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Rice (Oryza Sativa) Response to Low Glyphosate Rates as Influenced by Cultivar, Growth Stage, and Imazethapyr Applications

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RICE (*Oryza sativa*) RESPONSE TO LOW GLYPHOSATE RATES AS INFLUENCED BY CULTIVAR, GROWTH STAGE, AND IMAZETHAPYR APPLICATIONS

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ABSTRACT

Off-target movement of glyphosate onto rice is a perennial concern when rice is grown in close proximity to glyphosate-tolernat crops. If differential tolerance to sub-lethal rates of glyphosate exists among rice (*Oryza sativa*) cultivars, these cultivars could be utilized in breeding programs or glyphosate-drift sensitive areas. Field and greenhouse experiments were conducted in 2006 and 2007 to examine differences among rice cultivars in response to sub-lethal rates of glyphosate, and to examine imidazolinone-tolerant rice response to imazethapyr and sub-lethal rates of glyphosate applied sequentially to determine the potential for either herbicide to predispose rice to greater injury. In the field experiment, glyphosate was applied at 0, 45, and 90 g ae/ha at the 3- to 4-leaf, panicle initiation, and boot growth stages of 10 rice cultivars. Cultivars inherently differed in plant height, flag leaf length, and yield. Glyphosate applied at the 3- to 4-leaf stage reduced plant height and yield of cultivars less than 5%. Height of all cultivars was affected more by glyphosate at 90 g/ha applied at panicle initiation, but the greatest yield reduction across all cultivars was from 90 g/ha glyphosate applied at boot stage. Relative flag leaf length of some cultivars was reduced by glyphosate more at one location than the other, and this reduction differed by growth stage. In field and greenhouse experiments, glyphosate was applied at 0, 45, and 90 g ae/ha at 14, 7, 3, 1, and 0 d prior to applications of imazethapyr at 0, 105, or 210 g ai/ha to the imidazolinone-tolerant cultivar ‘CL 161’. Imazethapyr at those rates was also applied 14, 7, 3, 1, and 0 d prior to receiving glyphosate at the above rates to determine any predisposition of CL 161 to either herbicide. Glyphosate reduced relative plant height, relative dry mass, and relative yield of CL 161. There was no interaction between imazethapyr and glyphosate on relative plant height, relative dry mass, relative leaf chlorophyll content, or relative yield; therefore, influence is independent. There is

no evidence that sequential applications of imazethapyr or sub-lethal rates of glyphosate will predispose CL 161 to greater injury from either herbicide.

This thesis is approved for recommendation
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Chapter 2

Rice (*Oryza sativa*) Response to Low Glyphosate Rates as Influenced by Cultivar, Growth Stage, and Imazethapyr Applications

Jason R. Meier

Introduction

Many crops have natural tolerance to selective herbicides. This allows the use of these herbicides for weed control without effects on the crop itself. Resistance mechanisms in weedy plants are similar to those in crop plants. Some of the readily recognized mechanisms include: detoxification of the herbicide (Carey et al. 1997; Fuerst and Vaughn 1990; Leah et al. 1994), target site mutations (Hall et al. 1994; Llewellyn and Powles 2001; Tan et al. 2005), reduced translocation (Feng et al. 2004; Koger and Reddy 2005; Perez-Jones et al. 2007), and sequestration (Ge et al. 2009; Michitte et al. 2007; Yuan et al. 2007). Additional mechanisms are almost certain to be confirmed as biological systems evolve with continued use of herbicides as weed control tools. All of the early herbicide tolerance in crop plants, and some of the new, was accomplished through gene transfer by breeding when a related herbicide-tolerant plant species was identified (Kishore et al. 1992). Screening of soybean (*Glycine max* (L.) Merr.) germplasm resulted in identification of metribuzin-tolerant lines due to a detoxification mechanism that results in inactivation of metribuzin (Barrentine et al. 1982). Inbred lines of corn (*Zea mays* L.) showed differences in sensitivity to some sulfonylurea herbicides; one line was completely tolerant to primsulfuron whereas others were completely killed (Harms et al. 1990). The mechanism of tolerance for imidazolinone-tolerant rice is due to a target site mutation that reduces the sensitivity of the acetohyroxyacid synthase (AHAS) enzyme to imidazolinone herbicides (Tan et al. 2005). Imidazolinone-tolerant crops were developed through selection or mutagenesis, utilizing conventional plant breeding techniques and because of this are considered non-transgenic (Tan et al. 2005). A single surviving rice plant was identified and its progeny showed tolerance to several acetolactate synthase inhibiting (ALS) herbicides (Croughan 1994). Imazethapyr and imazamox (ALS inhibitors) are the herbicides recommended for use in imidazolinone-tolerant rice because of efficacy on red rice and crop tolerance (Scott et al. 2011; White and Hackworth 1999).

Interest in the development of glyphosate-resistant crops began in the early 1980s and the pursuit of other herbicide-resistant crops continues at the present time. Several methods were used to develop glyphosate-resistance in plants; however, the method that resulted in production of commercial glyphosate-resistant crops was the introduction of an insensitive 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene into the crop genome. The *Agrobacterium*-mediated gene transfer has been successful in many dicot plants; however, this method does not work well for monocots (Kishore, et al. 1992). The *Agrobacterium* technology appears to be the most efficient method for gene transfer and therefore is used in transformation of dicot crops such as canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.) and sugarbeet (*Beta vulgaris* L.) (Kishore, et al. 1992). Most of the glyphosate-resistant crops contain the bacterial EPSPS enzyme that occurs naturally in the *Agrobacterium* sp. strain CP4 that is tolerant to glyphosate (Padgette et al. 1995; Dill 2005).

In 1996, glyphosate-tolerant soybean and canola were introduced for commercial use followed by glyphosate-tolerant cotton in 1997 and glyphosate-tolerant corn in 1999. Since the introduction of glyphosate-tolerant crops, the use of glyphosate increased yearly as more and more acres of glyphosate-tolerant crops were planted, and issues concerning glyphosate drift onto non-target crops and weed resistance to glyphosate have also increased (Dill 2005). Although most glyphosate-tolerant crops can tolerate glyphosate at rates above what is labeled, there is the potential for other adverse effects from glyphosate applications. In glyphosate-tolerant cotton, applications of glyphosate 24 h prior to applications of 2,4-D and halosulfuron at drift rates increased cotton injury (Smith et al. 2005). Glyphosate applications can also predispose glyphosate-tolerant soybean to various diseases such as sudden death syndrome (*Fusarium solani* f. sp. *glycines*) and root rot (*Phytophthora megasperma*) (Johal and Huber 2009). Injury to glyphosate-tolerant soybean from a reduced rate of dicamba was also increased when the dicamba was applied with glyphosate (Kelley et al. 2005).

