is explained by the method of determination of imazethapyr movement in soil. Additionally, carryover problems with imazethapyr have also been reported (Bresnahan et al. 2000; Johnson et al. 1993; Kin et al. 1995; Moyer and Esau 1996; Zhang et al. 2002). Carryover is dependent on herbicide soil solution concentration since the amount of herbicide taken up by a plant is a function of the degree of plant transpiration and the herbicide concentration in soil water (Renner et al. 1988).

The problems of ground water contamination and carryover have been detected due to some distinguishing characteristics of imazethapyr that affect its environmental behavior. Imazethapyr is persistent in the environment with half-lives ranging from 53 to 122 d (Curran et al. 1992a; Mills and Witt 1989). Imazethapyr residues have been detected 3 years after herbicide application (Loux et al. 1989b). Due to its high water solubility (1415 mg L^{-1}) and weak soil adsorption, imazethapyr can be mobile in certain soils (Madani et al. 2003; O'Dell et al. 1992; Souza 1998). Knowledge of factors affecting imazethapyr adsorption and availability is important for understanding its environmental behavior and potential crop carryover.

There is a paucity of information about red rice control, red rice resistance and environmental fate of imazethapyr in the rice environment. For these reasons, the objectives of this dissertation were: (1) to study the effects of water management on red rice control by imazethapyr, (2) to assess the ALS resistance/tolerance in red rice ecotypes, (3) to study the photolytic degradation of imazethapyr, and (4) to study the adsorption and availability of imazethapyr as affected by soil moisture.

CHAPTER II

EFFECT OF FLOOD TIMING ON RED RICE (*Oryza* spp.) CONTROL WITH IMAZETHAPYR APPLIED AT DIFFERENT DRY-SEEDED RICE GROWTH STAGES*

Introduction

Red rice (*Oryza sativa* L.) is the most troublesome weed in commercial rice in the southern US (Webster 2000). Red rice interference causes reduction in commercial rice grain yield, with the degree of losses depending on the infestation level, duration of interference, and crop management (Diarra et al. 1985; Kwon et al. 1991; Montealegre and Vargas 1989; Souza and Fischer 1986).

Competition data showed that each seedhead of red rice m^{-2} caused reduction in commercial rice yield of 16 to 18 kg ha^{-1} (Montealegre and Vargas 1989; Souza and Fischer 1986). Combining yield losses with reductions in commercial rice quality results in significant economic loss at the farm level.

Red rice is from the same genus as commercial rice, *Oryza.* In the US, red rice biotypes can be classified as *Oryza sativa* spp. indica, *O. sativa* spp. japonica, *O. nivara* and *O. rufipogon* (Vaughan et al. 2001). Because of its similarities, controlling red rice in commercial rice with traditional rice herbicides has been difficult. The use of different rice cultivation systems and crop rotation are used to manage red rice (Avila and Marchezan 2000), but these alternatives have limited efficacy on red rice control.

---

*Reprinted with permission from "Effect of flood timing on red rice (*oryza spp*.) control with imazethapyr applied at different dry-seeded rice growth stages" by Avila, L. A., S. A. Senseman, G. N. McCauley, J. M. Chandler, and J. H. O'Barr. 2005. *Weed Technology*, In press. Copyright 2005 by AllenPress.

Imidazolinone-tolerant rice offers an opportunity to effectively control red rice with little effect on crop safety (Steele et al. 2002). Imidazolinone tolerant rice was developed employing either induced mutation by gamma radiation or chemical transformation by ethyl methanesulfonate (EMS) (Croughan 1998). A subsequent EMS seed mutation imparts even greater resistance of rice to imidazolinones (Gealy et al. 2003).

Imazethapyr is used to control important weeds in imidazolinone tolerant rice, in both drill seeded (Webster and Baldwin 1998) and pre-germinated water seeded systems (Masson and Webster 2001). Imazethapyr has been more effective when applied POST as compared to PRE (Masson and Webster 2001; Steele et al. 2002). Red rice often requires sequential applications to obtain desirable weed control (Dillon et al. 1999; Steele et al. 2002). In Texas, at least 96% red rice control was observed with single POST applications of imazethapyr at 70 g ha^{-1}; conversely, red rice control with sequential applications was better than any single application regardless of rate or timing (Steele et al. 2002). Similar results were obtained by Ottis et al. (2003) where 95% control of red rice was achieved with a split application of imazethapyr.

Herbicide-resistant varieties represent an effective weed management option, especially for difficult-to-control weeds such as red rice. However, there are likely to be substantial challenges to using these varieties (Gealy et al. 2003). Growing herbicide-resistant varieties in proximity with sexually compatible *Oryza* relatives provides an opportunity for outcrossing (Gealy et al. 2003; Langevin et al. 1990; Olofsdotter et al. 2000) and ultimately, for herbicide resistance. The extent of outcrossing can be variable. Most studies show values of less than 1% (Gealy et al. 2003), although 52% outcrossing has been reported for red rice in commercial rice (Langevin et al. 1990). For this reason, complete red rice control is needed for long-term use of this technology (Dillon et al. 1999).

Agricultural practices can have an important impact on red rice infestation (Olofsdotter et al. 2000). Therefore, researchers around the world are studying management practices to minimize the development of herbicide-resistant weed

populations (Gealy et al. 2003). One of the practices that impact weed control and growth is water management. Effective water management in commercial rice becomes especially critical in integrated herbicide weed control programs (Bhagat et al. 1996). Water depth and flood timing are key components of water management and are especially important for red rice control. Because moist soil enhances red rice emergence (Ferrero 2001) and weed growth (Bhagat et al. 1999), flood timing is critical for weed suppression. Generally, delayed flood after planting encourages weed emergence, while early flood reduces weed emergence through submersion (Bhagat et al. 1996). Early flood after rice emergence significantly increases red rice control and rice grain yield (Machado et al. 1998; Noldin 1988). Effective water management in imidazolinone-resistant rice can enhance red rice control and minimize outcrossing, ultimately, increasing the useful lifetime of the technology. The objective of this experiment was to determine the optimal flood timing after imazethapyr application to maximize red rice control and commercial rice grain yield.

Materials and Methods

Field studies were conducted in Texas, during 2002 and 2003 at the Texas Agricultural Experiment Station (TAES) Research and Extension Center located near Beaumont. Soil was a League clay soil (fine, smectitic, hyperthermic, oxyaquic dystruderts) with 1% organic matter content and pH 5.8. The research area was part of a rice – fallow rotation. In late summer, in the year prior to rice, the area was disked two or three times as needed. Then, the soil surface was graded using laser guided equipment[*] to re-establish the desired slope and remove existing levees. Levees were then reestablished in early fall on 7.3-m centers. By preparing levees in the fall, needed over-winter settling occurred to minimize seepage between plots. Weeds were

[*] Leveling equipment, Spectra Laser, 5200 Mitchelldale E14, Houston, TX 77092.

chemically controlled during the winter by glyphosate[†] at 1.12 kg a.i. ha^{-1} rate. Final seedbed preparation was completed using a Lely Roterra[‡] just prior to planting.

Imidazolinone tolerant variety CL-161 was drill-seeded[§] at rate of 90 kg ha^{-1} on April 19, 2002 and April 14, 2003. Plots consisted of six drilled rows of rice spaced 18 cm apart and measuring 5.5 m long in 2002 and 6.1 m long in 2003. The seeds were pretreated with the insecticide fipronil {5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1,*R,S*)-(trifluoromethyl)sulfonyl]-1-*H*-pyrozole-carbonitrile} each year to control rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) (Way et al. 2004). Strawhulled red rice was seeded perpendicular to the rice rows using a Planet Jr.[**] driller at rate of 56 kg ha^{-1} to guarantee a substantial and uniform red rice infestation. Soil moisture in both years was adequate to promote rice emergence and activation of herbicide applied PRE. Plots were surface irrigated as needed to maintain adequate moisture for optimum rice growth and herbicide activity. In drill-seeded rice production, this technique is called flushing, and consists of flood the area and draining as soon as the soil is completely wet. A flush or a rainfall is necessary to activate the herbicide (Williams et al. 2002). Fertilization consisted of an application of 34 kg ha^{-1} of P_2O_5 as triple superphosphate pre-plant-incorporated (PPI) followed by a split application of nitrogen as urea at the 2- to 3-leaf stage (56 kg ha^{-1}), tillering (78 kg ha^{-1}), and at panicle differentiation (61 kg ha^{-1}).

Imazethapyr was applied PRE immediately after seeding at 70 g ha^{-1} followed by 70 g ha^{-1} at EPOST (3- to 4-leaf stage) or LPOST (5-leaf stage), using a CO_2 pressurized backpack sprayer with four Teejet XR8002[††] flat-fan nozzles in a boom calibrated to deliver a volume of 187 L ha^{-1} of spray solution at 40 psi. Nonionic surfactant[‡‡] at 0.25% v/v was included in all POST applications. The experimental design was a randomized complete block with a factorial arrangement of flood timing and

---

[†] Roundup Ultra, Monsanto Compay, 800 N Lindbergh Boulevard, St. Louis, MO 63167.
[‡] Lely Rotera, Vermeer Manufacturing Company, Box 200, Pella, Iowa 50219-0200.
[§] Planter, Kincaid Equipment Manufacturing, 210 West First St, Haven, KS 67543.
[**] Power Manufacturing Company, Inc. P.O. 707, Bennettsville, SC, 29512-0707.
[††] Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189-7900.
[‡‡] Nonionic surfactant, Latron AG-98® is a misture of alkylaryl polyoxyethylene glycols. Rohm and Haas, 100 independence Mall West, Philadelphia, PA 19106.

imazethapyr application stages. Treatments were replicated four times. Imazethapyr application stages included early post (EPOST) applications made at the 3- to 4-leaf stage and late post (LPOST) applications made at the 5-leaf stage. Flood timings included 1, 7, 14, and 21 days after herbicide treatment (DAT). One herbicide non-treated control was included for each combination of flood timing and application stage.

Red rice control and rice injury were estimated visually 21, 28, and 35 days after imazethapyr POST application (DAPOST) and one day before harvest (pre-harvest) using a scale of 0 to 100% where 0 = no control or rice injury and 100 = complete weed control or rice death. Rice grains were harvested with a mechanical plot harvester[§§] when grain moisture was approximately 20%. Final grain yield was adjusted to 12% moisture.

The visual estimates of red rice control were subjected to arcsine transformation prior to analysis to normalize the data. The data were tested for equality of error variance, normality of distribution, and independence. Then, the data were subjected to analysis of variance (ANOVA) testing all the possible interactions of flood timing and application stages and year. Means for significant effects were separated using Tukey's test at $p \leq 0.05$.

Results and Discussion

For plant stand count and rice injury, analysis of variance revealed no treatment by year interaction and no water management by application stage interaction; thus, data were combined over year. The plant stand count averaged 310 plants m^{-2} and was similar among treatments showing no effects of the pre-emergence treatment on rice emergence. Consistently with previous research (Gealy et al. 2003), imazethapyr did not visually injured the imidazolinone variety CL-161.

For red rice control evaluated at 21 and 28 DAPOST, the analysis of variance revealed no treatment by year interaction. Therefore, data were combined over years. Statistical analysis did not reveal a flood timing by application stage interaction, so, data were

[§§] Kubota, Kubota Corporation, 1-2-47 Shikitsu-higashi, Naniwa-ku, Osaka 556-8601, Japan.

averaged across flood timings (Table 1) and across application stages (Table 2). Red rice control by imazethapyr at 21 DAPOST was lower when flood was established at 14 or 21 DAT (Table 1). At 28 DAPOST, the lowest red rice control was observed when flood was established 21 DAT. Red rice control evaluated at 21 and 28 DAPOST was higher in the EPOST application compared with LPOST (Table 2). Similar results were found by Masson et al. (2001) for barnyardgrass control. When imazethapyr was applied at a single late post application, barnyardgrass control was reduced compared with early herbicide application stage.

Table 1. Red rice control at 21 and 28 DAPOST in response to flood timing after imazethapyr application. Data were averaged across application stages.[a]

	Red rice control [b]	
Flood timing [c]	21 DAPOST	28 DAPOST
DAT	——— % ———	
1	88 a [d]	93 a
7	79 ab	93 a
14	77 b	88 a
21	68 c	78 b

[a] Abbreviations: DAT, days after treatment; DAPOST, refers to evaluation time in days after herbicide postemergence application.

[b] Red rice control in percentage, refers to visual red rice control based on a scale from 0-100% where 0= no control and 100= total red rice control.

[c] Flood timing refers to the treatments of flood timing in days after imazethapyr postemergence treatment.

[d] Means followed by different letter are significantly different according to the Tukey test at $p \leq 0.05$.

Table 2. Red rice control at 21 and 28 DAPOST, in response to imazethapyr application stage. Data were averaged across flood timing.[a]

Application stage[c]	Red rice control[b]	
	21 DAPOST	28 DAPOST
	%	
EPOST	84*[d]	92*
LPOST	72	83

[a] Abbreviations: DAPOST, refers to the evaluation time in days after herbicide postemergence application; EPOST, early postemergence application (three to four-leaf stage); LPOST, late postemergence application (five-leaf stage).

[b] Red rice control in %, refers to visual red rice control based on a scale from 0-100% where 0= no control and 100= total red rice control.

[c] Application stage, refers to the stage of rice on the time of application.

[d] Means followed by * are significantly different according to F-test at $p \leq 0.05$.

Red rice control evaluated at 35 DAPOST and at pre-harvest revealed no treatment by year interaction; therefore, data were combined over years. However, a significant interaction for flood timing and application stages were observed (Table 3). At 35 DAPOST, red rice control ranged from 75 to 97% (EPOST and LPOST). Similar results were noted for pre-harvest evaluations. These data are consistent with red rice control levels determined in earlier studies using sequential imazethapyr applications (Ottis et al. 2003; Steele et al. 2002). When imazethapyr was applied EPOST, late flood (21 DAT) resulted in 10 to 15% decrease in red rice control compared to control from earlier flood timings. When imazethapyr was applied LPOST, flood introduced at 14 and 21 DAT reduced the red rice control by as much as 8 to 13% when compared with the earlier flood timings. When flood was delayed, red rice control was reduced because early flood is important for enhancing imazethapyr soil activity (Williams et al. 2002) and reducing red rice emergence (Smith and Fox 1973). Early flood establishment reduces germination and emergence of red rice by creating anaerobic conditions in the soil (Roel

et al. 1999). Results from Ferrero (2001) showed that when the soil is kept moist the red rice emergence from the top 1cm of soil was 50%, but, and when kept flooded with 2-cm of water the emergence decreased to 18% (Ferrero 2001).

Table 3. Red rice control in percentage, at 35 DAPOST and before harvest in response to imazethapyr application stage and timing of flood. Data represent an interaction between flood timing and imazethapyr application stage.[a]

	Red rice control [b]			
	35 DAPOST		Pre-harvest	
	Application stage [c]		Application stage	
Flood timing [d]	EPOST	LPOST	EPOST	LPOST
DAT	%			
1	95 a [e]	92 a	97 a	95 a
7	97 a	92 a	98 a	96 a
14	96 a	84 b	99 a	84 b
21	85 b	79 b	84 b	80 b

[a] Abbreviations: DAT, days after treatment; POST, postemergent application; DAPOST, refers to the evaluation time, in days after herbicide postemergence application. EPOST, early postemergence application (three to four-leaf stage); LPOST, late postemergence application (five-leaf stage).

[b] Red rice control in percentage, refers to visual red rice control based on a scale from 0-100% where 0= no control and 100= total red rice control.

[c] Application stage, refers to the stage of rice on the time of application.

[d] Flood timing refers to the treatments of flood timing in days after imazethapyr postemergence treatment.

[e] Means followed by different letter are significantly different according to the Tukey test at $p \leq 0.05$.

Grain yield data analysis showed a significant treatment by year interaction. Therefore, data were presented separately for each year. Within years, there was no interaction between imazethapyr application stage and flood timing. In 2002, rice grain yield was lower when rice was flooded 21 DAT due to greater red rice competition (Table 4). In 2003, rice grain yield was not affected by flood timing. The lack of difference in the second year was probably due to severe wind and heavy rain that occurred at the end of the growing season causing excessive grain shattering. Plots where early flood was applied were more mature, therefore, making them more prone to late-season shattering from the severe weather. The lower grain yield found in the first year associated with the delayed flooding was due to lower red rice control.

Table 4. Rice grain yield in response to flood timing after imazethapyr application. Data were averaged across flood timing.[a]

	Rice grain yield	
Flood timing [b]	2002	2003
DAT	kg ha^{-1}	
1	7050 ab [c]	5110 [d]
7	7400 a	5160
14	6550 ab	5050
21	6020 b	5990

[a] Abbreviations: DAT, days after treatment.

[b] Flood timing refers to the treatments of flood timing in days after imazethapyr postemergence treatment.

[c] Means followed by different letter are significantly different according to the Tukey test at $p \leq 0.05$.

[d] Means were not different according to F-test $p \leq 0.05$.

In both years, controlling red rice with imazethapyr increased rice grain yield regardless of application timing (Table 5). Grain yield for both years was inversely correlated with respective weed densities. Red rice competition resulted in an 18% grain yield reduction in 2002 and a 55% grain yield reduction in 2003. Similar results were found for red rice competition in Arkansas rice (Diarra et al. 1985).

Table 5. Rice grain yield in response to imazethapyr application stage. Data were averaged across application stages.[a]

Application stage [b]	Rice grain yield	
	2002	2003
	kg ha^{-1}	
Nontreated control	5850 b [c]	2940 b
EPOST	7300 a	6410 a
LPOST	6970 a	6640 a

[a] Abbreviations: EPOST, early postemergence application (three to four-leaf stage); LPOST, late postemergence application (five-leaf stage).

[b] Application stage, refers to the stage of rice on the time of application.

[c] Means within a year followed by different letter are significantly different according to the Tukey test at $p \leq 0.05$; prior to analysis of variance and means separation.

Results of this study indicated that timing of flood and rice stage of imazethapyr application impact red rice control and rice grain yield. In general, earlier postemergence herbicide applications provided better red rice control. For EPOST applications, flood could be delayed until 14 DAT without adversely affecting red rice control. However, flood within 7 DAT was needed to control red rice at least 95% for LPOST applications of imazethapyr. The results of this experiment are in agreement to those found by Machado et al. (1998) where flood timing was one of the most important

aspects in red rice control. Delayed flood increased red rice emergence and reduced rice grain yield. Earlier flood after commercial rice emergence significantly increased red rice control in drill-seeded rice (Machado et al. 1998; Noldin 1988).

Although red rice was not controlled completely as desired, the control was enough to increase rice grain yield in an area with a high red rice infestation. The use of other red rice control techniques in combination with the imidazolinone-resistant rice system is important to effectively manage red rice. Practices such as crop rotation and fallow need to be included into the rice management strategy to reduce outcrossing and ultimately, prolonging the use of this technology. Also, it will be necessary to develop new herbicide-resistant or herbicide-tolerant varieties to allow rotation of herbicide modes of action to achieve long-term red rice control. Until new herbicide-resistant varieties are available, researchers need to study strategies that further enhance red rice control in the imidazolinone-resistant rice system.