It is not uncommon for rice to be grown near or adjacent to soybean, corn, and cotton fields. This creates potential issues concerning off-target movement of glyphosate onto rice. In imidazolinone-tolerant rice cultivars it is unknown if applications of imazethapyr will affect injury from glyphosate at sub-lethal rates associated with drift. No rice cultivars grown in the United States are tolerant to full rates of glyphosate. If rice cultivars exist that are more tolerant to low glyphosate rates commonly associated with herbicide drift they could be planted in more drift-sensitive areas, or used in breeding programs to develop cultivars with more tolerance to glyphosate. The focus of this research was to determine if rice cultivars respond differently to sub-lethal rates of glyphosate, and to examine the effects of imazethapyr applications on imidazolinone-tolerant rice before or after applications of sub-lethal rates of glyphosate.

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Chapter 1

Rice (*Oryza sativa*) Cultivar Response to Low Glyphosate Rates as Influenced by Growth Stage

Jason R. Meier

The off-target movement of glyphosate onto rice can cause adverse effects on growth and yield of rice. If differential tolerance to sub-lethal rates of glyphosate exists among rice cultivars, these cultivars could be utilized in breeding programs or glyphosate drift sensitive areas. Field experiments were conducted in 2006 and 2007 to evaluate the effects of low glyphosate rates on rice cultivars at different growth stages to determine if there are differences among rice cultivars in response to sub-lethal rates of glyphosate. Glyphosate was applied at 0, 45, and 90 g ae/ha at the 3- to 4-leaf (3-4 LF), panicle initiation (PI), and boot growth stages. Rice cultivars inherently differed in plant height, flag leaf length, and yield. Glyphosate applied at the 3-4 LF stage did not reduce plant height or yield of cultivars by more than 5%; however, glyphosate applied at PI and boot growth stages resulted in height and yield reductions, which increased with the rate of glyphosate. Height of all cultivars was affected more by glyphosate when applied at 90 g/ha at PI compared to 3-4 LF and boot growth stages, but the greatest yield reduction across all cultivars was from 90 g/ha glyphosate applied at boot. Flag leaf length of some cultivars was reduced by glyphosate more at one location than at the other and this reduction varied by growth stage at each location. Rice cultivars in this experiment responded differently to low rates of glyphosate and response varied by growth stage. Glyphosate exposure to rice at low rates during PI and boot can be detrimental to rice yield and should be avoided.

Nomenclature: Glyphosate; rice, *Oryza sativa* L.

Key words: Glyphosate drift, plant height, flag leaf length, yield.

Physical drift, the off-target movement of droplets of a pesticide, is most often the result of improper application (Wauchope et al. 1982). Herbicide drift can occur when herbicides are applied during drift-condusive conditions such as when wind speed is high or when conditions favor temperature inversion (Hatterman-Valenti et al. 1995). Other factors that influence off-target movement of herbicides are nozzle size, carrier volume, droplet size, and distance of nozzle to target. Simulated glyphosate drift from a helicopter, a fixed-wing aircraft, and ground equipment were evaluated by Yates et al. (1978). The least amount of drift was observed from a ground spray with low-pressure and deflector nozzles and the greatest amount of drift was observed from D-6 jet nozzles mounted on fixed-wing aircraft (Yates et al. 1978). The severity of damage to susceptible plants from drift depends on many factors, such as species, growth stage, herbicide formulation, environmental conditions, droplet size, herbicide concentration in drift particles, and plant coverage (Deeds et al. 2006). The concentration of off-target herbicide particles usually ranges between 1/10 and 1/100 of the initial concentration of the intended use rate (Al-Khatib and Peterson 1999; Al-Khatib and Tamhane 1999; Bode 1987; Maybank et al. 1978). Even though off-target exposure to herbicides may occur at sub-lethal rates, severe injury to susceptible crops may occur and depending on the susceptibility of plants to specific herbicides, injury can occur at a considerable distance from the target (Al-Khatib et al. 1992; Al-Khatib and Peterson 1999; Al-Khatib et al. 2003).

Injury from sub-lethal rates of glyphosate has been reported in many crops, including pea (*Pisum sativum* L.) (Al-Khatib and Tamhane 1999), corn (*Zea mays* L.) (Ellis and Griffin 2002; Matthews et al. 1998; Rowland et al. 1999), rice (Davis et al. 2011; Ellis et al. 2003;Hensley et al. 2009; Koger et al. 2005; Kurtz and Street 2003), soybean (*Glycine max* (L.) Merr.) (Al-Khatib and Peterson 1999; Ellis and Griffin 2002; Ellis et al. 2002), cotton (*Gossypium hirsutum* L.)

(Rowland et al. 1999; Ellis and Griffin 2002), wheat (*Triticum aestivum* L.) (Deeds et al. 2006; Roider et al. 2007), and grain sorghum [*Sorghum bicolor* (L.) Moench] (Al-Khatib et al. 2003). Yield of non-glyphosate-resistant (GR) corn was reduced by more than 50% from glyphosate applied at rates of 84 and 168 g ae/ha to corn 15- to 25-cm tall (Matthews et al. 1998) and yield reductions of 20 and 80% were reported for non-GR corn and cotton with glyphosate at 315 g/ha (Rowland et al. 1999). The height of wheat was progressively reduced with increasing rates of glyphosate applied at first node, boot stage, and early flowering (Roider et al. 2007). Glyphosate at sub-lethal rates can cause severe injury to rice and significantly reduce grain yield (Ellis et al. 2003; Hensley et al. 2009; Kurtz and Street 2003).

In most cases, injury symptoms from herbicide drift are more severe when drift occurs to the susceptible crop early in its development (Ghosheh et al. 1994; Hurst 1982). Wheat was more sensitive to glyphosate applied at first node than at boot stage or early flowering (Roider et al. 2007), and rice tolerance to acifluorfen, triclopyr, and fenoxaprop is dependent upon its growth stage at herbicide application (Smith 1988; Snipes and Street 1987; Street and Richard 1983). The most susceptible growth stages of rice associated with yield loss from glyphosate are panicle initiation and boot stage, although visible injury to rice from glyphosate was greater at the 3-4 LF stage and least at boot stage (Kurtz and Street 2003). Visible injury to corn, grain sorghum, wheat, and rice from glyphosate usually develops slowly and primarily consists of plant height reduction and slight yellowing of leaves (Al-Khatib et al. 2003; Ellis et al. 2003; Koger et al. 2005; Deeds et al. 2006; Roider et al. 2007). Plant height reduction and leaf chlorosis of Cocodrie and Priscilla from glyphosate increased with increasing glyphosate rates; however, injury to Priscilla was more severe when compared to Cocodrie (Koger et al. 2005). Rough rice yield also decreased more as glyphosate rate increased, but Cocodrie rice yield was reduced more than Priscilla rice yield at comparable rates (Koger et al. 2005). If some cultivars are more tolerant to sub-lethal rates of glyphosate than others, these cultivars could be used in areas with a high risk of glyphosate drift or potentially be used in breeding programs to develop new cultivars that are less susceptible to injury from glyphosate drift.

The objective of this research was to evaluate the effect of sub-lethal rates of glyphosate, applied at different growth stages, on growth and yield of 10 rice cultivars.