Summary and Conclusion

Field experiments were conducted in 2002 and 2003 in Beaumont, TX to evaluate the effect of flood timing on red rice control with imazethapyr applied at different commercial rice growth stages. Treatments included flood establishment at 1, 7, 14, and 21 days after postemergence herbicide treatment (DAT). Imazethapyr was applied preemergence (PRE) at 70 g ai ha^{-1} followed by 70 g ha^{-1} postemergence (POST) when imidazolinone tolerant rice variety CL-161 had 3- to 4-leaf stage (EPOST) or 5-leaf stage (LPOST). Flood needed to be established within 14 DAT to achieve at least 95% red rice control when imazethapyr was applied EPOST. However, flood needed to be established within 7 DAT to provide at least 95% red rice control when imazethapyr was applied LPOST. Delaying the flood up to 21 DAT reduced rice grain yield for both application timings.

CHAPTER III

ASSESSMENT OF ACETOLACTATE SYNTHASE (ALS) TOLERANCE TO IMAZETHAPYR IN RED RICE ECOTYPES (*Oryza* spp.) AND IMIDAZOLINONE TOLERANT/RESISTANT RICE VARIETIES (*Oryza sativa*)*

Introduction

One of the ten most troublesome weeds of commercial rice (*Oryza sativa* L.) in southern USA is red rice (Dowler 1994; Webster 2000) which belongs to the same genus as commercial rice (*Oryza* spp.) (Vaughan et al. 2001). It has long seed longevity (Goss and Brown 1939; Noldin 1995), and the ability to emerge from deep soil depths (Gealy et al. 2000b) making it a very aggressive weed. Grain yield reduction by red rice competition depends on several factors, including the severity of the infestation, duration of competition, and crop management (Montealegre and Vargas 1989; Kwon et al. 1991). Competition data showed that one red rice seedhead square meter caused a rice yield reduction of 16 kg ha^{-1} (Montealegre and Vargas 1989; Souza and Fischer 1986).

Red rice control has been partially achieved using cultural practices such as water-seeded rice, transplanted seedlings, stale seedbeds, crop rotation and fallow (Avila and Marchezan 2000). However, these systems are not applicable to all conditions, such as rice rotations in saline or hydromorphic soils (Ferrero et al. 1999). Controlling red rice with traditional rice herbicides has been mostly unsuccessful (Steele et al. 2002).

---

*Reprinted with permission from "Assessment of acetolactate synthase (ALS) tolerance to imazethapyr in red rice ecotypes (*Oryza* spp.) and imidazolinone tolerant/resistant rice varieties (*Oryza sativa*)" by Avila, L. A., S. A. Senseman, D. J. Lee, G. N. McCauley and J M. Chandler. 2005. *Pest Management Science*, 61:171-178.

Imidazolinone-tolerant rice varieties (CLEARFIELD*) have recently been released. Imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} is an imidazolinone herbicide used in conjunction with the CLEARFIELD* varieties to control several weeds including red rice. This herbicide controls many broadleaf weeds and several annual grasses in soybeans [*Glycine max* (L.) Merr.], peanut (*Arachis hypogaea* L.) and imidazolinone-tolerant crops, including rice (Vencill 2002). Imidazolinone herbicides inhibit acetolactate synthase (Devine et al. 1993) which is responsible for biosynthesis of the branched-chain amino acids leucine, isoleucine and valine (Anderson and Hibberd 1985; Devine and Shukla 2000). The use of imazethapyr in these tolerant varieties has been effective in controlling red rice with good crop safety (Steele et al. 2002). However, some red rice ecotypes have demonstrated tolerance to imazethapyr, including the blackhull TX 4 which has been controlled only up to at least 85% with imazethapyr at full field rate (Gealy and Black 1999). Red rice ecotypes differ in morphological characteristics (Diarra et al. 1985; Noldin et al. 1999b), growth pattern (Diarra et al. 1985; Noldin et al. 1999b), herbicide sensitivity (Noldin et al. 1999a, Gealy et al. 2000a), emergence (Gealy et al. 2000b) and genetic characteristics (Vaughan et al. 2001). Tolerant red rice ecotypes could diminish the benefit of the CLEARFIELD* system because the plants that are left uncontrolled in the field can outcross with the commercial rice variety (Langevin et al. 1990; Oka et al. 1961) resulting in tolerant offspring.

The intensive use of ALS inhibitors has led to weed resistance in several species (Devine and Shuckla 2000). Currently, there are 83 species that are resistant to ALS herbicides worldwide (Weed Science 2004). Imidazolinone tolerance or resistance is species dependent and has been attributed to many factors including differences in metabolism (Cole et al. 1989; Little and Shaner 1991; Masson and Webster 2001), foliar absorption, translocation (Ballard et al. 1995; Little and Shaner 1991; Shaner and Robson 1985), and an altered acetolactate synthase (ALS) (Al-Khatib et al. 1998; Subramanian et al. 1994). It is not known which mechanism is responsible for imazethapyr tolerance in red rice.

Methods used to evaluate ALS activity include whole plant bioassays, seed germination bioassays, *in vivo* ALS assays, and *in vitro* ALS assays. The use of *in vitro* ALS assays has been effective for determining ALS tolerance/resistance to ALS inhibitors when the mechanism of resistance is due partially or in whole to ALS activity. Compared with the other methods, the *in vitro* ALS assay is simple, rapid, inexpensive and reliable for identifying ALS tolerance/resistance (Kuk et al. 2003). Therefore, it is feasible that red rice ecotypes could be screened for ALS tolerance/resistance using *in vitro* ALS assays. By screening these ecotypes and determining the level of ALS tolerance/resistance, we may be able to more effectively use this technology. Based on this philosophy, the objective of this experiment was to determine if selected red rice ecotypes have ALS tolerance/resistance to imazethapyr using *in vitro* enzyme assay.

Materials and Methods

Plant Material

Three red rice ecotypes (LA 5, MS 5, and TX 4) were compared with a tolerant variety ('CL-121'), a resistant variety ('CL-161') and a conventional rice variety ('Cypress'). The red rice ecotypes were morphologically and physiologically characterized by Noldin et al. (1999b and genetically characterized by Vaughan et al. (2001). The red rice ecotypes were obtained from a collection of southern red rice ecotypes stored at Texas A&M University. These seeds were planted in the field in the summer of 2002 to increase seed numbers.

Whole Plant Bioassay

Pre-germinated seeds of the ecotypes and varieties were placed in 950-ml plastic cups filled with commercial growing medium (Grace Sierra Horticultural Products Company, 1001 Yosemite Drive, Milpitas, CA, USA). Cups were placed in a growth chamber programmed at 25 °C day and 20 °C night temperatures with a 12-hr photoperiod.

Hoagland solution as described by Silva (1980) was added to the media twice weekly and the plants were irrigated to saturation every other day. Imazethapyr was applied at the 3- to 4-leaf stage as a foliar postemergence application. The herbicide plus 0.25% 'Latron', a nonionic surfactant, was applied using a spray chamber calibrated to deliver 160 l ha^{-1} of herbicide solution. The imazethapyr rates used were 0, 0.14, 1.4, 14, 70, 140, 280, and 1400 g ha^{-1}. Plant height was taken at 14, 21, and 28 days after application and shoot dry weight was taken 28 DAT.

In Vitro Acetolactate Synthase Assay

The pre-germinated rice seeds were placed in rectangular plastic flats (52 x 25 cm) containing commercial potting media as designated previously and placed in a growth chamber at 25 °C day and 20 °C night temperature with a 12-hr photoperiod. The plants were sub-irrigated and harvested at the 3- to 4-leaf stage for enzyme extraction.

The ALS enzyme assay was conducted using a modification of the methods described by Singh et al. (1988) and Ray (1984). All the procedures were performed at 4 °C unless otherwise noted; low temperature was used to avoid the reactions to occur prematurely. Twelve g of plant shoots were pulverized in a prechilled mortar with pestle using liquid nitrogen. Shoots were homogenized in 30 ml of 0.1 M potassium phosphate buffer solution (pH 7.5) containing 50 mM $MgCl_2$, 50 mM thiamine pyrophosphate (TPP), 1 mM flavine adenine dinucleotide (FAD), 1 M sodium pyruvate and 10% glycerol. The homogenate was centrifuged at 20,000 x g for 20 min. The supernatant was decanted into a vial, containing 45% ammonium sulfate (w/v) and stored in ice for 30 min. The suspension was re-centrifuged at 20,000 x *g* for 20 min. The supernatant was discarded, and the resulting pellet was suspended with the assay buffer (pH 7.0), containing 1M sodium pyruvate, 50 mM $MgCl_2$, 50 mM TPP and 0.14 mM FAD. The enzyme was immediately assayed for activity.

The ALS assay reaction was conducted for 60 min in a final volume of 1.5 ml of reaction solution in 15-ml test tube in a constant temperature water bath at 37 °C. The

reaction solution consisted of 300 µl of the enzyme, 1185 µL of the assay buffer and 15 µL of herbicide solution. The enzyme was assayed at 0, 0.01, 0.1, 1, 3, 10, 100, 300, 1000, and 3000 µM of imazethapyr in the reaction vial. Herbicide stock solutions were prepared in a 1% methanol solution. Methanol (1%) was added in the blank treatment. The reaction was initiated by adding the pyruvate and terminated with 50 µl of 6 N H_2SO_4. The reaction product was allowed to decarboxylate at 60 °C for 15 min. Acetoin formed in the reaction was determined by incubation with a solution containing 0.5 ml of creatine 0.5% (w/v) and 1-napthanol 5% (w/v) dissolved in 10% NaOH, for color development. The solution was heated at 60 °C for 15 min. After cooling for 10 minutes, the sample was vortexed, and the absorbance was read at a wavelength of 530 nm, with a spectrophotometer. A standard curve was constructed using acetoin, which was subjected to color development procedure previously described. Specific enzyme activity was calculated from the standard curve and was based on micromoles of acetoin produced per mg of protein per hour. The protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Experimental Design and Analysis

Both the whole plant bioassay and the *in vitro* acetolactate synthase assay were conducted as a completely randomized design. Treatments were replicated three times and the experiment was repeated. The micromoles of acetoin produced per mg of protein per hour and percentage of ALS activity of the untreated control were calculated. The data were analyzed for homogeneity of error variance. The data were combined based on the determination of equal variances. The combined ALS activity data were subjected to analysis of variance and fit to log-logistic model (Seefeldt et al. 1995) using PROC NLIN (SAS 1999). The log-logistic mathematical expression used was:

$$Y = C + \left(\frac{D - C}{1 + e^{[b(\log(x) - \log(X_{50}))]}} \right) \qquad [1]$$

where C = lower limit, D = upper limit, b = slope at a point near the X_{50}; and X_{50} refers to GR_{50} and I_{50} doses resulting in 50% growth reduction (bioassay) or 50% inhibition of ALS activity (ALS assay), respectively.

The equation parameters are biologically meaningful. The upper limit D corresponds to the mean response of the untreated control; the lower limit C is the mean response at the higher doses; the parameter b describes the slope of the curve around the X_{50} (GR_{50} or I_{50}); and the values of X_{50} correspond to the dose that causes 50% response (Seefeldt et al. 1995). To run the model in SAS (1999) initial estimates for each parameter are required (Seefeldt et al. 1995). Initial estimates for I_{50} were obtained visually by plotting the data. Initial estimates for b were 1 and 2 as suggested from previous work (Seefeldt et al. 1995). Initial estimates of D and C represented mean responses of untreated plants and plants that received higher herbicide rates, respectively. The nonlinear routine used the initial estimates as a basis for obtaining a revised set of parameters that allowed the model to predict best fit of the data. This routine was iterated until stable parameter estimates were determined ultimately providing convergence to the procedure (Seefeldt et al. 1995).

The best model was chosen by comparing log-logistic models with different sets of assumptions using lack-of-fit F-test (Seefeldt et al. 1995). The models that were compared included data modeled with: 1) none of the parameters (C, D, b, I_{50} or GR_{50}) held constant; 2) upper limit (D) held constant; 3) lower limit (C) held constant; 4) slope (b) held constant; 5) upper and lower limits held constant; 6) upper limit and slope held constant; 7) lower limit and slope held constant; and 8) all variables held constant.

A resistance ratio (RR) was calculated based on X_{50} (I_{50} or GR_{50}) values with Cypress as a susceptible control. The resistant ratio was determined as follows:

$$RR = \frac{X_{50}V}{X_{50}Cypress} \qquad [2]$$

where RR= the resistance ratio; $X_{50}V$ is the estimated X_{50} value of a given variety or red rice ecotype (V); and $X_{50}Cypress$ is the X_{50} of the susceptible Cypress variety.

The following criteria were used to determine the level of tolerance/resistance. For the bioassay **Susceptible plants** were those that had GR_{50} within the 95% confidence interval of the susceptible Cypress variety. **Slightly tolerant plants** were those with GR_{50} that did not overlap the 95% confidence interval of either 'Cypress or CL-121. **Tolerant plants** were those with GR_{50} within the 95% confidence interval of the tolerant variety CL-121. **Resistant plants** were those with GR_{50} within the 95% confidence interval of the resistant variety CL-161. For the enzymatic tolerance/resistance the same criteria was used but, instead compared I_{50}*'s* .

Results and Discussion

Whole Plant Bioassay

Visible herbicide injury on susceptible plants was first observed at about 10 days after herbicide application (data not shown). The symptoms included chlorosis and stunting, followed by necrosis and eventual plant death at higher rates for susceptible plants. Resistant CL-161 plants did not show chlorosis, but had purple stems with stunting at higher imazethapyr rates.

Analysis of variance indicated that the plants responded differently to herbicide rate ($P<0.001$). Based on the lack-of-fit F-test (data not shown), the log-logistic equation provided a good overall fit to the data. The model that best described the data had upper and lower limits that were held constant; the equation parameters not held constant were the slope (b) and GR_{50} values. The observed values and the predicted values (Figure 2) showed that the model had a good overall fit of the data.

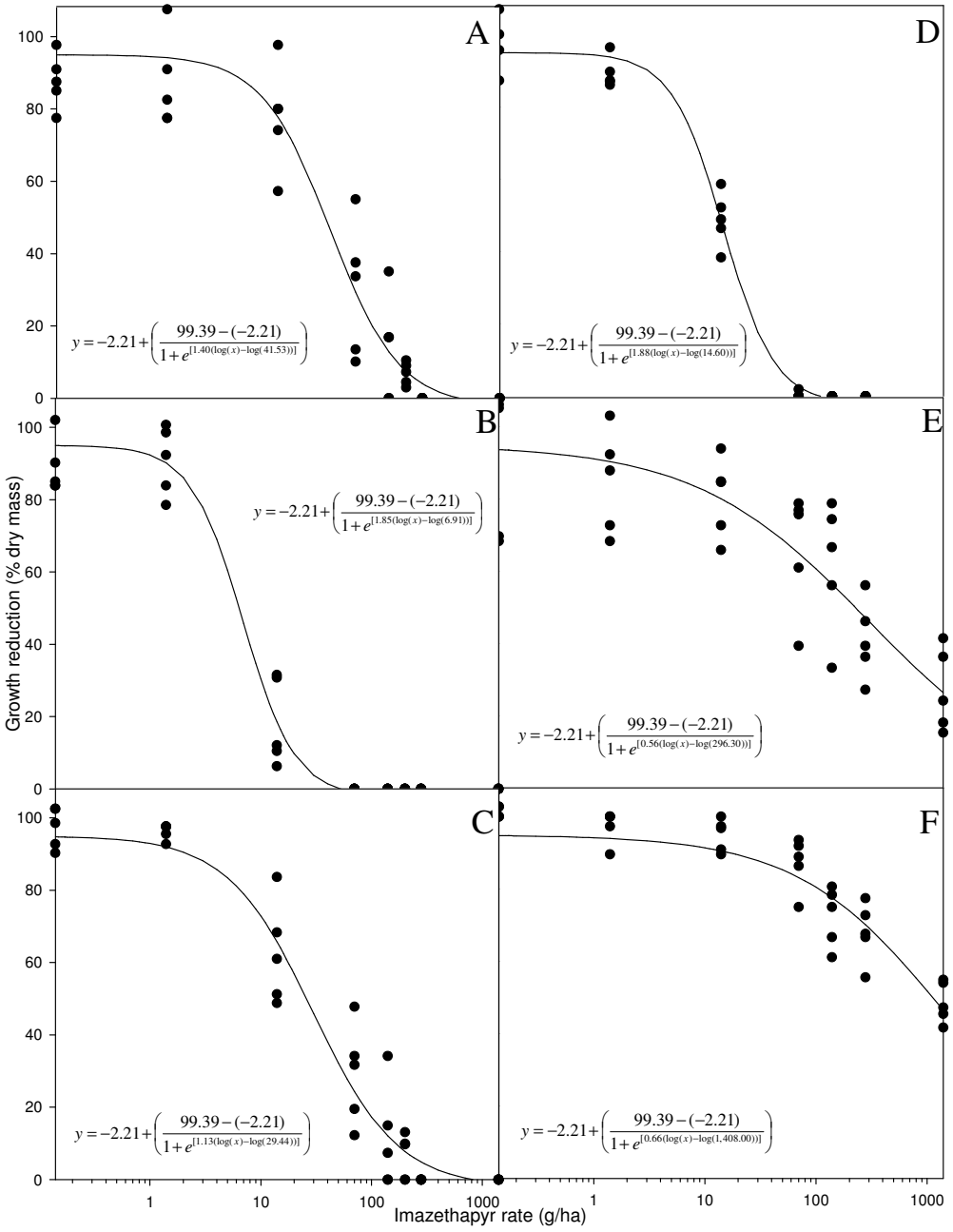

Figure 2. Fitted values (–) and observed values (●) of rice growth reduction in response to imazethapyr rates for red rice ecotypes (A = LA 5, B = MS 5, and C = TX 4) and rice varieties (D = Cypress, E = CL-121, and F = CL-161).

According to the predicted model (Figure 3), when imazethapyr was applied at the 10x rate (1400 g ha^{-1}), CL-121 and CL-161 did not reach 0% dry weight. The minimum dry weight values for CL-121 and CL-161 at the maximum rate tested (1400 g ha^{-1}) were 27 and 47%, respectively. Since these values never reached zero at this high rate, we predicted a greater imazethapyr resistance of these two varieties. Conversely, MS 5, Cypress, LA 5, and TX 4 reached 0% dry weight at 70, 140, 600, and 850 g ha^{-1}, respectively, showing differences in susceptibility.