Materials and Methods

Experiments were conducted in 2006 and 2007 at the Southeast Research and Extension Center (SEREC) near Rohwer, AR, on a Sharkey clay soil (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept) and at the University of Arkansas Pine Bluff Research Farm (UAPBRF) near Lonoke, AR, on a Calhoun silt loam (fine-silty, mixed, active, thermic Typic Glossaqualfs). Experiments were conducted under conventional production practices common in the area and flush-irrigated as needed until permanent flood. The experimental design was a split-split-plot with growth stage as the main plot, glyphosate rate as the sub-plot, and cultivar as the sub-sub-plot, replicated four times. The selected cultivars 'Banks', 'Bengal', 'CL 161', 'CL XL8', 'Cocodrie', 'Drew', 'Francis', 'LaGrue', 'Katy', and 'Wells' are currently, or were previously, used in Arkansas rice production. Katy, a mid-season long-grain released in 1989, is the oldest cultivar; Banks, a short-season long-grain released in 2004, is the most recent among these cultivars (Table 1). Katy and LaGrue (old cultivars), Francis and Wells, and Cocodrie and CL 161 have similar parents in their pedigree. Other cultivars were derived from older parents such as Drew, which was derived from Katy, and Banks, which was derived from LaGrue. The selected cultivars represent conventional and hybrid groups, short- and mid-season, long- and medium-grain, and semi-dwarf cultivars that are a cross-section of the rice phenotypes grown in

Arkansas (Table 1). All cultivars were drill-seeded 3 cm deep at 101 kg/ha in nine rows spaced 15 cm apart. Plots were 1.4 m wide and 7.6 m long with 1.5 m alleys separating the plots. All plots were managed for weed, insect, and disease control and fertilized according to the University of Arkansas Cooperative Extension Service recommendations (Slaton, 2001).

Glyphosate[1] was applied at 0, 45, and 90 g ae/ha, which represents 0, 1/20, and 1/10 of a recommended use rate (Scott et al. 2010). Applications were made using a CO_2 pressurized backpack sprayer equipped with AirMix 110015 nozzles[2] calibrated to deliver 112 L/ha at 186 kPa when the respective cultivar reached the 3-4 LF, PI, and boot stages of growth. The 3-4 LF growth stage timing was uniform among cultivars and applications were made accordingly. Since PI and boot growth stages can last for several days and differ among cultivars, PI applications were defined as internode elongation of 6 mm and boot applications were defined as swollen boot prior to boot split, and applications were made by cultivar.

Plant height (cm) was recorded 1, 2, and 3 weeks after each application (WAA) and prior to harvest from three plants at random locations in each plot based on measurement from the base of the plant at the soil surface to the extended tip of the uppermost leaf of each plant. Flag leaf length (cm) was also recorded from three plants at random locations in each plot prior to harvest. The entire plot was harvested for yield with a small plot combine at each location both years. Data were expressed in terms of percentage of the corresponding check for each cultivar ([treated/nontreated]*100). Data were analyzed by ANOVA, and means were separated using Fisher's protected LSD at the 5% level of probability.

Results and Discussion

Cultivar Potential. Cultivar potential expressed in terms of plant height at harvest, flag leaf length, and yield from untreated plants illustrate the similarity or diversity in growth habits among cultivars (Table 2). Cultivars with similar lineages expressed similar traits while others expressed improved traits from breeding, such as higher yield. For example, yield of Drew, a descendant of Katy, was greater than yield of Katy (Table 2). More importantly, differences among cultivars with different lineages were also expressed. Drew (129 cm) was taller than all other cultivars, and Cocodrie was the shortest (97 cm) of all cultivars. Cocodrie also had the shortest flag leaf length and, except for CL 161, the lowest yield of all cultivars (Table 2). The greatest difference in yield was between Francis (9019 kg/ha) and Katy (6478 kg/ha) (Table 2).

Plant Height. A reduction in relative plant height is reflective of the stunting that occurred to rice following glyphosate applications when compared to the non-treated control. Based on relative plant height, plants recovered slightly from 45 g/ha glyphosate when compared across cultivars and growth stages from 1 to 3 WAA at both locations (Table 3). Relative plant height was reduced more with glyphosate at 90 g/ha at both locations (Appendix 1) and did not recover with time, whereas plants treated with 45 g/ha glyphosate recovered slightly (Table 3). The growth stage of rice at the time of glyphosate application also affected the degree of plant height reduction with glyphosate treatment (Appendix 2 and 3). Relative plant height was reduced more with 45 and 90 g/ha of glyphosate applied at the 3-4 LF stage compared with PI and boot applications 1 WAA (Table 4).Rice recovered over time from the 3-4 LF applications, but not from the PI and boot stage applications. Glyphosate applied at 90 g/ha at the PI growth stage reduced relative plant height progressively from 1 to 3 WAA. Visual observations of a split culm of all plants treated with 90 g/ha glyphosate at the PI growth stage, regardless of cultivar, showed a necrotic spot at the growing point where panicle formation occurs. Glyphosate is translocated to the active growing point (Senseman 2007), which could explain why plant height was reduced progressively with time. Glyphosate applied to rice at the boot growth stage had little effect on plant height because most cultivars, except taller-mid-season cultivars Drew and Katy, had reached their height potential by this time.

The growth stage of rice at the time of glyphosate application also influenced relative plant height at harvest (Appendix 4). Relative plant height at harvest also differed among cultivars by glyphosate rate (Table 5). Reductions in relative plant height from applications of glyphosate at 90 g/ha to rice at the 3-4LF growth stage differed among cultivars, but were minimal (5% or less). Applications of 90 g/ha glyphosate at PI reduced relative plant height of all cultivars more compared with glyphosate at 45 g/ha. Relative plant height of Bengal was reduced more than all other cultivars at PI with glyphosate at 45 g/ha, and was reduced more than Banks, Cocodrie, Francis, Katy, LaGrue, and Wells with PI applications of glyphosate at 90 g/ha. Previous research has indicated that medium-grain rice cultivars are more sensitive to glufosinate, bispyribac, and clomazone compared to long-grain cultivars (Lanclos et al. 1999; Zhang and Webster 2002; Zhang et al. 2004; Mudge et al. 2005). At boot, relative plant height was also reduced more as glyphosate rate increased in cultivars Banks, CL 161, Drew, Francis, Katy, LaGrue, and Wells. Relative plant height of Bengal, a short-season, semi-dwarf cultivar, was not affected from boot applications of glyphosate because the maximum plant height was achieved prior to the boot application. Drew had the greatest reduction in relative plant height from boot applications of glyphosate at 90 g/ha, followed by Katy (Table 5). Drew, Katy, and CL 161 are all mid-season cultivars, but CL 161 is a semi-dwarf, and Drew and Katy had not reached their height potential at boot stage. Relative plant height of Banks, CL 161, CL XL8, Cocodrie, Francis, LaGrue, and Wells were reduced by no more than 6% from 90 g/ha of glyphosate applied at boot stage. Glyphosate at 45 g/ha had no effect on plant height at any growth stage of Banks, CL XL8, Drew, Francis, Katy, and Wells cultivars. The greatest reductions in plant height at harvest were from applications of glyphosate at 90 g/ha to rice at the PI growth stage. This growth stage is more sensitive to reductions in plant height from glyphosate because internode elongation begins at about the same time as PI and continues until full plant height is achieved (Slaton 2001). Although this growth stage is similar for all rice cultivars, and applications were made by cultivar, there were still differences among cultivars in response to glyphosate at 90 g/ha applied at PI.

Flag Leaf Length. Flag leaf assimilates are an important contributor to yield potential of small grains (Austin et al. 1977), and reductions in flag leaf length from glyphosate at sub-lethal rates has been observed in rice and wheat (Deeds et al. 2006; Davis et al. 2011) Glyphosate applied at the 3-4 LF growth stage had little effect (6% or less) on relative flag leaf length of rice cultivars at either location (Table 6). Similar to plant height, glyphosate at 45 and 90 g/ha had little effect on flag leaf length when applied at a vegetative growth stage because rice plants had recovered from injury before the flag leaf had begun to develop. As the flag leaf is beginning to develop at the PI stage, glyphosate reduces flag leaf growth causing emergence of a stunted, shortened leaf (Davis et al. 2011; Senseman 2007, Slaton 2001). At Lonoke, glyphosate applied at PI reduced the flag leaf length of Wells by 26%, which was more than any other cultivar. However, relative flag leaf length of Wells was only reduced 8% from PI applications of glyphosate at Rohwer. Relative flag leaf length of Bengal and CL XL8 at Rohwer was reduced more than other cultivars from PI applications of glyphosate. Applications were made when the flag leaf sheath had begun to swell. The flag leaf is not fully developed until full or late boot when the flag leaf has fully extended prior to emergence of panicles from the boot (Slaton 2001). Therefore, a reduction in flag leaf length is still possible. The greatest reduction in relative flag leaf length from boot applications at Rohwer was 7% in Francis, but was less than 3% in all other cultivars. Boot applications at Lonoke reduced relative flag leaf length of Banks, CL 161, CL XL8 and Cocodrie by more than 10%. Katy was the only cultivar that did not have a reduction in relative flag leaf length of more than 5% at any growth stage at either location.

Yield. Other parameters are often used as symptoms to determine glyphosate injury, but yield reduction is the most relevant and true indicator of injury. Relative yield was influenced by an interaction of rice cultivar, glyphosate rate, and growth stage. Either rate of glyphosate applied at the 3-4 LF growth stage, as well as 45 g/ha applied at PI, had little effect on relative yield (8% or less) of rice cultivars (Table 7). Reductions in relative yield among cultivars from PI applications of 90 g/ha glyphosate and boot stage applications of 45 and 90 g/ha glyphosate were more apparent. The greatest reduction in relative yield from 90 g/ha glyphosate applied at PI was 26% in Bengal and 25% in Drew, which were similar to that of CL XL8 (18%) but were lesser than those of other cultivars. Although glyphosate at 90 g/ha applied at PI greatly reduced relative yield of Bengal and Drew, Banks and Katy were reduced only by 2 and 5%, respectively, and relative yield of LaGrue increased by 5%. Of all growth stages, the greatest reductions in relative yield were observed from both rates of glyphosate applied at boot. Stress or injury during the boot stage can reduce grain yield (Slaton 2001). Boot applications of glyphosate at 45 g/ha reduced relative yield of Katy 10%, but reduced relative yield of Drew 51% and Cocodrie 53%. Even though relative yield of CL 161 increased 8% from applications of 45 g/ha made at 3-4LF and was not reduced from PI applications, relative yield was decreased by 34% from the boot application. Relative yield of Wells, Bengal, and Banks was reduced by less than 4% from 45 g/ha glyphosate applied at 3-4LF and PI, but was reduced 20, 21, and 27% from boot applications. Boot applications of 90 g/ha resulted in devastating yield reductions in all cultivars, and ranged between 64% and 95%. CL XL8 and Francis were reduced the least but were decreased by 64% and 69%. Relative yield of Drew was reduced 95%, which was not different than Cocodrie (90%), but was reduced more compared to other cultivars. Even Lagrue, which had a 5% increase in relative yield from 90 g/ha glyphosate applied at PI, was reduced 83% from 90 g/ha glyphosate applied at boot. Overall, glyphosate at 90 g/ha reduced relative yield more than 45 g/ha, and applications of glyphosate at boot growth stage were more detrimental to yield than 3-4 LF and PI applications (Appendix 5 and 6).

Reductions in plant height, flag leaf length, and yield are possible in rice from exposure to reduced rates of glyphosate, and these reductions are influenced by rice cultivar and the growth stage of rice at the time of exposure. The most concerning of these effects is yield. Rough rice yield of some cultivars was reduced more than others, but when yield potential is factored in, more sensitive high-yielding cultivars may still be more feasible than low-yielding cultivars. For example, Katy had the lowest yield potential of all cultivars, excluding CL 161, and was reduced only 10% with 45 g/ha glyphosate applied at boot stage. Yield of other cultivars such as CL XL8, Francis, LaGrue, and Wells was reduced by 16 to 20% but still had a higher yield than Katy. Although some cultivars appear to have more tolerance than others to the rates of glyphosate used in this experiment, exposure to glyphosate drift during PI and boot can be detrimental to rice yield and should be avoided. These more tolerant cultivars may have utility in identifying germplasm for breeding programs to produce higher yielding cultivars with increased tolerance.

Sources of Materials

[1] Glyphosate (Roundup Weathermax 4S), 540 g ae L^{-1}, potassium salt of glyphosate, Monsanto Company, 800 North Linbergh Boulevard, St. Louis, MO 63167.

[2] AirMix 110015 Nozzle, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

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Table 1. Background information of cultivars selected for trial.

Cultivar	Year released and developer	Pedigree	Highlights
Banks	2004 – AR	LaGrue//Lemont/RA73/3/LaGrue/4/LaGrue	Short-season, long-grain
Bengal	1992 – LA	Mars/M-201//Mars	Short-season, semi-dwarf, medium-grain
CL 161	2002 – BASF	Proprietary cultivar; developed from Cypress	Mid-season, semi-dwarf, long-grain
CL XL8	2003 – RiceTec	Proprietary hybrid	Short-season, long-grain, hybrid
Cocodrie	1997 – LA	Cypress//82CAY21/Tebonnet	Short-season, semi-dwarf, long-grain
Drew	1996 – AR	Newbonnet/Katy	Mid-season, long-grain
Francis	2002 – AR	Lebonnet/9902/3/Dawn/9695/Starbonnet/4/LaGrue	Very short-season, long-grain
Katy	1989 – AR	Bonnet 73/CI9722//Starbonnet/Tetep/3/Lebonnet	Mid-season, long-grain
LaGrue	1993 – AR	Bonnet73/Nova76/Bonnet73/3/Newrex	Short-season, long-grain
Wells	1999 – AR	Newbonnet/3/Lebonnet/CI9902//Labelle	Short-season, long-grain

(Moldenhauer et al. 1990; Wilson et al. 2004)

Table 2. Cultivar potential without glyphosate treatment, averaged over years and locations.