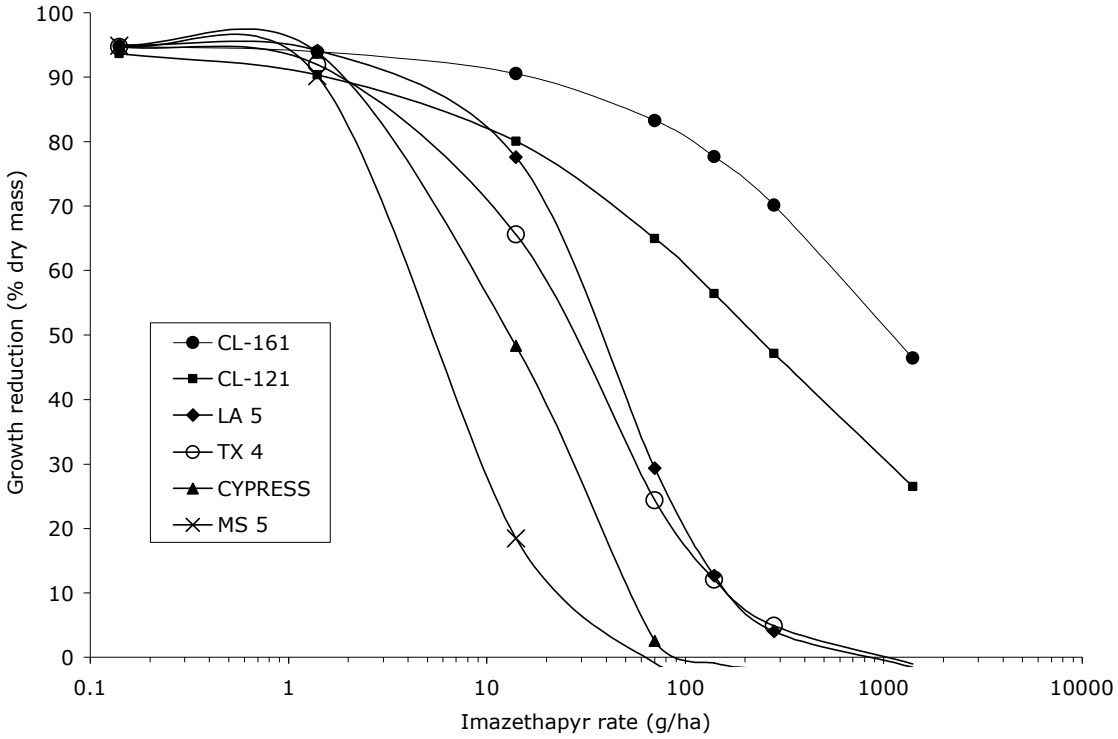

Figure 3. Modeled growth reduction of red rice ecotypes and rice varieties in response to imazethapyr rates using the log-logistic model (Seefeldt et al. 1995).

Table 6. Equation values, of C [a], D [b], b [c], growth reduction as described by GR_{50} values with confidence interval and resistance ratios for three red rice ecotypes and three rice varieties in response to imazethapyr application estimated by log-logistic analysis (Seefeldt et al. 1995).

Plant	b	GR_{50} [d] (g ha^{-1})	GR_{50} [d] (95% CI)	Resistance ratio [e]
Cypress	1.88	14.6	(12.0 - 17.2)	--
MS 5	1.85	6.9	(4.4 - 9.4)	0.5
TX 4	1.13	29.4	(21.9 - 37.0)	2.0
LA 5	1.40	41.5	(32.2 - 50.9)	2.8
CL-121	0.56	296.3	(189.6 – 403.0)	20.3
CL-161	0.66	1408.0	(722.5 - 2093.5)	96.4

[a] C (lower limit) = - 2.2 for all plants.

[b] D (upper limit) = 99.4 for all plants.

[c] b (slope around GR_{50}).

[d] Rate of imazethapyr (g ha^{-1}) that causes 50% growth reduction.

[e] Resistance ratio = $GR_{50}V$/ GR_{50}Cypress.

The predicted equation parameters for each set of plants tested are shown in Table 6. The C and D values are the same for all the plants tested while b and GR_{50} had different values for each plant. Based on overlapping 95% confidence intervals and the susceptible/tolerant/resistant criteria described in the materials and methods, the predicted GR_{50} showed that MS 5 was the most susceptible red rice ecotype. MS 5 was more susceptible than Cypress (susceptible control). Red rice ecotypes TX 4, and LA 5 were slightly tolerant because the GR_{50} values were between the susceptible (Cypress) and from the tolerant control (CL-121). Similar results for TX 4 tolerance to imazethapyr have been reported by others (Gealy and Black 1999). CL-161 exhibited the highest level of resistance and was considered the resistant control. Imazethapyr at 1400 g ha^{-1} was required to reach GR_{50}, which represented a 10x rate for rice (140 g ha^{-}

[1]). The results for CL-161 are in accordance with previous results that showed that a 5x rate did not injure CL-161 (Hackworth et al. 2002). However, CL-121 was injured with a 1x rate of imazethapyr (Malik et al. 2002). With Cypress used as a susceptible control, the resistance ratios were 96- and 20-fold greater for CL-161 and CL-121, respectively. TX 4 and LA 5 were both 2-fold greater, while MS 5 was less than 1-fold of Cypress.

In Vitro Acetolactate Synthase Assay

Differences existed in ALS response to herbicide concentrations among plants ($P<0.001$). Based on the lack-of-fit F-test the log-logistic equation, exhibited a good overall fit to the data (data not shown). The equation that best explained the data was the equation where C (lower limit), D (upper limit), and I_{50} were not held constant among plants. In this model, slope (b) was held constant for all plants indicating similar behavior as imazethapyr concentration increased. In Figure 4 it is showed the predicted values versus the observed values for each plant tested. To better evaluate data predictability by the log-logistic curves, the observed data were plotted against the fitted values (Figure 5). The calculated R^2 values for these linear plots were 0.98, 0.99, 0.97, 0.98, 0.94, and 0.88 for LA 5, MS 5, TX 4, Cypress, CL-121, and CL-161, respectively. At lower imazethapyr concentrations ALS activity varied considerably. This variability resulted with some ALS activity values being greater than the untreated control. Similar results for ALS activity have been shown by others (Seefeldt et al. 1995). However, the I_{50} values showed relatively low variability. Low variability in ALS activity in proximity of I_{50} is important because the I_{50} is the most accurate estimate of plant sensitivity to a herbicide (Seefeldt et al. 1995).

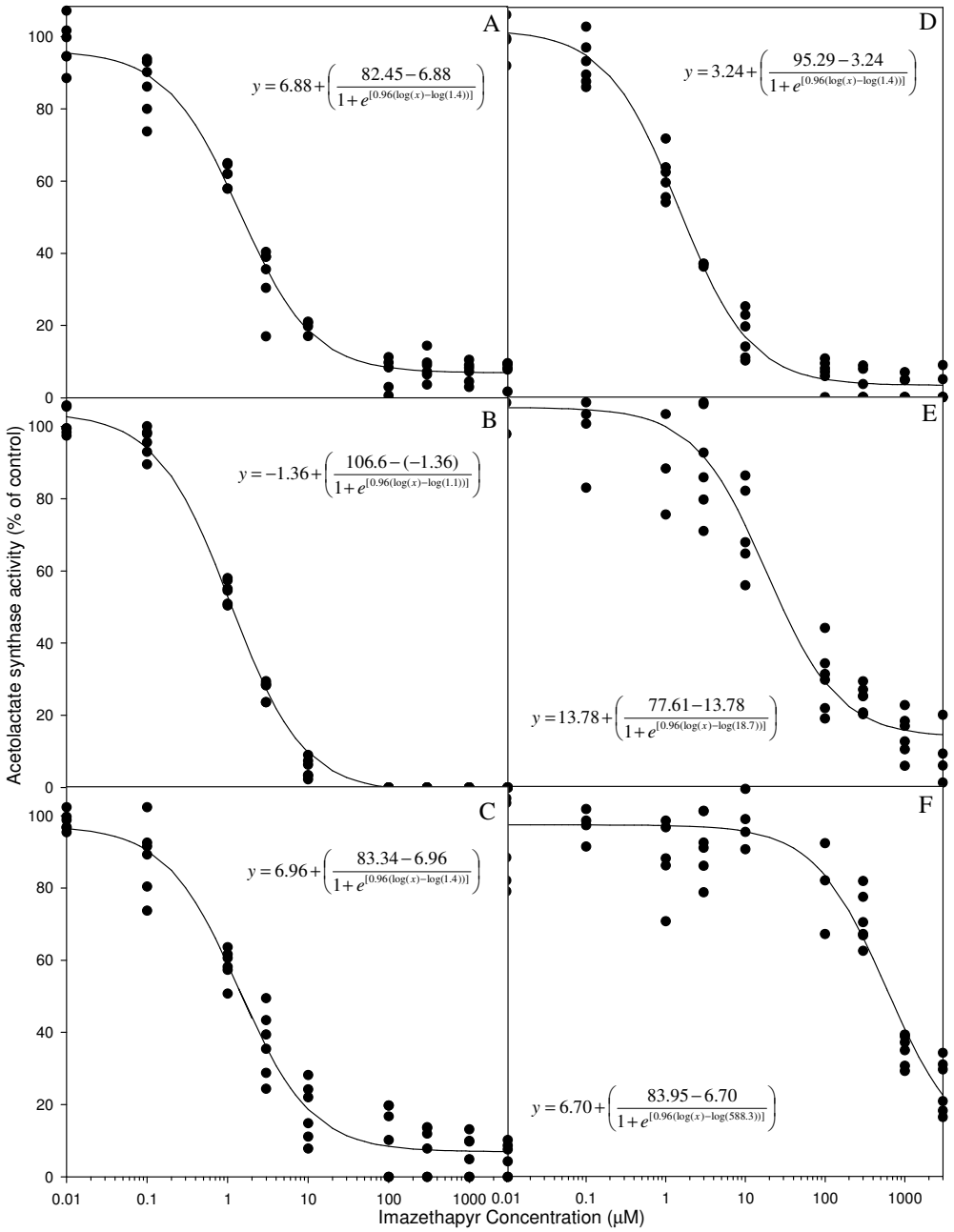

Figure 4. Fitted values (–) and observed values (●) of ALS activity in response to imazethapyr concentration for red rice ecotypes (A = LA 5, B = MS 5, and C = TX 4) and rice varieties (D= Cypress, E= CL-121, and F= CL-161).

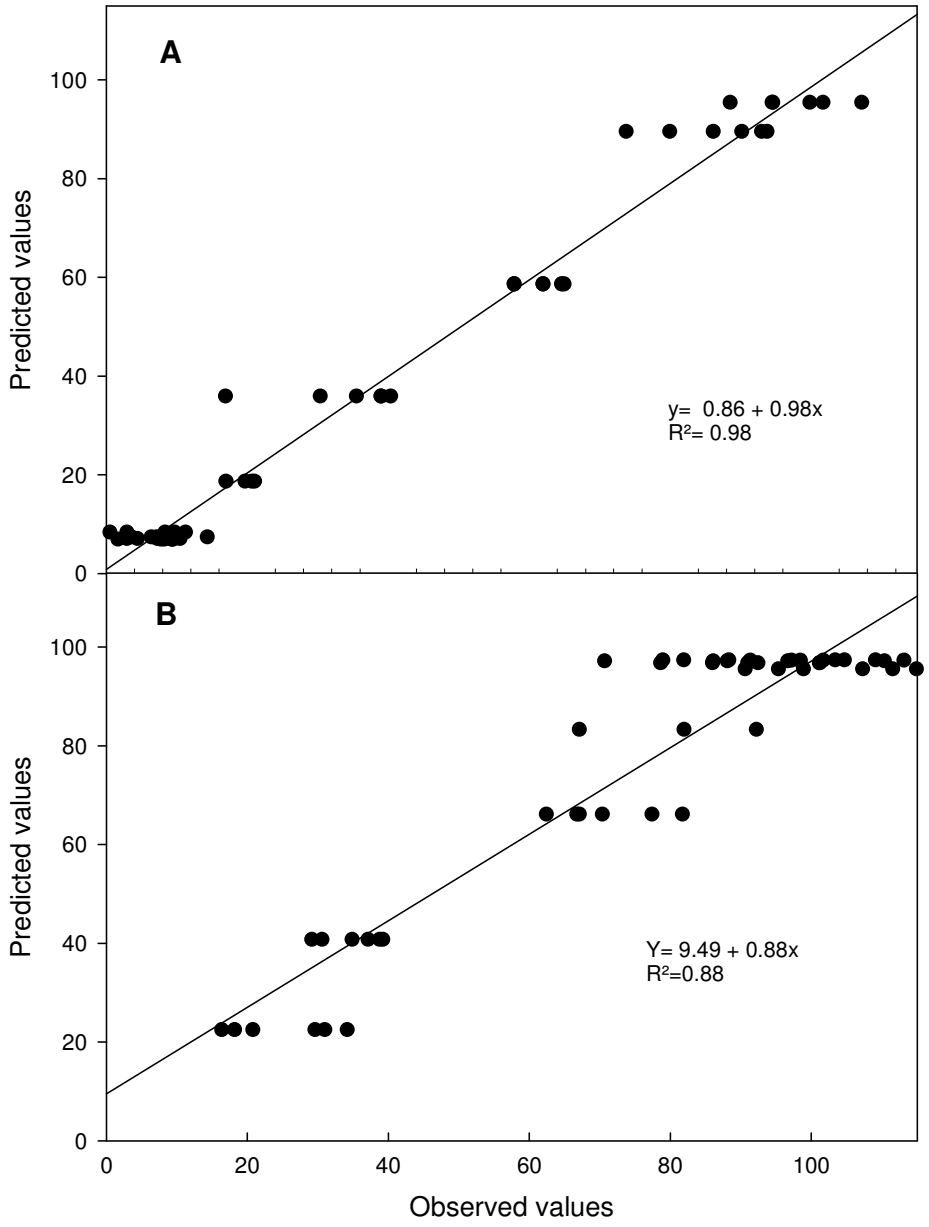

Figure 5. ALS activity observed versus predicted values for the red rice (A) red rice TX 4 and (B) variety CL-161.

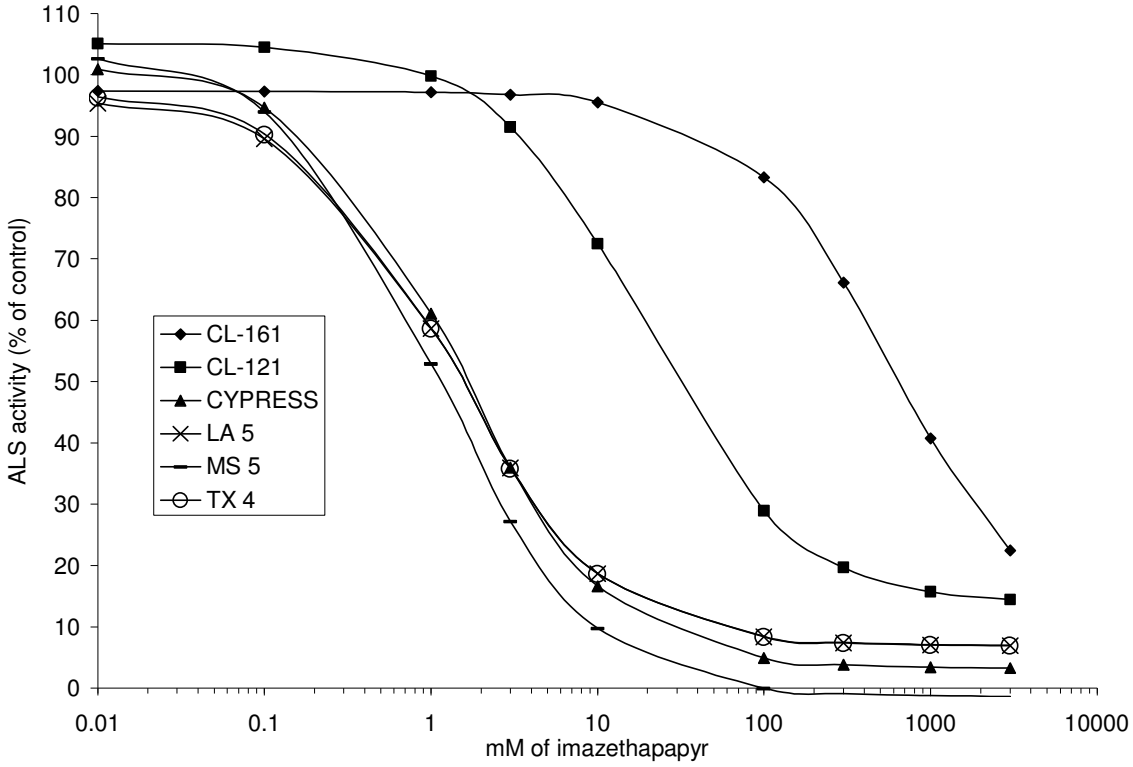

Figure 6. Modeled ALS enzyme activity as percentage of the untreated control in response to imazethapyr concentration using the log-logistic model (Seefeldt et al. 1995).

Based on the shape of the log-logistic curves (Figure 6) it is apparent that CL-161 contains an ALS enzyme that is most resistant to imazethapyr while the red rice ecotypes and Cypress were more susceptible. The decline in ALS activity commenced at approximately 0.1µM of imazethapyr for Cypress and the three red rice ecotypes, 1µM for CL-121, and 10µM for CL-161 variety. Consequently, higher rates of imazethapyr were necessary to adversely affect ALS activity of CL-161. The lower limit ALS activity for the three red rice ecotypes and for Cypress was achieved at approximately 100 µM while the CL-121 lower limit was approximately 1000 µM. CL-161 enzyme activity did not reach the lower limit with the highest imazethapyr concentration of 3000

μM. Solubility limitations of imazethapyr prevented the creation of higher concentrations needed to achieve lower limits of ALS activity for CL-161.

The predicted equation values for each set of plants tested are shown in Table 7. The slope (*b*) was the only parameter that was equal for all plants tested. Based on overlapping of the confidence intervals for the predicted I_{50} values, the plants were ranked as follows from most resistant to least resistant: CL-161 > CL-121 > Cypress = TX 4 = MS 5 = LA 5. The resistance ratios (RR) showed that CL-161 and CL-121 were 420- and 13-fold more tolerant/resistant, respectively, than Cypress; the red rice ecotypes were equally or more susceptible than Cypress. I_{50} values for the red rice ecotypes did not differ from Cypress and showed high susceptibility to imazethapyr when compared with the tolerant CL-121 and the resistant CL-161. Therefore, the basis for the tolerance observed in the bioassay and in the literature (Gealy and Black 1999) does not involve ALS tolerance to imazethapyr for these ecotypes. These observations demonstrate that the red rice ecotypes examined in this study have not developed ALS-based tolerance/resistance to imazethapyr. Most of the ALS studies have shown that resistance was due to target site mutation on ALS (Al-Katib et al. 1998). Other resistance mechanisms, such as increased herbicide metabolism or differential absorption and translocation, may have been responsible for this tolerance (Devine and Shukla 2000).

For the bioassay, CL-161 was 96-fold more resistant than Cypress (Table 6 column 5). However, in the ALS assay, CL-161 was 420-fold more resistant than Cypress (Table 7 column 6). Similar results for these parameters were found in the literature (Al-Khatib et al. 1998; Baumgartner et al. 1999). Because dose-response relationships of the whole plant bioassay and the ALS assay are not linear, a direct comparison cannot be made between GR_{50} and I_{50} (Al-Khatib et al. 1998; Baumgartner et al. 1999).

Based on the untreated control, ALS specific activity varied among plants. However, the variability was not correlated with I_{50} values (data not shown). For example, CL-161 had the highest I_{50} value, but a medium-range ALS activity. These results indicate that the CL-161 resistance to imazethapyr was probably due to altered ALS, rather than ALS overexpression (high enzyme activity). Similar results were found for *Amaranthus hybridus* (Manley et al. 1999), *Helianthus annuus* (Al-Khatib et al 1998), and *Monochoria vaginalis* (Hwang et al. 2001). The isolation of ALS was similar among plants examined based on the extractable protein (Table 7).

TX 4 and LA 5 showed slight tolerance to imazethapyr in the whole plant bioassay but did not show ALS tolerance in the ALS assay. Therefore, the tolerance reported in previous work may not be due to ALS tolerance but rather to differential metabolism, absorption, or translocation.

Table 7. Acetolactate synthase (ALS) inhibition as described by the log logistic equation values of *D* [a], *C* [b], *b* [c], and I_{50} [d] values, and confidence interval values for three red rice ecotypes and three rice varieties in response to imazethapyr application estimated by log-logistic analysis (Seefeldt et al. 1995) and ALS specific activity, protein concentration of the untreated enzyme and resistance ratio for three red rice ecotypes and two rice varieties.

			I_{50}			ALS specific activity [f,g]	Protein concentration [h]
Plant	*D*	*C*	(µM)	(95% CI)	Resistance ratio[e]	(µM acetoin mg^{-1} protein h^{-1})	(µg protein mg^{-1} fresh weight)
Cypress	95.3	3.2	1.4	(1.1 – 1.8) [e]	---	35.6 ab [g]	1.7 [h]
MS 5	106.6	-1.4	1.1	(0.8 – 1.3)	0.8	38.3 a	1.9
TX 4	83.3	7.0	1.4	(1.0 -1.7)	1.0	27.9 ab	2.0
LA 5	82.5	6.9	1.4	(1.1 - 1.75)	1.0	39.5 a	1.7
CL-121	77.6	13.8	18.7	(12.7 - 24.2)	13.4	14.4 c	2.3
CL-161	83.9	6.7	588.3	(347.6 - 829.1)	420.2	24.4 bc	2.1

[a] Upper limit or maximum ALS activity.

[b] Lower limit or minimum ALS activity.

[c] Slope of the curve around I_{50} (0.96 for all plants).

[d] Concentration of imazethapyr that causes 50% ALS inhibition.

[e] Resistance ratio: I_{50} *V*/ I_{50} Cypress.

[f] Based on the activity of the untreated control.

[g] Different letters following values indicated differences with the Tukey test (α=0.05).

[h] F-test not significant at α=0.05.

Summary and Conclusion

Three red rice ecotypes including LA 5, MS 5, and TX 4 were evaluated for acetolactate synthase resistance/tolerance to imazethapyr. The red rice ecotypes were compared with a tolerant line ('CL-121'), a resistant line ('CL-161') and a conventional rice variety ('Cypress'). Based on enzymatic activity, the mean I_{50} values were 1.5, 1.1, 1.5, 1.6, 20.8, and 590.6 μM of imazethapyr, respectively, for LA 5, MS 5, TX 4, Cypress, CL-121, and CL-161. CL-161 was 32 times more resistant than CL-121 and at least 420 times more resistant than the average of the red rice ecotypes and 'Cypress'. Results from the ALS assay showed that red rice ecotypes and Cypress had high susceptibility to imazethapyr when compared with the tolerant CL-121 and the resistant CL-161. Measurable enzymatic tolerance to ALS-inhibiting herbicides has not yet developed in these red rice ecotypes.

CHAPTER IV

QUANTUM YIELD AND AQUEOUS HYDROXYL RADICAL RATE CONSTANT OF IMAZETHAPYR HERBICIDE

Introduction

Imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} is an imidazolinone herbicide used to control broadleaf weeds and annual grasses in soybean and peanut (Vencill 2002). The recent introduction of imidazolinone-tolerant rice varieties allows it to also be used to control red rice and other weeds in commercial rice. Because the use of imazethapyr in commercial rice is relatively new, less is known about its environmental behavior in flooded rice culture. Imazethapyr is a weak organic acid (pK_a 3.9) having water solubility of 1400 mg L^{-1} (pH 7; 25^{o}C) and vapor pressure < 0.013 mPa (60^{o}C) (Vencill 2002). Its primary degradation mechanism in the environment is through microbial activity (Flint and Witt 1997) with imazethapyr having a half-life of 53 to 122 days in aerobic field soil (Curran et al. 1992a; Mills and Witt 1989). Hydrolysis is limited with none observed at pH 5 or 7 and only minimal degradation occurring at pH 9 ($t_{1/2} \approx$ 9.6 month at 25^{o}C) (Shaner and O'Conner 1991). Under anaerobic conditions as occur in flooded rice paddies, no significant degradation occurred over a two month period in a variety of soils and sediment (Shaner and O'Conner 1991). Soil residues of imazethapyr can injure succeeding crops (Bresnahan et al. 2000; Johnson et al. 1993; Kin et al. 1995; Moyer and Esau 1996).

Imazethapyr undergoes aqueous photolysis with half-lives ranging from 44 h (pH 5) to 57 h (pH 9) (Shaner and O'Conner 1991). Because imazethapyr is weakly absorbed to soil (Vencil 2002) and is hydrolytically stable in water and under anaerobic aquatic conditions (Shaner and O'Conner 1991), photolytic mechanisms could play an important role in the dissipation of imazethapyr. Photolysis is an important dissipation mechanism for certain other pesticides used in rice production (Armbrust 1999).

The rate of direct photolysis is determined by light intensity, and the extent of light absorption and quantum yield of the molecule (Zepp 1978; Zepp and Cline 1977). Quantum yield (ϕ) is used to estimate direct photolysis rates under different use scenarios (Mill 1999) and, therefore, is an important environmental parameter for photo-labile compounds such as imazethapyr (Wan et al. 1994). Schwarzenbach et al. (1992) defines quantum yield as:

$$\phi = \frac{No.\ of\ molecules\ transformed}{No.\ of\ photons\ absorbed\ due\ to\ presence\ of\ the\ molecule} \quad [3]$$

Indirect photolysis is another important contributor to pesticide degradation in rice paddies (Armbrust 2000; Mabury and Crosby 1996). Singlet oxygen, alkylperoxy radicals, triplet states, and hydroxyl radicals are highly reactive chemical species present in natural waters that acts as intermediates in indirect photolytic reactions (Mabury and Crosby 1996). The hydroxyl radical (·OH) is the most reactive species toward a wide variety of organic compounds (Buxton et al. 1988), and can be formed in surface water by several mechanisms, including the photolysis of nitrate or dissolved organic carbon, and by reactions between H_2O_2 with Fe(II) (Schwarzenbach et al. 1992). Inclusion of hydroxyl radical reactions in environmental models such as EXAMS often improves correlations between the observed and predicted behaviors of pesticides in natural waters (Armbrust 1999; Armbrust 2000).

The quantum yield and hydroxyl rate constant for imazethapyr have not been reported in the literature but are necessary to fully evaluate its environmental fate in aquatic systems such as rice paddies. For these reasons, laboratory experiments were conducted to determine the 1) photodegradation rates in three natural rice paddy waters, 2) quantum yield, and 3) hydroxyl radical rate constant for imazethapyr.

Materials and Methods

Materials

Imazethapyr (99% purity) and 2,4-D (99% purity) were obtained from Chem Service, Inc. (P.O. Box 599, West Chester, PA 19381-0599). Acetonitrile, acetophenone, Acrodiscs® 13-mm GHP syringe filters, borosilicate vials (1 mL), catalase, high purity HPLC grade water, hydrogen peroxide (30%), K_2HPO_4, p-nitroanisole (PNA) and pyridine (PYR) were obtained from Burgoon (PO Box 1168, Galveston, TX). High performance liquid chromatography/Diode array detector (HPLC/DAD) and the columns were obtained from Waters®(34 Maple Street, Milford, MA, 01757). The spectrophotometer was obtained from Beckman Coulter, Inc. (4300 N. Harbor Boulevard, Fullerton, CA 92834-3100). Cuvets used for molar absorptivity and formic acid were obtained from Fisher (200 Park Lane, Pittsburg, PA 15275). Lamps for simulating sunlight were obtained from Kelsun Distributors Inc. (13000 Bel-Red Road, Suite 206, Bellevue, WA, 98005).

Simulated Sunlight Equipment

Two 100W UV lamps (F72T12/VHO 5.0 Midday sun) were used to simulate the UV portion of sunlight emission (Armbrust 2000). The lamps were placed in a growth chamber where the temperature was kept constant at 25 ± 1 ° C.

Imazethapyr Photolysis in Rice Paddy Water

Paddy water samples were collected two to three weeks prior to study conduct from rice fields located in Beaumont, TX (BM), Clarksdale, MS (CD) and Eagle Lake, TX (EL) and stored at 4 C until use. Analyses performed on the waters included elemental analysis, total dissolved salts, hardness, conductivity, alkalinity, and pH. Buffered deionized water (pH 7.0) was included as a control.

A Beckman DU-640 Spectrophotometer and a 10-mm Suprasil 300 quartz cuvet were used to determine absorbance of each water source to check for possible light attenuation. The absorbance was measured between 290 and 800 nm using the spectrophotometer described above. Absorbance was also determined for imazethapyr in deionized water solution (pH 7.0) to compare with the paddy water absorbance. Only pH and light absorbance correlated well with imazethapyr photolysis rates. Values for pH and light absorbance for each water sample are shown in Table 8.

Table 8. Light absorbance and pH of rice paddy water samples collected from Eagle Lake, TX, Beaumont, TX and Clarksdale, MS.

Water sources	pH	Absorbance[1] % of control
Deionized Water	7.0	---
Eagle Lake	7.3	0
Beaumont	8.0	207
Clarksdale	8.2	314

[1] Based on the control (deionized water), summation of light absorbance of selected wavelengths between 290-400 nm.

Water samples were fortified with imazethapyr at 15 µg ml^{-1} (approximately 1x field rate) and placed into 1-ml borosilicate glass vials, capped and subjected to irradiation by UV lamps at a distance of 12-cm beneath the light source for periods of 0, 1, 2, 6, 12, 24, 48, 72, and 96 h. A dark control for each exposure time was included to check for chemical hydrolysis. After irradiation, the samples were filtered using Acrodisc® syringe filters to remove particulates prior to chromatographic analysis. Filtration reduced imazethapyr recovery by <1% (data not shown).

After exposure and filtration, imazethapyr was quantified by high performance liquid chromatography (HPLC) equipped with a photodiode array detector (DAD). A Waters® Symmetryshield™ RP8 3.5-µm particle size 2.1 x 150-mm column with a mobile phase of 39.4% deionized water, 1.5% formic acid and 60% acetonitrile was used for chemical analysis. Isocratic elution at 0.3 ml min^{-1} was performed and imazethapyr was detected at a wavelength of 245 nm. The natural log of the remaining imazethapyr concentration [ln (C/Co)] was calculated and the first-order plots were constructed. Photolysis half-life values were calculated using the equation:

$$t_{1/2} = \frac{\ln(2)}{k_P} \quad [4]$$

where k_p is the absolute value of the slope and first-order rate constant for imazethapyr.

Photolysis half-lives were corrected for the sunlight equivalent using mid-day sunlight measured in College Station, TX on March 27th, 2005. Correction for cylindrical test tube effect was also performed (Schwarzenbach et al. 1992). The experiment was conducted as a randomized block design with three replications. Imazethapyr first-order rate constant data were subjected to analysis of variance and Fisher's protected LSD test at $p \leq 0.05$.

Imazethapyr Quantum Yield (ϕ)

The quantum yield of imazethapyr (ϕ) was estimated using chemical actinometry (USEPA 1985) where ϕ_I is estimated by comparison with an actinometer having a known quantum yield. The chemical actinometer system used was the p-nitroanisole (PNA)/pyridine (PYR) system developed by Dulin and Mill (1982).

The molar absorptivity of imazethapyr (7.85×10^{-5} M in pH 7.0 buffer) and PNA (1×10^{-5} M in high purity water) were measured by spectrophotometry, as described earlier. The blank for imazethapyr was pH 7 phosphate buffer while the blank for PNA was high purity water. Absorbance was measured between 300 and 400 nm and absorptivity calculated for each wavelength using the Beer-Lambert law:

$$\varepsilon = \frac{A}{bc} \quad [5]$$

where ε is molar absorptivity in M^{-1} cm^{-1}, A is absorbance at wavelength λ, b is the cell path length (cm), and c is the molar concentration of imazethapyr or PNA. Maximum calculated molar absorptivity of PNA (ε_{314nm} = 10,835 M^{-1} cm^{-1}) was within 2% of reported values (ε_{314nm} = 10,965 M^{-1} cm^{-1}) (Dulin and Mill 1982). For imazethapyr, the maximum molar absorptivity occurred at 224 nm and was calculated as ε_{224nm} = 5,898 M^{-1} cm^{-1}. Molar absorptivities for both imazethapyr and PNA were used to calculate the quantum yield.

Using discreet samples for each compound, imazethapyr (7.85 x 10^{-5} M in pH 7.0 buffer) and PNA (1 x 10^{-5} M) were simultaneously exposed to the UV light source. In pilot studies, PNA was treated with various concentrations of pyridine (0.2, 0.1, 0.05, 0.005, 0.0025, and 0.0005 M) to regulate the PNA degradation rate so that it would closely match that of imazethapyr. The experiment was performed as described for the imazethapyr photolysis study, with regards to light source, exposure time, temperature, and experimental design.

Quantum yield was calculated using the equation:

$$\phi_I = \phi_{PNA}\left[\frac{k_I}{k_{PNA}} x \frac{\sum I_\lambda \varepsilon_{\lambda PNA}}{\sum I_\lambda \varepsilon_{\lambda I}}\right] \quad [6]$$

where ϕ_{PNA} is the quantum yield for PNA; k_I and k_{PNA} are the first-order rate constants for imazethapyr and PNA, respectively; I_λ is the irradiance at wavelength λ (watts m^{-2}); and $\varepsilon_{\lambda I}$ and $\varepsilon_{\lambda PNA}$ are the molar absorptivity (M^{-1} cm^{-1}) at each wavelength λ for imazethapyr and PNA, respectively. The quantum yield of the PNA actinometer was calculated using the PYR molar concentration and the equations described by Dulin and Mill (1982):

$$\phi_{PNA} = 0.00028 + 0.44\ [PYR] \quad [7]$$

The PYR concentration that regulated the PNA degradation rate to best match imazethapyr's degradation rate was 0.0005 M. Therefore, in this experiment, the calculated ϕ_{PNA} = 0.0005.

Imazethapyr Hydroxyl Radical Rate Constant ($k^{I}_{\bullet OH}$)

The imazethapyr hydroxyl radical rate constant was determined by the method used by Armbrust (2000). For this experiment, the herbicide 2,4-D was included as a benchmark to compare with other literature results. Equimolar concentrations of herbicide (either imazethapyr or 2,4-D) and acetophenone at 0.02 mM were dissolved with high purity water and transferred into clear borosilicate glass vials with no other co-solvents. Hydrogen peroxide was dissolved in each vial to generate hydroxyl radicals through the photolytic cleavage of hydrogen peroxide. Prior to light irradiation, aqueous hydrogen peroxide solution was added to generate a final concentrations of 0.5, 1, 3, 5, 10, and 20 mM. Additional controls included: 1) herbicide + acetophenone (no peroxide) in amber vial; 2) herbicide + acetophenone + 20 mM peroxide in amber vial; and 3) herbicide + acetophenone in clear vial. The vials where capped and placed 12 cm beneath the light source and irradiated for 10 min. After irradiation, the excess hydroxyl radicals were quenched with 15 µl of 500 µg ml^{-1} catalase solution (pH 7.0 phosphate buffer) as reported by Armbrust (2000). A 150-µl volume of acetonitrile and formic acid was added to the vials prior to analysis to make the samples more compatible with the mobile phase.

Acetophenone, 2,4-D, and imazethapyr were quantified by HPLC with an isocratic mobile phase consisting of 60% deionized water, 39% acetonitrile and 1% formic acid using a 0.3 ml min^{-1} flow rate. Detection wavelengths were 245- nm for both imazethapyr and acetophenone and 281 nm for 2,4-D. The experiment was conducted as a randomized block design with four replications.

The hydroxyl radical rate constant was determined for each herbicide using competitive kinetics (Haag and Yao 1992) and was calculated using the equation:

$$k^{M}_{OH^*} = \frac{\ln([M]_o / \ln[M]_\infty)}{\ln([C]_o / \ln[C]_\infty)} k^{C}_{OH^*} \qquad [8]$$

where, $k^{M}_{OH^*}$ and $k^{C}_{OH^*}$ are the rate constant for the herbicide and the reference compound (acetophenone), respectively.

Results and Discussion

Photolysis Rate Constant Determination in Rice Paddy Waters

Imazethapyr degradation in dark controls was < 1% and corrections for dark reactions were not performed. Linear regression analysis of the natural log of concentration remaining/initial concentrations (C/Co) against time (h) for each water source is shown in Figure 7. The first-order rate kinetics, half-lives, and coefficients of determination are shown in Table 9.

Although half-lives were relatively short for imazethapyr in all water sources, analysis of variance showed differences in imazethapyr half-life between water sources. Significant faster photodegradation of imazethapyr was observed in deionized water and Eagle Lake paddy water. Slower photolysis was observed in the paddy waters of Beaumont and Clarksdale. Half-life values found in this experiment are similar to the value found by Curran et al. (1992b) for imazethapyr photolysis in aqueous solution (4 h). These results demonstrated that imazethapyr has a relatively short aqueous photolysis half-life in rice paddy water. But, in rice paddy fields several factors may affect photolysis. Adsorption to colloids may affect photolysis of imazethapyr applied pre-emergence. Imazethapyr photolysis was faster in moist soil and sandy soil compared to silty clay loam soils (Curran et al. 1992a). Greater adsorption and reduced herbicide availability may reduce photolysis rates (Curran et al. 1992a). Another factor that may affect the photolysis rate is depth of water (Beretvas et al. 2000). The deeper the water profile, the lower the UV intensity (Beauclerc and Gunn 2001). Photolytic half-lives for the compound ammonium dinitramide (ADN) for a summertime irradiation, ranged from 6 min at the surface to 15 years at a depth of 2 m (Beretvas et al. 2000). Quantum yield can be used to predict photolysis at different water depths using programs such as the US EPA's GC-SOLAR . Other factor that may be very important in controlling imazethapyr photolysis is the movement of the herbicide deeper in the soil profile. Light penetration is minimal in soil depths greater than >1mm in the profile (Frank et al. 2002). Flushing

the rice field to incorporate imazethapyr may reduce its dissipation by photolysis. Therefore, longer photolysis half lives in the field are possible for imazethapyr.