Cultivar	Plant height	Flag leaf length	Yield
	cm	cm	Kg/ha
Banks	123	30	8520
Bengal	110	36	8270
CL 161	106	32	7130
CL XL8	114	36	8320
Cocodrie	97	26	7970
Drew	129	35	8520
Francis	113	28	9020
Katy	124	32	6480
LaGrue	123	30	8320
Wells	118	36	8770
LSD (0.05)	4	2	780

Table 3. Height of rice relative to no herbicide treatment as affected by interaction of location, glyphosate rate, and weeks after application, averaged over cultivar, growth stage, and years (P=0.0098)[a].

	Relative plant height					
	1 WAA		2 WAA		3 WAA	
Location	45 g	90 g	45 g	90 g	45 g	90 g
	%					
Lonoke	95	89	95	86	97	89
Rohwer	95	91	97	92	97	91

LSD (0.05) to compare means at the same rate and location over time = 1.
LSD (0.05) to compare means between rates at the same location = 2.
LSD (0.05) to compare means between rates and locations = 5.
[a] Abbreviations: WAA, wk after application; g, g ae/ha.

Table 4. Height of rice relative to no herbicide treatment as affected by interaction of growth stage, glyphosate rate, and weeks after application, averaged over cultivars, locations, and years (P=<0.0001)[a].

	Relative plant height					
	1 WAA		2 WAA		3 WAA	
Growth Stage	45 g	90 g	45 g	90 g	45 g	90 g
	%					
3-4 LF	88	78	94	81	99	92
PI	97	93	95	89	94	84
Boot	100	99	99	95	99	94

LSD (0.05) to compare means at the same growth stage and rate over time = 1.
LSD (0.05) to compare means between rates at the same growth stage = 2.
LSD (0.05) to compare means between rates and growth stages = 6.
[a] Abbreviations: WAA, wk after application; g, g ae/ha; LF, leaf; PI, panicle initiation.

Table 5. Height of rice at harvest relative to no herbicide treatment as affected by interaction of cultivar, glyphosate rate, and growth stage, averaged over locations and years (P=<0.0001)[a].

	Relative plant height					
	3-4 LF		PI		Boot	
Cultivar	45 g	90 g	45 g	90 g	45 g	90 g
	%					
Banks	101	101	99	94	101	95
Bengal	99	97	91	85	104	100
CL 161	101	102	96	86	99	95
CL XL8	98	99	98	84	100	98
Cocodrie	101	102	99	88	95	94
Drew	99	100	96	86	97	88
Francis	98	97	99	93	101	96
Katy	101	100	98	91	100	91
LaGrue	100	100	96	91	97	94
Wells	98	97	98	93	100	96

LSD (0.05) to compare means between cultivars at the same rate and growth stage = 3.
LSD (0.05) to compare cultivar means between rates at the same growth stage = 3.
LSD (0.05) to compare cultivar means between rates and growth stage = 4.
[a] Abbreviations: LF, leaf; PI, panicle initiation; g, g ae/ha.

Table 6. Flag leaf length of rice at harvest relative to no herbicide treatment as affected by interaction of cultivar, location, and growth stage, averaged over glyphosate rates and years (P=<0.0001)[a].

	Relative flag leaf length					
	3-4 LF		PI		Boot	
Cultivar	Lonoke	Rohwer	Lonoke	Rohwer	Lonoke	Rohwer
	%					
Banks	102	102	103	95	82	102
Bengal	105	94	94	75	92	101
CL 161	95	101	93	88	86	99
CL XL8	96	99	86	76	86	98
Cocodrie	106	103	94	88	87	98
Drew	101	94	94	84	95	104
Francis	96	96	104	97	97	93
Katy	99	108	104	95	102	102
LaGrue	96	98	96	98	91	97
Wells	94	98	74	92	104	102

LSD to compare means between cultivars at the same location and growth stage = 8.
LSD to compare cultivar means at the same location but different growth stages = 21.
LSD to compare cultivar means at different locations and growth stages = 21.
[a] Abbreviations: LF, leaf; PI, panicle initiation.

Table 7. Yield of rice relative to no herbicide treatment as affected by interaction of cultivar, glyphosate rate, and growth stage, averaged over locations and years (P=0.0002)[a].

	Relative yield					
	3-4 LF		PI		Boot	
Cultivar	45 g	90 g	45 g	90 g	45 g	90 g
	%					
Banks	99	102	103	98	73	23
Bengal	96	92	96	74	79	23
CL 161	108	107	100	85	66	16
CL XL8	94	96	93	82	82	36
Cocodrie	101	102	102	89	47	10
Drew	100	97	96	75	49	5
Francis	93	98	107	91	82	31
Katy	99	98	102	95	90	20
LaGrue	103	98	103	105	84	17
Wells	99	99	96	85	80	22

LSD (0.05) to compare means between cultivars at the same rate and growth stage = 9.
LSD (0.05) to compare cultivar means between rates at the same growth stage = 10.
LSD (0.05) to compare cultivar means between rates and growth stages = 21.
[a] Abbreviations: LF, leaf; PI, panicle initiation; g, g ae/ha.

Appendix

App. 1. Height of rice over time relative to no herbicide treatment as affected by interaction of location, growth stage, and glyphosate rate averaged over years and cultivars (P=0.0325)[a].

		Glyphosate rate	
Location	Growth Stage	45 g	90 g
		%	
Lonoke	3-4 LF	92	79
	PI	96	88
	Boot	100	97
Rohwer	3-4 LF	95	89
	PI	96	89
	Boot	99	95

LSD (0.05) to compare means between rates at the same location and growth stage = 3.
LSD (0.05) to compare means between rates at the same location but different growth stages = 8.
LSD (0.05) to compare means between rates at different locations and growth stages = 8.
[a] Abbreviations: g, g ae/ha; LF, leaf; PI, panicle initiation.

App. 2. Height of rice over time relative to no herbicide treatment as affected by interaction of location, growth stage, and weeks after application averaged over years and cultivars ($P = < 0.0001$)[a].

		Relative height		
Location	Growth Stage	1 WAA	2 WAA	3 WAA
			%	
Lonoke	3-4 LF	80	83	93
	PI	94	91	90
	Boot	101	98	96
Rohwer	3-4 LF	85	95	97
	PI	96	93	88
	Boot	98	97	97

LSD (0.05) to compare means over time at the same location and growth stage = 1.
LSD (0.05) to compare means between growth stages at the same location = 2.
LSD (0.05) to compare means between locations and growth stages = 2.
[a] Abbreviations: WAA, wk after application; LF, leaf; PI, panicle initiation.