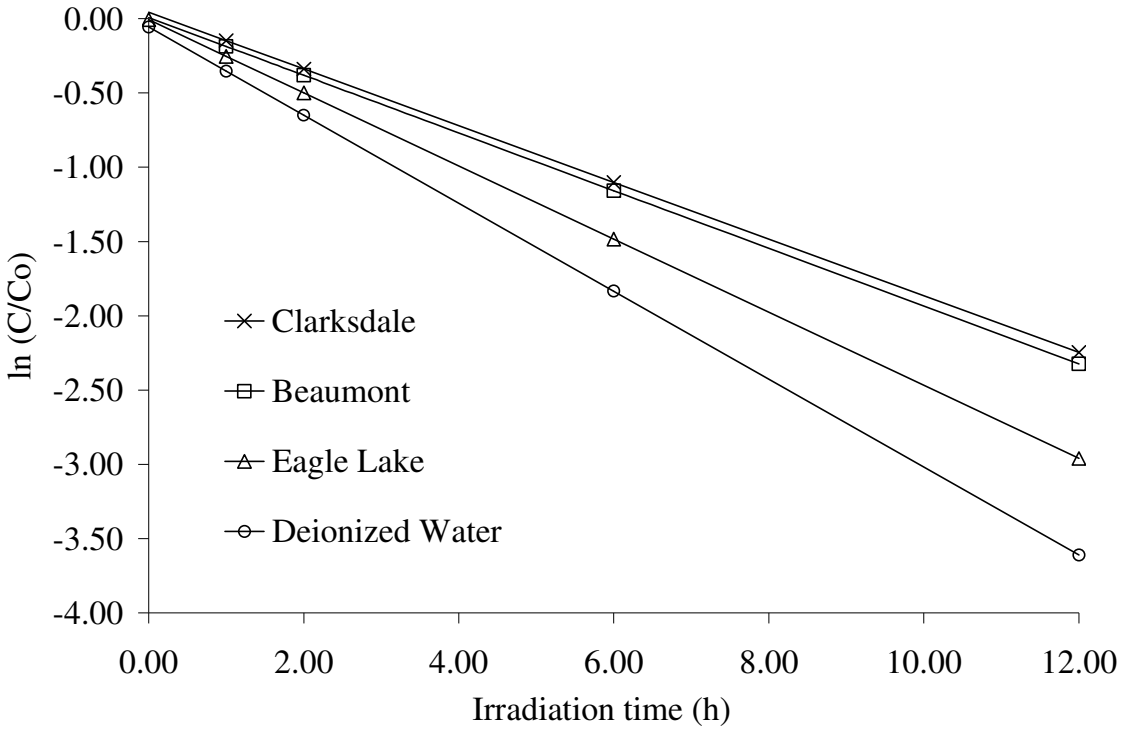

Figure 7. First-order rate plots for degradation of 15 µg ml^{-1} imazethapyr in deionized water and water collected from rice paddies at Eagle Lake, TX; Beaumont, TX, and Clarksdale, MS. Fitted equations for imazethapyr in each water source were: (Clarksdale) y = 0.0427 - 0.1908x (R^2=0.96), (Beaumont) y = 0.0065 - 0.1941x (R^2=0.98), (Eagle Lake) y = - 0.0074 - 0.2467x (R^2=0.95) and (Deionized water) y = - 0.0576 - 0.2961x (R^2=0.98).

Table 9. First-order constant (k), half-life (t ½), and coefficient of determination (R^2) for imazethapyr photolysis in deionized water and water collected from rice paddies at Eagle Lake, TX, Beaumont, TX, and Clarksdale, MS.

Water source [a]	Rate constant (k)	t ½ [b]	t ½ [c]	Coefficient of determination (R^2)
	M^{-1} h^{-1}	h	h	
Deionized	-0.296 a [d]	2.3	3.3	0.98
Eagle Lake	-0.247 a	2.8	4.0	0.95
Beaumont	-0.194 b	3.6	5.1	0.98
Clarksdale	-0.191 b	3.6	5.1	0.96

[a] Water sources: Deionized (pH 7.0), Eagle Lake rice paddy (pH 7.3), Beaumont rice paddy (pH 8.0), and Clarksdadle rice paddy (pH 8,2).

[b] Non-corrected values, values determined with UV-light exposure.

[c] Corrected for sunlight equivalent based on the light emission of unobstructed midday sunlight at College Station on March 27th 2005 and corrected for the effect of a cylindrical vial (Schwarzenbach 1992).

[d] Means within a column followed by different letters are significantly different at $P \leq 0.05$ according with LSD test.

Faster degradation of imazethapyr observed in deionized water and Eagle Lake paddy water may have been due to the low turbidity that allowed more light penetration. Conversely, the Beaumont and Clarksdale paddy waters were more turbid. Relative absorbance of each water sample at selected wavelengths is shown in Table 8. It is apparent that BM and CD paddy waters absorb more light compared to DW and EL. The largest absorbance was observed around the same wavelengths where imazethapyr effectively absorbs (data not shown). Therefore, light attenuation may have caused photolysis differences between water samples. It is known that humic acids in water delay photodegradation of imazethapyr by light attenuation (Elazzouzi et al. 2002). Dissolved inorganic substances may hinder degradation due to light attenuation or

accelerate degradation due to mediation of indirect photoprocesses (Miller and Zeep 1979).

The Pearson's correlation between half-life and water pH (0.955) was significant at α=0.05. This relatively high correlation suggested that pH significantly affected the degradation of imazethapyr in these natural waters. However, an experiment testing three deionized water pH (4, 7, and 9) showed that imazethapyr photolysis was not dependent on water pH (Figure 8). This probably happened because there was a positive correlation between turbidity and pH within the rice paddy water sources (data not shown). When turbidity was removed as a factor in the pH experiment, pH did not affect imazethapyr photolysis. However, in a field situation, pH may affect imazethapyr photolysis, because herbicide adsorption control photolysis rate, control herbicide availability (Si et al. 2004) and pH controls imazethapyr soil adsorption (Madani et al. 2003; Renner et al. 1988; Stougaard et al. 1990). Imazethapyr adsorption is promoted by low pH values (Gennari et al. 1998; Loux et al. 1989a; Renner et al. 1988; Stougaard et al. 1990). In theory, the pH effect should be noticed only in the first few weeks of flooding, because after this period, regardless of the original pH before flooding the soil pH should approach neutrality (Snyder and Slaton 2002). The change in pH upon flooding may take up to several weeks, depending on the soil type, organic matter levels, microbial population, temperature, and other soil chemical properties (Snyder and Slaton 2002).

Quantum Yield (ϕ) Determinations

The UV-visible absorbance spectra for imazethapyr and PNA in phosphate buffer (pH 7.0) are shown in Figure 9. The measured molar absorptivities and UV-irradiance values were used to calculate the quantum yield for imazethapyr, resulting in a value of ϕ_I = 0.023 ± 0.002 (n=4). This value is similar in magnitude to those reported for other pesticides (Wan et al. 1994; Wong and Chu 2003) which are often $\leq$ 0.1. This value

indicates that approximately 2% of the absorbed light energy actually goes towards disrupting chemical bonds in imazethapyr.

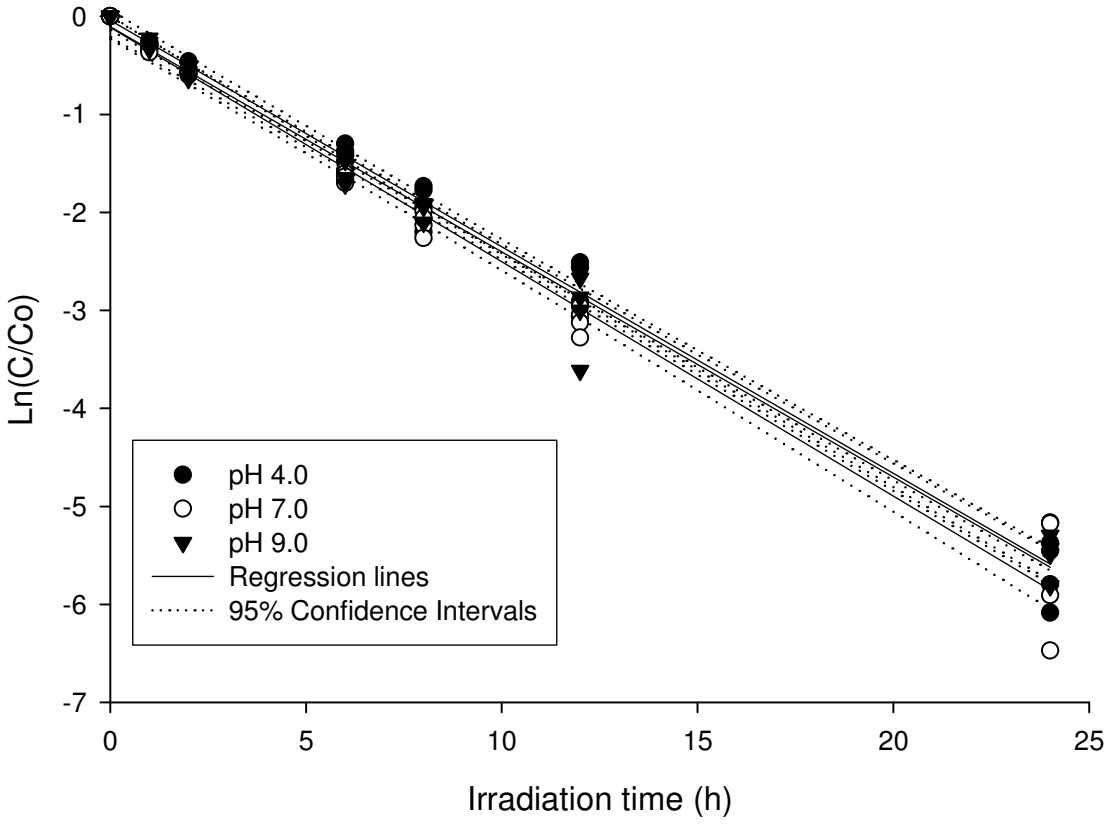

Figure 8. Natural log of remaining concentration of imazethapyr [Ln (C/Co)] after UV light exposure under three water pHs.

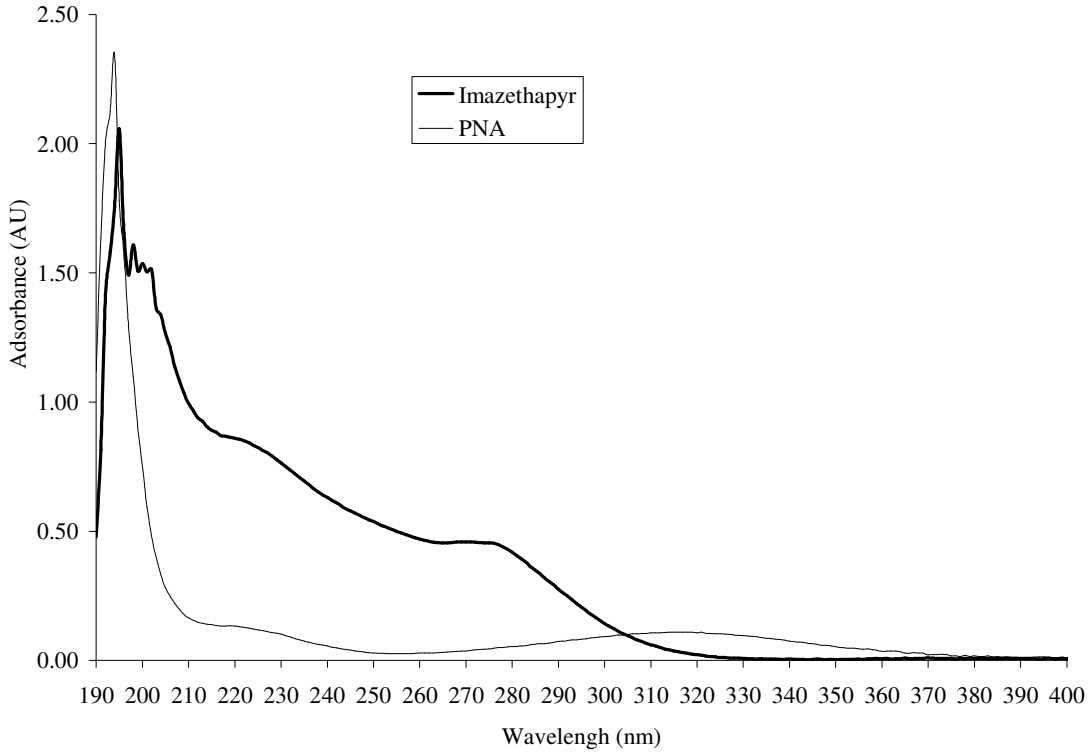

Figure 9. Light absorbance by the herbicide imazethapyr and by the chemical actinometer p-nitroanisole (PNA).

Hydroxyl Radical Rate Constant ($k^{I}_{\bullet OH}$) Determinations

During the 10 min study duration, imazethapyr losses due to direct photolysis, chemical hydrolysis were <1, <1, and 1% for acetophenone, imazethapyr and 2,4-D. The losses due to reaction with hydrogen peroxide in absence of light were <1, 3, and 2% for acetophenone, imazethapyr and 2,4-D, respectively. Therefore, corrections for these losses were not made. The measured hydroxyl radical rate constant for 2,4-D herbicide, $3.5 \pm 0.91 \times 10^{13}$ M^{-1} h^{-1}, and was within 80% of literature values (Table 10). The hydroxyl radical rate constant for imazethapyr was calculated to be $2.8 \pm 0.44 \times 10^{13}$ M^{-1} h^{-1}. The magnitude of this value is similar to those reported for other pesticides

(Armbrust 2000) and indicates that imazethapyr reacts with hydroxyl radicals at nearly diffusion-controlled rates.

Table 10. Measured and previously reported hydroxyl radical rate constant for imazethapyr and 2,4-D.

Chemical	Rate constant k_{OH*}^{M} ($M^{-1}\,h^{-1}$)	
	Measured[a]	Reported
Imazethapyr	$2.8 \pm 0.44 \times 10^{13}$	---
2,4-D	$3.5 \pm 0.91 \times 10^{13}$	1.8×10^{13} (Haag and Yao 1992) 5.8×10^{12} (Mabury and Crosby 1996) 8.4×10^{12} (Armbrust 2000)

[a] The competitor (acetophenone) hydroxyl radical rate constant used to calculate the measured rate constant was $2.1 \times 10^{13}\ M^{-1}\,h^{-1}$ (Haag and Yao 1992).

Summary and Conclusion

The recent introduction of imidazolinone-tolerant rice varieties allow imazethapyr to be used in commercial rice. Little is known about imazethapyr photodegradation in the rice field. Laboratory studies were conducted to determine the direct and indirect photolysis rates for imazethapyr and to evaluate the photolysis of imazethapyr in three rice paddy waters. The quantum yield (ϕ_I) for imazethapyr was determined to be 0.023 ± 0.002 while the hydroxyl radical rate constant ($k_{\bullet OH}^{I}$) was $2.8 \pm 0.44 \times 10^{13}\ M^{-1}\,h^{-1}$. These results show that imazethapyr is susceptible to both direct and indirect photolysis reactions in water. The results also show that imazethapyr photolysis in paddy water will be affected by turbidity due to its impact on the availability of sunlight to drive direct and indirect photolysis reactions.

CHAPTER V
IMAZETHAPYR ADSORPTION AND AVAILABILITY IN THREE SOILS AS AFFECTED BY SOIL MOISTURE CONTENT

Introduction

Imazethapyr {2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid} is an imidazolinone herbicide used to control many broadleaf weeds and several annual grasses in soybean and peanut (Vencill 2002). Imazethapyr is relatively safe to the environment due to its low mammalian toxicity (Vencill 2002). Imazethapyr dissipates in the environment mainly by biodegradation (Flint and Witt 1997). Studies have demonstrated little downward movement of imazethapyr in the field under normal application conditions (Gan et al. 1994). It was postulated that net water flow in soil during the growing season is upward, and hence limits the downward movement of the weakly adsorbed imazethapyr residues (Gan et al. 1994). Upward movement of imazethapyr has been detected in course soil (Wyk and Reinhardt 2001). Furthermore, the soil surface becomes more acidic as moisture levels decrease, thus further immobilizing the residues due to increased sorption at the lower soil pH values (Gan et al. 1994). Contrasting with those results, imazethapyr was the most frequent herbicide detected in rivers and ground water in the Midwest US (Battaglin et al. 2000) and when studied with undisturbed soils, imazethapyr moved in a 30-cm soil column (O'Dell et al. 1992). Results have indicated that imazethapyr could leach in course soil up a 30-cm depth, depending on rainfall amounts (Wyk and Reinhardt 2001).

Additionally, carryover problems with imazethapyr have also been reported (Bresnahan et al. 2000; Johnson et al. 1993; Kin et al. 1995; Moyer and Esau 1996; Zhang et al. 2002). Carryover is dependent on herbicide soil solution concentration since the amount of herbicide taken up by a plant is a function of the degree of plant transpiration and the herbicide concentration in soil water (Renner et al. 1988).

The problems of ground water contamination and carryover have been detected due to some distinguishing characteristics of imazethapyr (Table 11) that affect its environmental behavior. Imazethapyr is persistent in the environment with half-lives ranging from 53 to 122 d (Curran et al. 1992a; Mills and Witt 1989). Imazethapyr residues have been detected 3 years after herbicide application (Loux et al. 1989b). Due to its high water solubility (1415 mg L^{-1}) and weak soil adsorption, imazethapyr can be mobile in certain soils (Madani et al. 2003; O'Dell et al. 1992; Souza 1998).

Table 11. Imazethapyr characteristics.

Melting Point[a]	Vapor pressure[a,b]	Solubility (H_2O)[a,c]	pKa_1[d]	pKa_2[d]	Kow[e]		
	(mPa)				pH	pH	pH 7
(ºC)		mg L^{-1}			3	5	
169-173	<0.013	1400	2.1	3.9	14.5	0.08	<0.01

[a] Source: Vencill 2002.

[b] Vapor pressure at 60ºC.

[c] Solubility in water pH 7.0.

[d] Source: Gennari et al. (1998).

[e] Octanol – water partitioning coefficient (Gennari et al. 1998).

Soil pH affects imazethapyr sorption (Renner et al. 1988) and desorption in soil (Aichele and Penner 2005). As soil pH decreases toward the pK_a, sorption tends to increase because the hydrophobic neutral form predominates and sorbs more strongly than the anionic form (Cleveland 1996). For this reason, imazethapyr is strongly adsorbed (Che et al. 1992; Gennari et al. 1998; Loux et al. 1989a; Renner et al. 1988; Stougaard et al. 1990), less mobile, less efficacious (Stougaard et al. 1990) and less desorbed (Aichele and Penner 2005) and more persistent at a lower soil pH (Loux and Reese 1993). Imazethapyr dissipation is slower in pH 5 soils compared pH-7 soils

(Aichele and Penner 2005). This may be due to increased adsorption at lower pH (Aichele and Penner 2005). Results have shown that imazethapyr K_d differed between areas with soil pH above 6.2 compared to those values from soil pH's below 6.2 (Koskiken et al. 2003). Imazethapyr sorption was dependent on pH and organic matter (Wei and Weip 1998).

Herbicide adsorption to the soil is the major contributing factor related to herbicide mobility in the soil profile (Koskinen et al. 2003; Oliveira et al. 2004), environmental fate (Koskinen et al. 2003; Peter and Weber 1985) and herbicide efficacy (Peter and Weber 1985).

The most common method used to determine the relative adsorption of a compound to soil is the batch equilibrium method (Wauchope et al. 2002). But, this method uses slurries with high solution to soils ratios (often 2:1 or higher) and often short equilibration times that may not accurately predict mobility of acidic herbicides in the field where moisture content is relatively low (Johnson et al. 2000). Olso, when sorption is examined across a large range of herbicide concentrations, the partitioning coefficient of most herbicides is not linear. As a result, the linear K_d usually under-predicts pesticide sorption on soil at low solution concentrations and over-predicts pesticide sorption at high solution concentrations (Smith et al. 2003). Therefore, it is necessary to develop and use a method that is based on field moist levels to determine herbicide availability and adsorption in soil. A method using centrifugal force to extract available water from the soil at field-relevant moistures is an alternative to determining both herbicide adsorption to the soil and availability in soil solution (Lee et al. 2004). This method is called the double-tube centrifugation method as described by Lee et al. (2004). The apparatus consists of a specially machined 20 i.d. x 75 mm stainless steel inner tube with a perforated end. A 25-mm glass microfiber filter is placed at the bottom of each tube prior to the soil being placed inside such that the soil solution would be free of particulates after centrifugation. At the opposite end of the tube, the outer diameter of the tube is 28 mm such that the tube could be placed inside a 26-mm i.d., 33-mm o.d.

metal washer so as to suspend the stainless steel tube on top of a 28.6 i.d. x 114 mm centrifuge tube when the samples were centrifuged.

Knowledge of imazethapyr adsorption and availability is important for understanding its environmental behavior and potential crop carryover. Therefore, an experiment was conducted 1) to determine the relative amounts of imazethapyr availability in soil solution in three soils and in two water contents and 2) to compare K_d values obtained by the batch equilibrium method and the double centrifuge tube method for imazethapyr.

Materials and Methods

Soil Collection, Preparation and Treatment

Samples were collected from the top 5 cm of soil in three locations. These soils were Houston Black clay (fine, smectitic thermic Udic Haplusterts), Weswood silt clay loam (Fine-silty, mixed, superactive, thermic Udifluventic Haplustepts), and a Tremona loamy fine sand (thermic Aquic Arenic Palenstalfs). The soil samples were air dried at room temperature and passed through a 2-mm sieve. The soil characteristics are shown in Table 12.

Determination of Available Imazethapyr and Kd.

Double centrifuge method. Moisture levels at each water potential for each soil were determined using the method described by Romano et al. (2002). Water potential included: 0 kPa to simulate a saturated soil and -33 kPa to simulate field capacity. These two moisture levels were chosen because they represent estimates of field moist soil at field capacity and a saturated soil representing a flooded rice field.

The experiment was conducted at 18ºC unless otherwise noted. For each water potential and soil, a solution containing imazethapyr was prepared to achieve a final concentration in soil of 125 mg g^{-1}. This concentration corresponded to a 1x field rate of imazethapyr (140 g a.i. ha^{-1}), assuming a 7.5-cm furrow slice. The solution was a mixture of ^{14}C-imazethapyr (pyridine labeled) 777 kBq mg^{-1} specific activity and

technical-grade imazethapyr (99% purity). Radiolabeled imazethapyr was added to the water solution to obtain 35.9 Bq g^{-1} of soil, which accounted for approximately 39% of the total amount of imazethapyr in soil. Methanol, used for initial dilution of the herbicide was adjusted to be < 0.5% of the total amount of the final solution. Twenty-five g of soil was placed in 100-mL amber glass vials and treated with water containing imazethapyr. The amount of solution was the amount needed to achieve 0 and -33 kPa in each soil (Table 13). Herbicide solution and soil were mixed using a laboratory spatula until soil moisture was homogenous throughout the sample. After treatment, the samples were left to equilibrate for 48 h. Prior to the beginning of the experiment an equilibration test was done to determine the optimum equilibration time. Herbicide was equilibrated for 2, 4, 6, 12, 24, 48, 72, and 96 h. Results indicated that imazethapyr reaches equilibration 48 h after herbicide treatment.

Table 12. Properties for Houston Black clay (Houston Black), Weswood silty clay loam (Weswood) and Tremona loamy fine sand (Tremona).

Soil	Location	Sand	Silt	Clay	OC [b]	pH (1:1)[a]
		——————— % ———————				
Houston Black	Temple	19	38	43	1.51	8.1
Weswood	College Station	11	68	31	1.05	8.1
Tremona	Yoakum	81	10	9	0.42	7.0

[a] Soil:H_2O

[b] Percentage of organic carbon, calculation based on organic matter (OM): %OC=0.58*OM (Weber et al. 2004).

Table 13. Amount of water (mL), necessary to bring 25 g of soils Houston Black clay (Houston Black), Weswood silty clay loam (Weswood) and Tremona loamy fine sand (Tremona) to field capacity (-33 kPa) and to a saturated soil (0 kPa).

	Water potential (kPa)[a]	
Soil	-33	0
	ml	
Houston Black	5.4	9.9
Weswood	5.0	9.4
Tremona	0.9	5.9

[a]Based on water retention determination.

To determine the amount of imazethapyr available in soil solution after equilibration, 20 g of treated soil was placed in a double-tube centrifuge apparatus described by Lee et al. (2004). Samples were than centrifuged at 13,000 X *g* for 30 min at 20º C. This centrifugal force was necessary to represent plant-available water in soil based on soil water potential of -1500 kPa or the permanent wilting point (Kobayashi et al. 1994; Lee et al. 2004). After centrifugation, the volume of the extracted water collected in the outer centrifuge tube was determined by the difference in weight of the tube before and after centrifugation. Extracted soil solution was vortexed to homogenize the herbicide concentration. Depending on initial soil water content, a minimum of 400 µl of solution was pipetted and placed into a 7-ml liquid scintillation vial, and filled with liquid scintillation cocktail Ecolite (+). Radioactivity was determined in each vial using liquid scintillation spectroscopy (LSC). A portion of the centrifuged soil was taken (~ 1 g), placed in paper envelopes and dried at 50º C for 48 h. After drying, a 100-mg dry soil sample was oxidized and the trapped CO_2 was captured with scintillation cocktail and analyses were performed by LSC. A quality control calculation was performed to evaluate the mass balance of imazethapyr in soil and in extracted water.

Based on the amount of radioactivity in the soil solution, radioactivity concentration (RC) in dpm ml^{-1} was calculated as done by Lee et al. (2004). The total amount of available imazethapyr in water solution (TASS) was calculated using the following equation:

$$TASS = \frac{\left\{(RC)(VSSE)\left[\frac{PNR}{PR}\right]\right\}}{[(SA)(MCS)]} \quad [9]$$

where, RC is the radioactivity concentration (dpm ml^{-1}), VSSE is the volume of soil solution extracted from the samples (ml), PNR is the percentage of nonradiolabeled imazethapyr added to the treatment (%), PR is the percentage of radiolabeled imazethapyr added to the treatment (%), SA is the specific activity of imazethapyr (dpm ng^{-1}), and MCS is the mass of soil centrifuged (g).

The available concentration of imazethapyr (µM) in soil solution (ACSS) was calculated by the following equation:

$$ACSS = \frac{\left\{(RC)\left[\frac{PNR}{PR}\right]\right\}}{[(SA)(MW)]} \quad [10]$$

where RC is concentration of radioactivity (dpm l^{-1}), PNR is percentage of nonradiolabeled imazethapyr (%), PR is the percentage of radiolabeled imazethapyr added to the treatment (%), SA is the specific activity of imazethapyr (dpm μg^{-1}), MW is the molecular weight of imazethapyr (289.3 µg μM^{-1}).

The partitioning coefficient (K_d) was calculated using the following equation:

$$K_d = \frac{\left[\frac{(RA_i - RA_{ac})(SA)}{MCS}\right]}{[(ACSS)(SA)]} \quad [11]$$

where K_d is the partitioning coefficient (ml g^{-1}), RA_i is amount of initial radioactivity (dpm), RA_{ac} is amount of radioactivity in soil solution after centrifugation (dpm), SA is specific activity (µg dpm^{-1}), MCS is the mass of soil that was centrifuged (g), and ACSS is the available concentration of clomazone in soil solution (dpm ml^{-1}).

Batch equilibrium method. Batch equilibrium experiment was also determined to compare relative affinity of imazethapyr to the soils using conventional adsorption method. The study was conducted at 18º C. To determine $K_{d(BE)}$, a single point K_d was determined as suggested by Cleveland (1996). A imazethapyr stock solution was prepared in 0.01 M $CaCl_2$, to achieve final concentration in soil of 125 mg g^{-1}, using the same radioactivity amount as in the double centrifuge method. One g of soil was placed in a 100-ml centrifuge tube, and 5 ml of herbicide solution was added to the soil. The centrifuge tubes were placed in a reciprocal shaker for 48 h for equilibration and than centrifuged at 2000 X *g* for 20 min. After centrifugation, a 3-mL aliquot of supernatant was drawn from the sample and placed in 7-ml liquid scintillation vials. Three ml of liquid scintillation cocktail (Ecolite (+)) was then added to each sample. For each sample, radioactivity was quantified using a liquid scintillation counter (LSC). For purpose of quality assurance, a blank was included to account for herbicide adsorption to the glass wall of the centrifuge tubes. Prior to the beginning of the experiment an equilibration test was done to determine the equilibration time. Herbicide was equilibrated for 4, 6, 12, 24, 48, 72, and 96 h. Results indicated that imazethapyr reaches equilibration 48 h after herbicide treatment.

Experimental Design and Data Analysis

The experiment was conducted as a randomized complete block design in a two-factor factorial arrangement with 4 replications. The factors were soil series and water potential. Data were analyzed by ANOVA and means were compared by LSD (0.05).

Results and Discussion

Analysis of variance showed no significant interactions between soil and water potential for TASS, $K_{d(DC)}$, or PAS_{DC}. Only main effects were significant (Table 14). There was a significant interaction between soil and water potential for ACSS. However, for $K_{oc(DC)}$ there were no significant interactions or main effects. The analysis

of variance for $K_{d(BE)}$ showed no difference between soils (data not shown). Analysis of variance showed a significant difference between the batch equilibrium method and the double centrifuge method (Table 15).

Effect of Soil

The order of decreasing TASS was Tremona > Weswood > Houston Black (Table 16). Values of TASS ranged from 17 to 34 ng imazethapyr g^{-1} soil. Imazethapyr concentration in soil has been reported to vary from 4 to 18 ng g^{-1} of soil 30 days after herbicide application (Mills and Witt 1989). The sandier soil Tremona had higher TASS values. This shows that the total availability of imazethapyr was dependent on available soil water (data not shown), showing that in soil with higher amount of water in solution the herbicide can promote greater injury to plants (weed control or carry over).

Table 14. Analyzes of variance (ANOVA) table for the dependent variables total imazethapyr available in soil solution (TASS), $K_{d(DC)}$ values, percentage of imazethapyr adsorbed to soil (PAS_{DC}) and available concentration of imazethapyr in soil solution ACSS (µM) in response to the factors soil and water potential (kPa).

Source	df[a]	TASS	ACSS	$K_{d(DC)}$	PAS_{DC}[b]	$K_{oc(DC)}$
Rep	2	NS[c]	NS	NS	NS	NS
Soil	2	<0.001	<0.001	0.004	NS	NS
kPa	1	<0.001	<0.001	0.043	0.005	NS
Soil * kPa	2	NS	<0.001	NS	NS	NS
Means Square Error		17.9	0.011	0.001	0.007	54.6
CV %		16.7	9.1	10.5	13.8	54.2

[a] df = Degree of freedom.

[b] Values in percentage, prior to analysis were transformed using arcsine.

[c] NS= Not significant at 0.05.

Table 15. Analysis of variance (ANOVA) table for percentage of imazethapyr adsorbed to soil (PAS) in response to the factors soil and method of determination (double centrifuge at 0, double centrifuge at -33 and batch equilibrium).

Source	df [a]	PAS [b]
Soil	2	NS [c]
Method	2	<0.001
Rep	2	NS
Soil * Method	4	NS
Error	16	
Mean square error		0.06
CV (%)		11.3

[a] df = Degree of freedom.

[b] Values in percentage, prior to analysis were transformed using arcsine.

[c] NS= Not significant at 0.05.

Sorption coefficient $K_{d(BE)}$ determined by the standard slurry method demonstrated no differences among soils (data not shown). No differences were observed, probably, because the soil pHs were above 7.0 (Table 12). It is known that soil pH has a remarkable effect on imazethapyr adsorption, but little or no difference on imazethapyr adsorption between soils were observed when pH values were above 6.0 (Ahmad et al. 2001; Loux et al. 1989a; Renner et al. 1988; Stougaard et al. 1990). Imazethapyr binding to soil is promoted by lower pH (Madani et al. 2003) because the carboxylic acid on the molecule tends to be protonated which interacts more strongly with the organic matter. In this experiment, the average $K_{d(BE)}$ was 0.8 ml g^{-1}, a value similar to values found in other work where K_d values averaged 1.0 ml g^{-1} for soil with pH 7.1 (Johnson et al. 2000). A broad range of $K_{d(BE)}$ values have been reported for imazethapyr: 0.02 to 6.9 ml g^{-1} (Ahmad et al. 2001) 0.2 to 3.8 ml g^{-1} (Koskiken et al. 2003) and 0.1 to 0.8 ml g^{-1} (Oliveira et al. 2001) and 0.5 to 13.8 ml g^{-1} (Johnson et al. 2000).

Table 16. Total imazethapyr available in soil solution (TASS), partitioning coefficients ($K_{d(BE)}$, $K_{d(DC)}$), partitioning coefficient with organic carbon ($K_{oc(BE)}$ and $K_{oc(DC)}$) and percentage of imazethapyr adsorbed to soil (PAS_{BE} and PAS_{DC}) for Houston Black clay (Houston Black), Weswood silty clay loam (Weswood) and Tremona loamy fine sand (Tremona).[a]

Soil	TASS	$K_{d(BE)}$	$K_{oc(BE)}$[b]	$K_{d(DC)}$	$K_{oc(DC)}$	PAS_{BE}	PAS_{DC}
	ng g^{-1} soil	ml g^{-1}				% [d]	
Tremona	33.9 a [c]	0.7 [ns]	164.5 a	0.1 b	14.4[ns]	15.8[ns]	36.5[ns]
Weswood	19.3 b	1.0	90.5 b	0.1 b	9.6	12.1	27.0
Houston Black	16.6 c	0.8	54.1 b	0.7 a	10.6	13.7	35.2

[a] Nomenclature: Variables followed by (BE) underscore were determined using batch equilibrium and variables followed by (DC) underscore were determined using the double centrifuge method.

[b] $K_{oc} = \frac{K_d * 100}{\%OC}$ (Weber et al. 2004).

[c] Values fallow by the different letter in the column differ by LSD at 0.05.

[d] Values in percentage, prior to analysis were transformed using arcsine, values showed are untransformed.

[ns] Data on column, do not differ significantly at 0.05 probability level.

Contrasting with results found for $K_{d(BE)}$, $K_{d(DC)}$ was different among soils, with the values in decreasing order being Houston Black>Tremona=Weswood. Values for $K_{d(DC)}$ were 10-fold smaller than values for $K_{d(BE)}$. Lee et al. (2004) also found that clomazone partitioning by the double centrifuge method was less than values found in the literature that determined K_d using the batch equilibrium method. This difference was attributed to the fact that $K_{d(BE)}$ does not take into account field moisture conditions (Lee et al. 2004), and uses too much water making the $K_{d(BE)}$ values unrealistic regarding to field conditions.

$K_{oc(BE)}$ values in decreasing order were Tremona>Weswood=Houston Black and ranged from 54 and 165 ml g^{-1}. However, $K_{oc(DC)}$ showed no difference between soils, with values ranging from 9.6 to 14.4 ml g^{-1}.

The percentage of herbicide adsorbed to the soil calculated by the batch equilibrium method (PAS_{BE}) ranged from 12 to 16%. Values were not different between soils. The same trend was found for the percentage of herbicide in soil solution calculated by the double centrifuge method (PAS_{DC}) but the values had a higher range (27 to 37%).

Effect of Water Potential on Herbicide Adsorption

For water potential effect, more imazethapyr was available in soil solution at 0 kPa compared to -33 kPa (Figure 10a). Similar results were found for clomazone in rice soils using the double centrifuge technique (Lee et al. 2004). Wetter soil provided greater $K_{d(DC)}$ values as well (Figure 10b). There was a strong correlation between TASS and $K_{d(DC)}$ at both water contents (Figure 10c).

Availability Concentration of Imazethapyr in Soil Solution

The ACSS values ranged from 0.5 to 3.4 μM (Table 17). Tremona soil had the highest ACSS when compared with the Weswood and Houston Black at -33 kPa water potential. There were no differences between ACSS for the soils tested at 0 kPa. Similar trend were found for clomazone (Lee et al. 2004) and atrazine (Green and Obien 1969). This was probably caused by herbicide dilution in the wetter soils (Table 13).

To further investigate the percentage of herbicide adsorbed to the soil (PAS) PAS_{DC} (double centrifuge method) was compared with PAS_{BE} (batch equilibrium method (Table 18). When determined by batch equilibrium, imazethapyr shows less adsorption when compared with to PAS_{DC} at both water potential (-33 kPa and o kPa). Due to the excessive amounts of water, $K_{d(BE)}$ tended to underestimate herbicide sorption to the soil and overestimate mobility (Johnson et al. 2000). Furthermore, PAS_{DC} with lower water potential (-33 kPa) had a higher values of PAS_{DC} when compared to higher water potential (0 kPa), indicating that the less water is in soil solution, the higher the amount of herbicide adsorbed. Soil moisture decreased herbicide sorption to the soil (Moyer

1987). This is particularly important for soil or herbicides with low adsorption capacity (Green and Obien 1969) such as the herbicide imazethapyr.

Table 17. Available concentration of imazethapyr in soil solution ACSS (µM) for soils Houston Black clay (Houston Black), Weswood silty clay loam (Weswood) and Tremona loamy fine sand (Tremona) at two water potentials.

	Water potential (kPa)	
Soil	-33	0
	µM	
Tremona	3.4 a[a] A[b]	0.9 a B
Weswood	0.8 b A	0.5 b B
Houston Black	0.8 b A	0.5 b B

[a] Values fallowed by a different lower case letter in the column differ by LSD at 0.05.

[b] Values fallowed by a different capital letter in the row differ F-test at 0.05.

Table 18. Percentage of imazethapyr adsorbed to the soil at -33 and 0kPa using the double centrifuge method and the more conventional batch equilibrium method at 1:5 soil:water ration. [a]

Soil	Imazethapyr adsorbed to the soil % [b]
Double centrifuge method (-33 kPa)	39.2 a [c]
Double centrifuge method (0 kPa)	26.6 b
1:5 batch equilibrium method	13.9 c

[a] Double centrifuge method, method adapted from Lee et al. 2004.