App. 3. Height of rice over time relative to no herbicide treatment as affected by interaction of cultivar, location, and growth stage, averaged over glyphosate rates and years (P=<0.0001)[a].

	Relative height					
	3-4 LF		PI		Boot	
Cultivar	Lonoke	Rohwer	Lonoke	Rohwer	Lonoke	Rohwer
	%					
Banks	84	95	92	90	97	97
Bengal	89	93	95	96	102	101
CL 161	88	91	90	94	99	99
CL XL8	87	92	83	87	99	100
Cocodrie	83	91	94	92	96	95
Drew	86	91	91	95	94	94
Francis	87	91	96	93	99	98
Katy	83	93	92	92	97	96
LaGrue	84	90	91	92	96	94
Wells	84	94	95	92	105	97

LSD (0.05) to compare cultivar means at the same location and growth stage = 3.
LSD (0.05) to compare cultivar means at between locations or growth stages = 8.
LSD (0.05) to compare cultivar means between locations and growth stages = 8.
[a] Abbreviations: LF, leaf; PI, panicle initiation.

App. 4. Height of rice at harvest relative to no herbicide treatment as affected by interaction of cultivar, location, and growth stage, averaged over glyphosate rates and years (P=<0.0001)[a].

	Relative height					
	3-4 LF		PI		Boot	
Cultivar	Lonoke	Rohwer	Lonoke	Rohwer	Lonoke	Rohwer
	%					
Banks	100	101	98	96	99	97
Bengal	99	97	88	88	102	102
CL 161	101	102	95	87	96	97
CL XL8	100	98	94	88	98	101
Cocodrie	103	100	99	88	93	96
Drew	99	99	92	90	93	92
Francis	97	98	98	93	100	97
Katy	100	101	98	91	96	95
LaGrue	102	98	96	91	96	95
Wells	96	100	96	95	100	95

LSD (0.05) to compare cultivar means at the same location and growth stage = 3.
LSD (0.05) to compare cultivar means between locations or growth stages = 5.
LSD (0.05) to compare cultivar means between locations and growth stages = 5.
[a] Abbreviations: LF, leaf; PI, panicle initiation.

App. 5. Yield of rice relative to no herbicide treatment as affected by interaction of cultivar, location, and growth stage, averaged over glyphosate rates and years (P=<0.0001)[a].

	Relative height					
	3-4 LF		PI		Boot	
Cultivar	Lonoke	Rohwer	Lonoke	Rohwer	Lonoke	Rohwer
	%					
Banks	99	103	106	94	33	63
Bengal	88	100	87	82	50	50
CL 161	112	103	101	85	32	50
CL XL8	100	91	84	91	52	66
Cocodrie	104	99	100	91	19	39
Drew	95	102	88	84	22	31
Francis	93	98	102	96	47	66
Katy	97	100	103	94	51	59
LaGrue	96	105	103	105	44	58
Wells	95	103	99	82	45	58

LSD (0.05) to compare cultivar means at the same location and growth stage = 9.
LSD (0.05) to compare cultivar means between locations or growth stages = 28.
LSD (0.05) to compare cultivar means between locations and growth stages = 28.
[a] Abbreviations: LF, leaf; PI, panicle initiation.

App. 6. Yield of rice relative to no herbicide treatment as affected by interaction of location and glyphosate rate, averaged over cultivars, locations, and years (P=0.0199)[a].

	Relative Yield	
Location	45 g	90 g
	%	
Lonoke	87	69
Rohwer	94	69

LSD to compare means between rates at the same location = 4.
LSD to compare means between rates and locations = 15.
[a] Abbreviations: g, g ae/ha.

Chapter 2

Glyphosate Injury to Imidazolinone-Tolerant Rice Before and After Imazethapyr Applications

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Applications of imazethapyr at labeled rates can injure imidazolinone-tolerant rice and the symptoms may appear similar to those produced by sub-lethal doses of glyphosate. Field and greenhouse experiments were conducted in 2007 to examine imidazolinone-tolerant rice response to imazethapyr and low rates of glyphosate applied sequentially and to determine the potential for either herbicide to predispose rice to greater injury when applied sequentially. Glyphosate was applied at 0, 45, and 90 g ae/ha at 14, 7, 3, 1, and 0 d prior to applications of imazethapyr at 0, 105, or 210 g ai/ha. Imazethapyr at those rates was also applied 14, 7, 3, 1, and 0 d prior to glyphosate treatment. In the greenhouse experiment, glyphosate reduced relative dry mass and relative plant height of rice at all intervals regardless of application sequence and was not influenced by imazethapyr. Imazethapyr applied 7 d before glyphosate reduced relative plant height but did not influence the reduction in relative plant height from glyphosate. Relative chlorophyll content of rice leaves increased following glyphosate applications and was not influenced by imazethapyr; therefore, the chlorophyll meter was not a reliable indicator of herbicide injury. When conducted under field conditions, imazethapyr did not reduce relative plant height; however, glyphosate at 90 g/ha reduced relative plant height of rice at all intervals regardless of application sequence and was not influenced by imazethapyr. Glyphosate at 45 and 90 g/ha applied 14 d before imazethapyr had no effect on relative yield, but glyphosate at 90 g/ha reduced relative yield when applied 7 to 0 d before imazethapyr and when applied at all intervals after imazethapyr. There was no interaction between glyphosate and imazethapyr and thus no evidence that imazethapyr applications will predispose CL 161 to greater injury from glyphosate or that glyphosate will predispose CL 161 to injury from imazethapyr.

Nomenclature: Glyphosate; imazethapyr; rice, *Oryza sativa* L. 'CL 161'

Key words: Glyphosate drift, plant height, yield.

Imazethapyr is used in imidazolinone-tolerant (IT) rice systems for control of red rice (*Oryza sativa* L.), as well as other grass weed species common to rice production (White and Hackworth 1999). Steele et al. (2002) reported that sequential imazethapyr treatments provided up to 98% control of red rice and that uncontrolled plants were stunted and did not produce viable seed by harvest; however, rates above 52 g ai/ha applied POST did not increase red rice control and may contribute to minor yield reduction. Tolerance to imidazolinone herbicides was developed from a single rice plant that survived a chemically induced mutation (Sanders et al. 1998). Although IT cultivars are tolerant to imazethapyr, injury to these cultivars is still possible from imazethapyr (Levy et al. 2006; Masson et al. 1999; Ottis et al. 2003; Ottis et al. 2004; Steele et al. 2002; Webster and Masson 2001), and IT cultivars have differential tolerance to imazethapyr (Levy et al. 2006; Wenefrida et al. 2004).

According to Steele et al. (2002), average injury from imazethapyr to the research cultivar 93-AS-3510, in the form of chlorosis and stunting, was less than 5% 20 days after treatment (DAT) regardless of rate or application timing, and among POST rates, visual injury was highest with 70 g/ha imazethapyr, the highest rate tested. Similarly, 4% injury was observed 28 DAT with 70 g/ha imazethapyr applied POST to 93-AS-3510 (Hackworth et al. 1998). Levy et al. (2006) reported that as the rate of imazethapyr increased from 70 to 280 g/ha, injury to 'CL 121' 3 weeks after treatment (WAT) increased from 18 to 38%; however, at 3 WAT 'CL 161' was injured 11% by 280 g/ha imazethapyr and less than 5% from the lower rates. The difference in tolerance between CL 121 and CL 161 is due to the IT parent lines used to develop these cultivars. PCW-16, the original IT germplasm for CL 161, is eight times more tolerant than the male parent, 93-AS-3510, of CL 121 (Levy et al. 2006, Wenefrida et al. 2004). Visual injury to IT rice from imazethapyr appears to decrease as the growth stage at application increases and visual injury diminishes over time (Levy et al. 2006; Steele et al. 2002). Although most glyphosate-tolerant crops can tolerate glyphosate at rates above what is labeled, there is the potential for other adverse effects from glyphosate applications. In glyphosate-tolerant cotton, applications of glyphosate 24 h prior to applications of 2,4-D and halosulfuron at drift rates increased cotton injury (Smith et al. 2005). Glyphosate applications can also predispose glyphosate-tolerant soybean to various diseases such as sudden death syndrome (*Fusarium solani* f. sp. *glycines*) and root rot (*Phytophthora megasperma*) (Johal and Huber 2009). Injury to glyphosate-tolerant soybean from a reduced rate of dicamba was also increased when the dicamba was applied with glyphosate (Kelley et al. 2005).

Injury to IT rice from imazethapyr has been observed in the form of general stunting, height reduction, and chlorosis from applications of imazethapyr at labeled rates (Bond et al. 2006; Ottis et al. 2003; Ottis et al. 2004; Steel et al. 2002). Injury to rice from sub-lethal rates of glyphosate has also been observed as general stunting, height reduction, and chlorosis (Ellis et al. 2003; Koger et al. 2005). At 7 DAT, rice height was reduced by 16 to 37% from 18 to 140 g ai/ha glyphosate applied at the two- to three-leaf rice stage (Ellis et al. 2003). Kurtz and Street (2003) also observed injury to rice at early rating intervals from glyphosate at 140 g/ha applied at the three- to four-leaf growth stage. Crop injury, in the form of chlorosis and necrosis, is usually assessed by visual ratings or quantitatively by biomass reduction. A quantitative, non-destructive method that produces rapid results would be advantageous to determine herbicide injury. One method to measure relative plant vigor or healthiness in response to injury that has been of interest among investigators is the use of a chlorophyll meter (Diaz-Montano et al. 2007;

Jemison and Utsch 1999). The chlorophyll meter makes rapid and non-destructive measurements to provide a relative indication of leaf chlorophyll concentration (Marquard and Tipton 1987; Yadava 2006). The chlorophyll meter determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in the red and near-infrared regions. Using these two transmittances, the meter calculates a numerical value that is proportional to the amount of chlorophyll present in the leaf (Anonymous 2008). Several investigators have demonstrated that leaf chlorophyll content can be estimated using a chlorophyll meter on various plant species including wheat (*Triticum aestivum* L.) (Uddling et al. 2007; Singh et al. 2002), corn (*Zea mays* L.) (Peterson et al. 1993), and rice (Peng et al. 1995; Singh et al. 2002). The numerical value given by the chlorophyll meter is referred to as a SPAD (soil plant analysis development) value (Singh et al. 2002). Monje and Bugbee (1992) observed that extracted samples rice, wheat, and soybean [*Glycine max* (L.) Merr.] produced a regression coefficient (r^2) of 0.93 between SPAD and extracted chlorophyll. Increasing SPAD values have been correlated with increased chlorophyll present in the leaf and higher nitrogen content commonly associated with healthier plants or new leaves (Azia and Stewart 2001; Himelrick et al. 1992; Uddling et al. 2007), and a decrease in the SPAD value indicates a decrease in the chlorophyll content of the leaf (Anonymous 2008). In rice, leaf nitrogen is closely related to photosynthesis rate and grain yield (Peng et al. 1995). Because yellowing of leaves is a common symptom associated with glyphosate and imazethapyr injury, a chlorophyll meter could perhaps be used to help determine or quantify rice injury.

IT rice cultivars are often grown in close proximity to glyphosate-resistant crops; therefore, the potential for off-target movement of glyphosate is of concern. The objectives of this research were to determine if sub-lethal rates of glyphosate will predispose CL 161 to imazethapyr injury or if imazethapyr applications will predispose CL 161 to greater injury from sub-lethal rates of glyphosate and to determine if a chlorophyll meter can be used to quantify herbicide injury.

Materials and Methods

Greenhouse study. Greenhouse experiments were conducted at the University of Arkansas at Monticello in Monticello, AR, in 2007. Four seeds of the cultivar CL 161 were hand-seeded into 12.7-cm-tall pots with a 10.1 cm^2 diameter and filled with a commercial potting mix[1]. Plants were exposed to 12 h day:night periods with an average temperature of 35 C and were watered daily. At the one- to two-leaf growth stage, plants were thinned to two plants per pot, and a 26-8-16 fertilizer[2] was applied weekly after the two- to three-leaf growth stage.

The experimental design was a randomized complete block with six replications, and the test was duplicated. Glyphosate[3] was applied at 0, 45, and 90 g ae/ha at 14, 7, 3, 1, and 0 d prior to applications of imazethapyr[4] at 0, 105, or 210 g ai/ha plus a non-ionic surfactant[5] at 0.25% v/v. Imazethapyr plus NIS was also applied at the above rates 14, 7, 3, 1, and 0 d prior to receiving glyphosate to determine predisposition of plants to injury from the herbicides applied in sequence. At 0 d, both herbicides were applied to the same plot in separate applications. Initial treatments were applied at the four- to five-leaf growth stage in a spray chamber[6] calibrated for a 258 L/ha output volume. Plant height (cm) and leaf chlorophyll content (SPAD) were measured 1, 2 and 3 wk after the final herbicide applications (WAFA). Plant height was measured from the base of the plant at the soil surface to the extended leaf tip of the uppermost leaf. Leaf chlorophyll content was measured from a random location on leaves of one plant with a SPAD-502 chlorophyll meter[7]. At 3 WAFA, both plants from each plot were cut at the soil surface and dried, and dry weight (g) was recorded. Data were expressed in terms of percentage of the corresponding check for each cultivar ([treated/nontreated]*100). For plant height and SPAD measurements the statistical model was a split-plot where the whole plot was a four-factor factorial (sequence of herbicides by interval between applications by glyphosate rate by imazethapyr rate) and WAFA was the split-plot factor. The four-factor factorial model was the same for the dry mass measurements, but there was no time factor, hence no split-plot factor. Data were subjected to ANOVA, and means were separated using Fisher's protected LSD at the 5% level of probability.