[b] Values in percentage, prior to analysis were transformed using arcsine, values showed are untransformed.

[c] Values fallow by the different letter in the column differ by LSD at 0.05 level of probability.

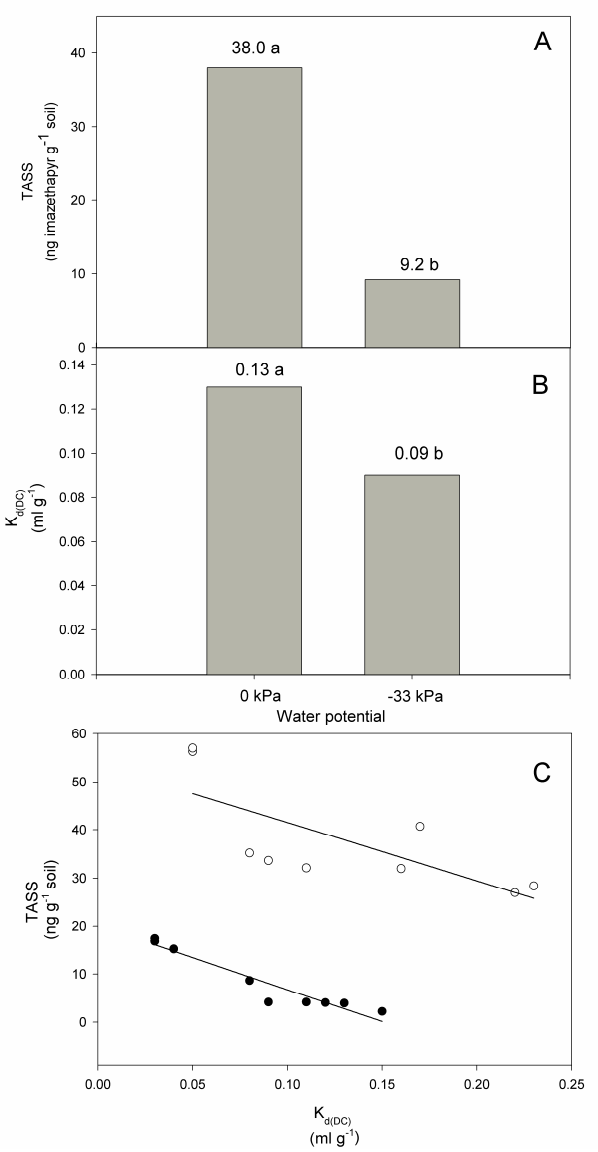

Figure 10. Effect of water potential on (A) TASS, (B) $Kd_{(DC)}$ and (C) on the relationship between total available imazethapyr in soil solution (TASS) and sorption coefficient determined by double centrifuge method ($K_{d(DC)}$) after 48-hours equilibration as a function of water potentials 0 kPa (○) and -33 kPa (●). Fitted equations (—) for each water potential were: y = 53.59 - 120.8x (R^2=0.55) for 0 kPa and y = 19.96 - 131.85x (R^2=0.92) for -33 kPa. In Figure 10a and b, columns with different letters differ by F-test at 0.05.

The relationship between available imazethapyr concentration in soil solution (ACSS) and the total available amount of imazethapyr in soil solution (TASS) is presented in Figure 11. There was a significant relationship for these two variables at each water potential, with the coefficient of determination of 0.99 for -33 kPa and 0.94 for 0 kPa. As TASS increased, ACSS increased, with -33 kPa providing the steepest slope. As water decreased, ACSS increased. Similar results were found with clomazone (Lee et al. 2004).

The results for TASS and ACSS indicated that imazethapyr was more available and more concentrated in soil solution in the sandier Tremona soil compared to Houston Black and Weswood. This indicates that imazethapyr may be more prone to injure sensitive crops in the sandier soil. Because TASS has a positive correlation with injury to crops as found for other herbicides (Lee et al. 1998; Lee et al. 2004). However, if more herbicide is in solution, less carryover may occur, due to increased biodegradation (Cantwell et al. 1989; Goetz et al. 1990), increasing in mobility (Moyer 1987) and in photolysis (Si et al. 2004).

Summary and Conclusion

Herbicide availability in soil solution is an important factor regulating herbicide fate and efficacy. An experiment was conducted to determine imazethapyr availability and adsorption to three soils at two water contents. Soil samples were collected from three locations, including the USDA Blackland Research Center in Temple, TX (Houston Black clay), from the Texas A&M University Field Lab in College Station, TX (Weswood silty clay loam), and from the Texas Agricultural Experiment Station, Yoakum, TX (Tremona loamy fine sand). The total amount of available imazethapyr in soil solution (TASS), imazethapyr concentration (μM) in soil solution (ACSS), partitioning coefficient (Kd(DC)), and percentage of imazethapyr adsorbed to soil (PASDC) were obtained using a double-centrifuge method at two water potentials (-33 and 0 kPa). Kd(DC) and PASDC were compared to results determined by the standard batch equilibrium method (Kd(BE) and PASBE) on the same soils. Imazethapyr was

more available and more concentrated in sandy soil. With higher amounts of water in soil there was greater amount of imazethapyr in soil solution and a lower concentration of herbicide due to dilution. The double centrifuge method provided a better estimate of plant available herbicide compared to the batch equilibrium method.

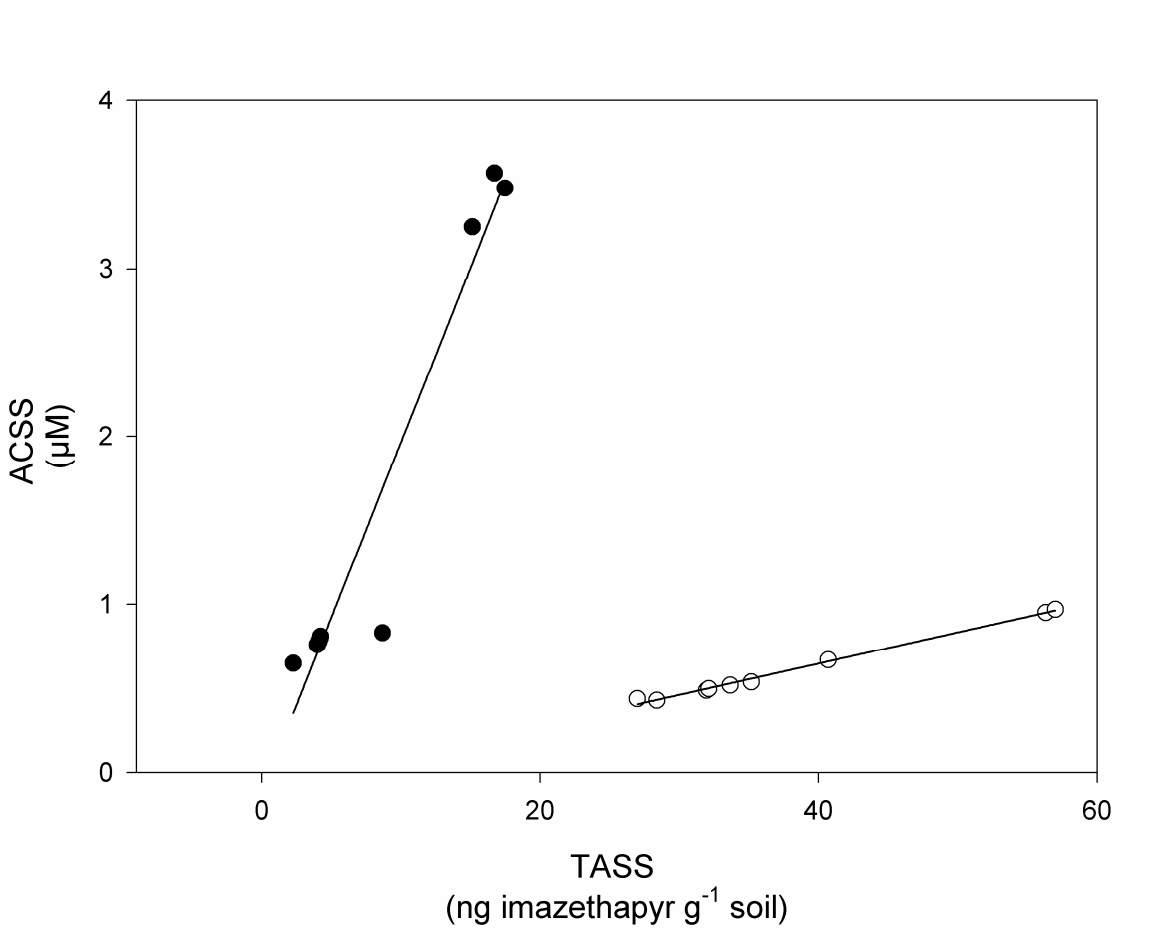

Figure 11. Relationship between available imazethapyr concentration in soil solution (ACSS) and total available imazethapyr in soil solution (TASS) after a 48-h equilibration as a function of water potentials 0 kPa (○) and -33 kPa (●). Fitted equations (——) for each water potential were y = -0.0916 + 0.0195x (R^2=0.99) for 0 kPa and for y = -0.1166 + 0.2082x (R^2=0.94) for -33 kPa. (R^2=0.99) for 0 kPa and for y = -0.1166 + 0.2082x (R^2=0.94) for -33 kPa.

CHAPTER VI

SUMMARY AND CONCLUSION

To answer the four objectives described earlier, a series of experiments were carried out to: 1) determine the effect of flooding time and stage of imazethapyr application on red rice control, 2) assess acetolactate synthase resistance to imazethapyr on selected red rice ecotypes, 3) determine the relative photolysis of imazethapyr, and 4) determine the effect of soil and moisture on imazethapyr adsorption and availability.

When imazethapyr was applied in sequential application of PRE followed by a POST application, the flood needed to be established within 14 DAT when imazethapyr was applied EPOST and 7 DAT when imazethapyr was applied LPOST to achieve >95% red rice control. Delaying the flood 21 DAT reduced rice grain yield for both EPOST and LPOST imazethapyr application timings.

Although ecotypes TX 4 and LA 5 were tolerant to imazethapyr in the whole plant bioassay, the ALS assay showed that all red rice ecotypes and Cypress were susceptibile to imazethapyr when compared with tolerant CL-121 and resistant CL-161. Measurable enzymatic tolerance to ALS-inhibiting herbicides has not yet developed in these red rice ecotypes. Other mechanisms may be responsible for the tolerance found in some of the ecotypes studied in the bioassay. For this reason, further studies are necessary to determine relative absorption, translocation or metabolism differences among the red rice ecotypes.

Imazethapyr photolyisis was different among water samples tested (Eagle Lake, Beaumont, Clarksdale and deionized water). The half-life for imazethapyr in two of the water samples tested (Beaumount and Clarksdale) were 5.1 h and for Eagle lake water was 4.0 h. The results showed that imazethapyr photolysis in paddy water was affected by turbidity due to its impact on the availability of sunlight to drive direct and indirect photolysis reactions. The results for direct photolysis and indirect photolysis showed that imazethapyr was susceptible to both direct and indirect photolysis. Calculated imazethapyr quantum yield (ϕI) was 0.023 ± 0.002 while the hydroxyl radical rate

constant ($k^{I}_{\bullet OH}$) was 2.8 ± 0.44 x 10^{13} M^{-1} h^{-1}. Quantum yield can be used to estimate photolysis rates under different scenarios and the hydroxyl radical rate constant can be used in models to predict indirect photolysis of imazethapyr.

Soil moisture and soil type affected imazethapyr availability in the soil. Imazethapyr was more available and more concentrated in the sandier Tremona soil. Using the double centrifuge method, there was a greater amount of imazethapyr in soil solution and a lower concentration of herbicide due to dilution when greater water volumes were applied to the soils. The double centrifuge method provided a better estimate of plant available herbicide particularly based on total amounts of imazethapyr in soil solution (TASS). Future studies are necessary to identify imazethapyr availability across a broader range of soil characteristics as well as the availability of other pesticides.

LITERATURE CITED

Ahmad, R., R. S. Kookana, and A. M. Alston. 2001. Sorption of ametryn and imazethapyr in twenty-five soils from Pakistan and Australia. J Environ. Sci. Heal. B. 36:143-160.

Aichele, T. C. and D. Penner. 2005. Adsorption, desorption, and degradation of imidazolinones in soil. Weed Technol. 19:154-159.

Al-Khatib, K., J. R. Baumgartner, D. E. Peterson, and R. S. Currie. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci. 46:403-407.

Anderson, P. C. and K. A. Hibberd. 1985. Evidence for the interaction of an imidazolinone herbicide with leucine, valine and isoleusine metabolism. Weed Sci. 33:479-483.

Armbrust, K. L. 1999. Photochemical processes influencing pesticide degradation in rice paddies. J. Pest. Sci. 24:69-73.

Armbrust, K. L. 2000. Pesticide hydroxyl radical rate constant: measurements and estimates of their importance in aquatic environments. J. Environ. Toxicol. Chem. 19:2175-2180.

Avila, L. A., D. J. Lee, S. A. Senseman, G. N. McCauley, J. M. Chandler, and J. T. Cothren. 2005a. Assessment of acetolactate synthase (ALS) tolerance to imazethapyr in red rice ecotypes (*Oryza* spp.) and imidazolinone tolerant/resistant rice (*Oryza sativa*) varieties. Pest. Manag. Sci. 61:171-178.

Avila, L. A. and E. Marchezan. 2000. Control of red rice seed banks under different lowland management systems. Int. Rice Res. Notes. 25:30-31.

Avila, L. A., S. A. Senseman, G. N. McCauley, J. M. Chandler, and J. H. O'Barr. 2005b. Effect of flood timing on red rice (*Oryza* spp.) control with imazethapyr applied at different dry-seeded rice growth stages. Weed Technol. In press.

Ballard, T. O., M. E. Foley, and T. T. Bauman. 1995. Absorption, translocation, and metabolism of imazethapyr in common ragweed (*Ambrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*). Weed Sci. 43:572-577.

Battaglin, W. A., E. T. Furlong, M. R. Burkhardt, and C. J. Peter. 2000. Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Sci. Total Environ. 248:123-133.

Baumgartner, J. R., K. Al-Khatib, and R. S. Currie. 1999. Cross-resistance of imazethapyr-resistant common sunflower (*Helianthus annuus*) to selected imidazolinone, sulfonylurea, and triazolopyrimidine herbicides. Weed Technol. 13:489-493.

Beachell, H. M., C. R. Adair, and N. E. Jodon. 1936. Extent of natural crossing in rice. J. Amer. Soc. Agron. 30:743-753.

Beauclerc, K. B. and J. M. Gunn. 2001. Ultraviolet absorbance in lakes near the metal smelters in Sudbury, Canada. J. Environ. Monitor. 3:575-579.

Beretvas, M. K., J. P. Hassett, S. E. Burns, and T. M. Basford. 2000. Modeling the photolysis of ammonium dinitramide in natural waters. Environ. Toxicol. Chem. 19:2661-2665.

Bhagat, R. M., S. I. Bhuiyan, and K. Moody. 1996. Water, tillage and weed interactions in lowland tropical rice: a review. Agr. Water Manage. 31:165-184.

Bhagat, R. M., S. I. Bhuiyan, K. Moody, and L. E. Estorninos. 1999. Effect of water, tillage and herbicide on ecology of weed communities in intensive wet-seeded rice system. Crop Prot. 18:293-303.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248:254.

Bresnahan, G. A., W. C. Koskinen, A. G. Dexter, and W. E. Lueschen. 2000. Influence of soil pH-sorption interactions on imazethapyr carry-over. J. Agr. Food Chem. 48:1929-1934.

Buxton, B. V., C. L. Greenstock, W. P. Helman, and A. B. Ross. 1988. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (·OH/·O-) in aqueous solution. J. Phys. Chem Ref. Data. 17:513-886.

Cantwell, J. R., R. A. Liebl, and F. W. Slife. 1989. Biodegradation characteristics of imazaquin and imazethapyr. Weed Sci. 37:815-819.

Che, M., M. M. Loux, S. J. Traina, and T. J. Logan. 1992. Effect of pH on sorption and desorption of imazaquin and imazethapyr on clays and humic acid. J. Environ. Qual. 21:698-703.

Cleveland, C. B. 1996. Mobility assessment of agrochemicals: current laboratory methodology and suggestions for future directions. Weed Technol. 10:157-168.

Cole, T. A., G. R. Wehtje, J. W. Wilcut, and T. V. Hicks. 1989. Behavior of imazethapyr in soybeans (*Glycine max*) peanuts (*Arachis hypogaea*), and selected weeds. Weed Sci. 37:639-644.

Croughan, T. P. 1994. Application of tissue culture techniques to the development of herbicide resistant rice. La. Agric. 37:25-26.

Croughan, T. P. 1998. Herbicide resistent rice. [5,773,704]. 6-30-1998. U.S. Patent.

Curran, W. C., R. A. Liebl, and F. W. Simmons. 1992a. Effects of tillage and application method on clomazone, imazaquin, and imazethapyr persistence. Weed Sci. 40:482-489.

Curran, W. S., M. M. Loux, R. A. Liebl, and F. W. Simmons. 1992b. Photolysis of imidazolinone herbicides in aqueous solution and soil. Weed Sci. 40:143-148.

Devine, M. D., S. O. Duke, and C. Fedtke. 1993. Physiology of Herbicide Action. Englewood Cliffs: Prentice-Hall, NJ.

Devine, M. D. and A. Shukla. 2000. Altered target sites as a mechanism of herbicide resistance. Crop Prot. 19:881-889.

Diarra, A., R. J. Smith, and R. E. Talbert. 1985. Interference of red rice (*Oryza sativa*) with rice (*Oryza sativa*). Weed Sci. 33:644-649.

Dillon, T. L., F. L. Baldwin, and R. E. Talbert. 1999. Control of red rice and other difficult weeds in imidazolinone tolerant rice. Proc. South. Weed Sci. Soc. 52:15.

Dowler, C. C. 1994. Weed survey-southern states. Proc. South Weed Sci. Soc. 47:279-299.

Dulin, D. and T. Mill. 1982. Development and evaluation of sunlight actinometers. Environ. Sci. Technol. 16:815-820.

Elazzouzi, M., M. Mekkaoui, S. Zaza, M. El Madani, A. Zrineh, and J. M. Chovelon. 2002. Abiotic degradation of imazethapyr in aqueous solution. J. Environ. Sci. Heal.. B. 37:445-451.

Ferrero, A. 2001. Red rice control in rice [On-line], pages 1-8. 2001. in J. Chataigner, Ed. Cahiers The new development in rice agronomy and its effects on yield and quality in Mediterranean areas. Montpellier : CIHEAM-IAMM.

Ferrero, A., F. Vidotto, P. Balsani, and G. Airoldi. 1999. Mechanical and chemical control of red rice (*Oryza sativa* L. var. sylvatica) in rice (*Oryza sativa* L.) pre-planting. Crop Prot. 18:245-251.

Flint, J. L. and W. W. Witt. 1997. Microbial degradation of imazaquin and imazethapyr. Weed Sci. 45:586-591.

Frank, M. P., G. Phillip, and J. S. Chib. 2002. Effect of soil moisture and sample depth on pesticide photolysis. J. Agric. Food Chem. 50:2607-2614.

Gan, J., M. R. Weimer, W. C. Koskinen, D. D. Buhler, D. L. Wyse, and R. L. Becker. 1994. Sorption and desorption of imazethapyr and 5-hydroxyimazethapyr in Minnesota soils. Weed Sci. 42:92-97.

Gealy, D. R. and H. L. Black. 1999. Effect of imazethapyr on several red rice (*Oryza sativa* L.) accessions and rice lines. Proc. South. Weed Sci. Soc. 52:211.

Gealy, D. R., H. L. Black, and R. J. Smith Jr. 2000a. Sensitivity of red rice (*Oryza sativa*) ecotypes to molinate. Proc. Rice Tech. Working Group. 29:158.

Gealy, D. R., D. H. Mitten, and J. N. Rutger. 2003. Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): Implications for weed management. Weed Technol. 17:627-645.

Gealy, D. R., N. E. Saldain, and R. E. Talbert. 2000b. Emergence of red rice (*Oryza sativa*) ecotypes under dry-seeded rice (*Oryza sativa*) culture. Weed Sci. 14:406-412.

Gennari, M., M. Négre, and D. Vidrola. 1998. Adsorption of the herbicides imazapyr, imazethapyr and imazaquin on soils and humic acids. J. Environ. Sci. Heal. B. 33:547-567.

Goetz, A. J., T. L. Lavy, and E. E. Gbur. 1990. Degradation and field persistence of imazethapyr. Weed Sci. 38:421-428.

Goss, W. L. and E. Brown. 1939. Buried red rice seed. J. Am. Soc. Agron. 31:633-637.

Green, R. E. and S. R. Obien. 1969. Herbicide equilibrium in soils in relation to soil water content. Weed Sci. 17:514-519.

Haag, W. R. and C. C. D. Yao. 1992. Rate constants for reaction of hydroxyl radicals with several drinking water contaminants. Environ. Sci. Technol. 26:1005-1013.

Hackworth, H. M., P. Bruno, J. B. Guice , J. S. Harden, and A. Rhodes. 2002. Weed control and crop tolerance in a drill-seeded Clearfield rice production system. Proc. Rice Tech. Working Group. 29:168.

Hwang, I. T., K. H. Lee, S. H. Park, B. H. Lee, K. S. Hong, S. S. Han, and K. Y. Cho. 2001. Resistance to acetolactate synthase inhibitors in a ecotypes of *Monochoria vaginalis* discovered in Korean Pest. Biochem. Phys. 71:69-76.

Johnson, D. H., D. L. Jordan, W. G. Johnson, R. E. Talbert, and R. E. Frans. 1993. Nicosulfuron, primisulfuron, imazethapyr, and DPX-PE350 injury to succeeding crops. Weed Technol. 7:641-644.

Johnson, D. H., D. L. Shaner, J. Deane, L. A. Mackersie, and G. Tuxhorn. 2000. Time-dependent adsorption of imazethapyr to soil. Weed Sci. 10:769-775.

Kin, J., J. A. Ivany, and R. R. King. 1995. Persistence of imazethapyr in two atlantic Canada soils. Can. J. Soil Sci. 75:525-527.

Kobayashi, K., M. Onoe, and H. Sugiyama. 1994. Thenylchlor concentration in soil and water and its herbicidal activity. Weed Res. Japan. 39:160-164.

Koskinen, W. C., D. J. Mulla, R. S. Oliveira, B. R. Khakural, and P. C. Robert. 2003. Spatial variability of herbicide sorption on soil. Terrestrial field dissipation studies: purpose, design and interpretation. ACS Symposium Series. 842:88-101.

Kuk, Y. I., H. I. Jung, O. D. Kwon, D. J. Lee, N. R. Burgos, and J. O. Guh. 2003. Rapid diagnosis of resistance of resistance for sulfonylurea herbicides in monochoria (*Monochoria vaginalis*). Weed Sci. 51:305-311.

Kwon, S. L., R. J. Smith, and R. E. Talbert. 1991. Interference durations of red rice (*Oryza sativa*) in rice (*Oryza sativa*). Weed Sci. 39:363-368.

Langevin, S. A., K. Clay, and J. B. Grace. 1990. The incidence and effects of hybridization between cultivated rice and its related weed red rice (*Oryza sativa* L). Evolution. 44:1000-1008.

Lee, D. J., K. Kobayashi, and H. Ishizuka. 1998. Effect of soil moisture condition on the activity of soil-applied herbicides. Weed Res. Japan. 43:162-163.

Lee, D. J, S. A. Senseman, J. H. O'Barr, J. M. Chandler, L. J. Krutz, G. N. McCauley, and Y. I. Kuk. 2004. Soil characteristics and water potential effects on plant-available clomazone in rice. Weed Sci. 52:310-318.

Little, D. L. and D. L. Shaner. 1991. Absorption and Translocation of the Imidazolinone Herbicide, pages 53-69. in: D L Shaner and S L O'Conner, Eds. The Imidazolinone Herbicides, Boca Raton, FL: CRC Press.

Loux, M. M., R. A. Liebl, and F. W. Slife. 1989a. Adsorption of imazaquin and imazethapyr on soils, sediments, and selected adsorbents. Weed Sci. 37:712-718.

Loux, M. M., R. A. Liebl, and F. W. Slife. 1989b. Availability and persistence of imazaquin, imazethapyr, and clomazone in soil. Weed Sci. 37:259-267.

Loux, M. M. and K. D. Reese. 1993. Effect of soil type and pH on persistence and carryover of imidazolinone herbicides. Weed Technol. 7:452-458.

Mabury, S. A. and D. G. Crosby. 1996. Pesticide reactivity and its relationship to field persistence. J. Agr. Food Chem. 44:1920-1924.

Machado, S. L. O., H. Kummer, A. A. B. Mainardi, L. A. Avila, and E. Marchezan. 1998. Red rice seed bank: seed bank survey and re-infestation potential in rice commercial field in Rio Grande do Sul state, Brazil. Proc. Reunão Nacional do Arroz Irrigado. 6:384-386.

Madani, M., M. Azzouzi, A. Zrineh, D. Martens, and A. Kettrup. 2003. pH effect and kinetic studies of the binding behaviour of imazethapyr herbicide on some Moroccan soils. Fresen. Environ. Bull. 12:1114-1119.

Malik, M. S., R. E. Talbert, E. F. Scherder and M. L. Lovelace. 2002. Use of imazethapyr for barnyardgrass control in conventional and stale seedbed production of Clearfield rice. Proc. Rice Tech. Working Group. 29:167.

Mangel, G. 1991. Behavior of the imidazolinones herbicides in the aquatic environment. Pages 183-190. in: D. L. Shaner and S. L. O'Conner, eds. The Imidazolinone Herbicides. Boca Raton, FL: CRC Press.

Manley, B. S., B. K. Singh, D. L. Shaner, and H. P. Wilson. 1999. Imidazolinone resistance in Smooth pigweed (*Amaranthus hybridus*) is due to an altered acetolactate synthase. Weed Technol. 13:697-705.

Masson, J. A. and E. P. Webster. 2001. Use of imazethapyr in water-seeded imidazolinone-tolerant rice (*Oryza sativa*). Weed Technol. 15:103-106.

Masson, J. A., E. P. Webster, and J. Williams. 2001. Flood depth, application timing, and imazethapyr activity in imidazolinone-tolerant rice (*Oryza sativa*). Weed Technol. 15:315-319.

Mill, T. 1999. Predicting photoreaction rates in surface waters. Chemosphere. 38:1379-1390.

Miller, G. C. and R. Zeep. 1979. Photoreactivity of aquatic pollutants sorbed on suspended sediments. Environ. Sci. Technol. 13:860-873.

Mills, J. A. and W. W. Witt. 1989. Efficacy, phytotoxicity, and persistence of imazaquin, imazethapyr, and clomazone in non-till double-crop soybeans (*Glycine max*). Weed Sci. 37:353-359.

Montealegre, F. A. and J. P. Vargas. 1989. Effect of some cultural practices in the red rice population and commercial rice grain yield. Arroz. 38:19-24.

Moyer, J. R. 1987. Effects of soil moisture on the efficacy and selectivity of soil-applied herbicides. Rev. Weed Sci. 3:19-34.

Moyer, J. R. and R. Esau. 1996. Imidazolinone herbicide effects on following rotation crops in Southern Alberta. Weed Technol. 10:100-106.

Noldin, J. A. 1988. Red rice control in the water seeded system. Lavoura Arrozeira, 41:377.

Noldin, J. A. 1995. Characterization, seed longevity, and herbicide sensitivity of red rice (*Oryza sativa* L) ecotypes, and red rice control in soybeans [*Glycine max* (L.) Merr.], Ph.D. Dissertation, Texas A&M University, College Station.

Noldin, J. A., J. M. Chandler, M. L. Ketchersid, and G. N. McCauley. 1999a Red rice (*Oryza sativa*) biology. II. Ecotype sensitivity to herbicides. Weed Technol. 13:19-24.

Noldin, J. A., J. M. Chandler, and G. N. McCauley. 1999b Red rice (*Oryza sativa*) biology. I. Characterization of red rice ecotypes. Weed Technol. 13:12-18.

Oard, J., M. A. Cohn, S. Linscombe, D. Gealy, and K. Gravois. 2000. Field evaluation of seed production, shattering, and dormancy in hybrid population of transgenic rice (*Oryza sativa*) and the weed red rice (*Oryza sativa*). Plant Sci.157:13-22.

O'Dell, J. D., J. D. Wolt, and P. M. Jardine. 1992. Transport of imazethapyr in undisturbed soil columns. Soil Sci. Soc. Am. J. 56:1711-1715.

Oka, H. I. and W. T. Chang. 1961. Hybrid swarms between wild and cultivated rice species, *Oryza perennis* and *O. sativa*. Evolution. 15:418-430.

Oliveira, M. F., I. Colonna, H. T. Prates, E. C. Mantovani, R. L. Gomide, and R. S. Oliveira. 2004. Imazaquin herbicide sorption by an oxisol with till and no-till management. Pesqui. Agropecu. Bras. 39:787-793.

Oliveira, R. S., W. C. Koskinen, and F. A. Ferreira. 2001. Sorption and leaching potential of herbicides on Brazilian Soils. Weed Res. 41:97-110.

Olofsdotter, M., B. E. Valverde, and K. H. Madsen. 2000. Herbicide resistant rice (*Oryza sativa* L.): Global implications for weedy rice and weed management. Ann. Appl. Biol. 137:279-295.

Ottis, B. V., J. M. Chandler, and G. N. McCauley. 2003. Imazethapyr application methods and sequences for imidazolinone-tolerant rice (*Oryza sativa*). Weed Technol. 17:526-533.

Peter, C. J. and J. B. Weber. 1985. Adsorption, mobility and efficacy of alachlor and metholachlor as influenced by soil properties. Weed Sci. 33:874-881.

Pulver, E. 1986. Economical damage caused by red rice. Lavoura Arrozeira, 39:20-23.

Ray, T. B. 1984. Site of action of chlorsulfuron, Inhibition of valine and isoleusine biosynthesis in plants. Plant Physiol. 75:827-831.

Renner, K. A., W. F. Meggitt, and D. Penner. 1988. Effect of soil pH on imazaquin and imazethapyr adsorption to soil and phytotoxicity to corn (*Zea mays*). Weed Sci. 36:78-83.

Roel, A., J. L. Heilman, and G. N. McCauley. 1999. Water use and response in two rice irrigation methods. Agr. Water Manage. 39:35-46.

Romano, N., L. A. Hopmans, and J. H Dane. 2002. Suction tables. Pages 692-698 in Daneand, J. H.; Topp, G. C. eds. Methods of Soil Analysis. Part 4. Physical Methods, Madison, WI: Soil Science Society of America.

[SAS] Statistical Analysis System. 1999. SAS/STAT User's Guide. Version 8. Cary, NC: Statistical Analysis System Institute.

Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imboden. 1992. Environmental Organic Chemistry. New York: Wiley Interscience.

Seefeldt, S. S., J. E. Jensen, and E. P. Fuerst. 1995. Log-logistic analysis of herbicide dose-response relationship. Weed Technol. 9:218-227.

Shaner, D. L. and S. O'Conner. 1991. The Imidazolinone Herbicides. Boca Raton, FL: CRC Press.

Shaner, D. L. and P. A. Robson. 1985. Absorption, translocation, and metabolism of AC 252,214 in soybean (*Glycine max*), common cocklebur (*Xanthium strumarium*), and velvetleaf (*Abutilon theophrasti*). Weed Sci. 33:469-471.

Si, Y. B., S. Q. Wang, D. M. Zhou, and H. M. Chen. 2004. Adsorption and photo-reactivity of bensulfuron-methyl on homoionic clays. Clay. Clay. Miner. 52:742-748.

Silva, P. R. F. 1980. Nitrogen volatilization from rice leaves as affected by genotype, temperature, rate and source of nitrogen applied, Ph.D. Dissertation, The University of Arkansas, Fayetteville.

Singh, B. K., M. A. Stidham, and D. L. Shaner. 1988. Assay of acetohydroxyacid synthase. Anal. Biochem. 171:173-179.

Smith, M. C., D. R. Shaw, J. H. Massey, M. Boyette, and W. Kingery. 2003. Using nonequilibrium thin-disc and batch equilibrium techniques to evaluate herbicide sorption. J. Environ. Qual. 32:1393-1404.

Smith, R. J. and W. T. Fox. 1973. Soil water and growth of rice and weeds. Weed Sci. 21:61-63.

Snyder, C. S. and D. N. Slaton. 2002. Effects of soil flooding and drying on phosphorus reactions. News and Views. April:1-3.

Souza, A. P. 1998. Mobility and degradation of glyphosate and imazethapyr in soils with different textures and chemical compositions. Ph.D. Dissertation, Universidade de Viçosa, Viçosa, MG, Brazil.

Souza, P. R. and M. M. Fisher. 1986. Red rice: damage caused in the Gaucho's rice industry. Lavoura Arrozeira. 39:19-20.

Steele, G. L., J. M. Chandler, and G. N. McCauley. 2002. Control of red rice (*Oryza sativa*) in imidazolinone-tolerant rice (*O. sativa*). Weed Technol. 16:627-630.

Stougaard, R. N., P. J. Shea, and A. R. Martin. 1990. Effect of soil type and pH on adsorption, mobility, and efficacy of imazaquin and imazethapyr. Weed Sci. 38:67-73.

Subramanian, M. V., H. Y. Hung, J. M. Dias, V. W. Miner, J. H. Butler, and J. J. Jachetta. 1994. Properties of mutant acetolactate synthase resistant to triazolopyrimidine sulfonanilide. Plant Physiol. 94:239-244.

[USEPA] U.S. Environmental Protection Agency. 1985. 40 Code of Federal Regulations, Part 796.3700, Toxic Substances Control Act Test Guidelines; Final Rules, Photolysis in Aqueous Solution in Sunlight, Washington, DC.

Vaughan, L. K., B. V. Ottis, A. M. Prazak-Havey, C. A. Bormans, C. Sneller, J. M. Chandler, and W. D. Park. 2001. Is all red rice found in commercial rice really *Oryza sativa*? Weed Sci. 49:468-476.

Vencill, V. K. 2002. Herbicide Handbook, 8th ed.; Lawrence, KS: Weed Science Society of America.

Wan, D. C., M. K. Wong, and C. Y. Mok. 1994. Comparative study on the quantum yields of direct photolysis of organophosphorus pesticides in aqueous solution. J. Agr. Food Chem. 42:2625-2630.

Wauchope, R. D., S. Yeh, J. B. H. J. Linders, R. Kloskowski, R., K. Tanaka, B. Rubin, J., A Katayama, W. Kördel, Z. Gerstl, M. Lane, and J. B. Unsworth. 2002. Pesticide soil sorption parameters: theory, measurement, uses, limitations and reliability. Pest. Manag. Sci. 58:419-445.

Way, M. O., J. K. Olson, and B. M. Drees. 2004. Insect management alternatives. Pages 29-43. In D. Bowen ed. Rice Production Guidelines, Beaumont, TX: Texas A&M University System.

Weber, J. B., G. G. Wilkerson, C. F. Reinhardt. 2004. Calculating pesticide sorption coefficient (K_d) using selected soil properties. Chemosphere, 55:157-166.

Webster, E. P. and F. L. Baldwin. 1998. Weed control systems for imidazolinone-rice. Proc. Rice Tech. Working Group. 29:215.

Webster, T. M. 2000. The southern states 10 most common and troublesome weeds in rice. Proc. South. Weed Sci. Soc. 53:247-274.

Weed Science. 2004. Herbicide resistant weeds summary table. http://www.weedscience.org. (accessed on 04/02/2004).

Wei, Z. and L. Weip. 1998. Adsorption-desorption and photolysis of the herbicide imazethapyr in soil-water environment. China Environ. Sci. 18:476-480.

Williams, B. J., R. Strahan, and E. P. Webster. 2002. Weed management systems for Clearfield rice. La. Agr. 45:16-17.

Wong, C. C. and W. Chu. 2003. The direct photolysis and photocatalytic degradation of alachlor at different TiO_2 and UV sources. Chemosphere, 50:981-987.

Wyk, L. J. and C. F. Reinhardt. 2001. A bioassay technique detects imazethapyr leaching and liming-dependent activity. Weed Sci. 15:1-6.

Zepp, R. G. 1978. Quantum yields for reaction of pollutants in dilute aquatic solutions. Environ. Sci. Technol. 12:327-329.

Zepp, R. G. and D. M. Cline. 1977. Rates of direct photolysis in aquatic environment. Environ. Sci. Technol. 11:359-366.

Zhang, W., E. P. Webster, and M. P. Braverman. 2002. Rice (*Oryza sativa*) response to rotational crop and rice herbicide combinations. Weed Technol. 16:340-345.

VITA

Luis Antonio de Avila, son of Ataliba and Almerinda Pedrotti de Avila, was born in 1973 in Cruz Alta, a small city in southern Brazil as the 12th child of 13 children. In 1996 he received his B.S. degree in agronomy at the Universidade Federal de Santa Maria (UFSM). During the bachelors degree (1995) he met his beloved Marcia Vizzotto. In March 1999 he received his M.S. degree in agronomy from the same university.

In 1999, Mr. Avila was hired as Junior Professor by the Crop Sciences Department (Departamento de Fitotecnia) UFSM. In 2000, he was promoted to Assistant Professor and got married. In 2001 he was released in the faculty leave program to pursue his Ph.D. at Texas A&M University. His wife Marcia came to TAMU to pursue her Ph.D. as well. At the end of their Ph.D., they had the joy of their first child, a baby girl called Carolina Vizzotto de Avila.

At the completion of the degree Dr. Avila went back to his home country to resume his position at UFSM, expecting to be promoted to Associate Professor. His main duty will include teaching, research and extension in rice plant management and environmental fate of pesticides.

His permanent address is: Departamento de Fitotecnia, Centro de Ciências Rurais, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, RS, Brazil.