Field study. Treatments in the field experiment were the same as those in the greenhouse experiment. The experiment was established in 2007 at the Southeast Research and Extension Center (SEREC) near Rohwer, AR, on a Sharkey clay soil (very fine, montmorillonitic, nonacid, thermic Vertic Haplaquept). This experiment was conducted under conventional-tillage practices normal for the area and flush-irrigated as needed until permanent flood. The cultivar CL 161 was drill-seeded 3 cm deep at 101 kg/ha in nine rows spaced 19 cm apart. Plots were 1.7 m wide by 9 m long with 1.5 m alleys, and treatments were arranged in a randomized complete block design with four replications. Treatments in this study were also initiated at the four- to five-leaf growth stage, and applications were made using a CO_2 pressurized backpack sprayer equipped with AirMix 110015 nozzles[8] calibrated to deliver 112 L/ha at 186 kPa. All plots were managed for weed, insect, and disease control and fertilized according to the University of Arkansas Cooperative Extension Service recommendations (Slaton, 2001). Plant height (cm) was measured 1 and 2 WAFA and again prior to harvest in the same manner as in the greenhouse experiment, and rice was harvested with a small-plot combine for yield data. Data were expressed in terms of percentage of the corresponding check for each cultivar ([treated/nontreated]*100). For plant height measurements the statistical model was a split-plot where the whole plot was a four-factor factorial (sequence of herbicides by interval between applications by glyphosate rate by imazethapyr rate) and WAFA was the split-plot factor. The four-factor factorial model was the same for the yield measurements but there was no time factor hence no split-plot factor. Data were subjected to ANOVA, and means were separated using Fisher's protected LSD at the 5% level of probability.

Results and Discussion

Greenhouse Experiment. There was no interaction between imazethapyr and glyphosate for relative plant height, relative dry mass, or relative leaf chlorophyll content; therefore, there is no evidence from experiments conducted under greenhouse conditions that sequential applications of imazethapyr and sub-lethal rates of glyphosate will predispose CL 161 to greater injury from either herbicide.

Plant Height. An interaction of herbicide sequence, application interval, and glyphosate rate occurred for plant height. Glyphosate reduced plant height at all evaluations regardless of application sequence or interval (Table 1). Time after the final applications (WAFA) had more influence on glyphosate applied before imazethapyr. Measurements began 1 wk after the 0 d applications; therefore, glyphosate applied 14 d before imazethapyr and imazethapyr applied 14 d before glyphosate was active 1 to 2 wk longer than the same products applied at intervals of 7 d to 0 d before sequential products. While plants treated with glyphosate at 45 and 90 g/ha 14 d before imazethapyr were recovering, plants treated with these rates of glyphosate 3 d before and on the same day as imazethapyr were still showing reductions in plant height. Reductions in relative plant height in plants that did not receive glyphosate suggest that there was injury from imazethapyr applications (Bond et al. 2006; Ottis et al. 2003; Ottis et al. 2004; Steel et al. 2002). Imazethapyr applied 7 d before glyphosate at 0 g/ha reduced relative plant height 1, 2, and 3

WAFA. Imazethapyr is generally absorbed rapidly into foliage and plant growth is usually inhibited within a few hours, but injury symptoms usually appear 7 to 14 d after application (Senseman 2007).

Plant height was also influenced by an interaction of herbicide sequence, application interval, and imazethapyr rate (Table 2). Imazethapyr plus imazapyr are often used at sub-lethal rates to suppress growth of turfgrass (Senseman 2007). Relative plant height was reduced 4 to 16 percentage points more from imazethapyr at 210 g/ha applied 7 d before glyphosate compared to applications made 14, 3, 1, and 0 d before glyphosate 1, 2, and 3 WAFA. Relative plant height was reduced 4 to 9 percentage points more from glyphosate applied 14 to 1 d before no imazethapyr (0 g/ha) compared with applications of glyphosate before imazethapyr at 105 and 210 g/ha 1, 2, and 3 WAFA. This appears to be a safening effect but may possibly be due to increased injury of older leaves present at the time of imazethapyr application that may have increased the growth of newer leaves and compensated relative plant height. Further evidence to support this is that there was no increase in plant biomass from imazethapyr applications before or after glyphosate (Table 3). The differences in relative plant height from applications of glyphosate before imazethapyr can be attributed to the difference in time that the glyphosate was active in the treated plants since all imazethapyr was applied on the same day (Tables 1 and 2). Although there were differences between intervals when imazethapyr was applied at 0 g/ha before glyphosate 1, 2, and 3 WAFA, these differences were 5 percentage points or less and may be attributed to underlying effects of the glyphosate applications (Table 2). When imazethapyr was applied at 105 and 210 g/ha 7 d before glyphosate, plant height was reduced from 12 to 16 percentage points more compared to applications made 14, 1, and 0 d before glyphosate 1 WAFA, which again may be attributed to the amount of time before injury from imazethapyr appears after application (Senseman 2007). However, by 3 WAFA, differences in plant height from imazethapyr applied at different intervals before glyphosate were 7 percentage points or less which shows that plants were able to recover from plant height reductions over time (Webster and Masson 2001).

Dry Mass. An interaction of herbicide sequence, application interval, and glyphosate rate also occurred for relative dry mass. Glyphosate at 45 and 90 g/ha reduced relative dry mass of rice at all intervals regardless of application sequence and relative dry mass was reduced more as the rate of glyphosate increased when applied 14 and 7 d before imazethapyr (Table 3). Glyphosate at 90 g/ha applied 14 d before imazethapyr reduced relative dry mass more compared to applications 3 d after imazethapyr, but this may be attributed to the glyphosate being applied 14 d earlier and applied to smaller plants. The rate of imazethapyr had no influence on the reduction of dry mass by glyphosate (Table 4).

Leaf Chlorophyll Content. An interaction of herbicide sequence, application interval, and glyphosate rate occurred for relative leaf chlorophyll content as well. Glyphosate at 45 and 90 g/ha applied 14 d before imazethapyr reduced relative leaf chlorophyll content 1 WAFA (Table 5). But by 2 WAFA, there was no reduction in relative leaf chlorophyll content from glyphosate applied 14 d before imazethapyr, and by 3 WAFA, an increase in relative leaf chlorophyll content was observed. Glyphosate applied 14 d before imazethapyr and imazethapyr applied 14 d before glyphosate were the initial applications and were applied to rice at the four- to five-leaf growth stage. The glyphosate applied to these younger, smaller plants appeared to have more influence on leaf chlorophyll content 1 WAFA. Glyphosate at 45 and 90 g/ha applied 7 to 0 d before imazethapyr and at all intervals applied after imazethapyr increased relative leaf chlorophyll content 6 to 19 percentage points compared to no glyphosate 3 WAFA. Relative plant height was reduced 12 to 30 percentage points and relative dry mass 32 to 76 percentage points from glyphosate at 45 and 90 g/ha applied 7 to 0 d before imazethapyr, and at all intervals applied after imazethapyr, 3 WAFA (Table 1 and Table 3). Leaf chlorophyll measurements were taken from random leaves and random locations from plants at each evaluation, and as new leaves emerged, they were sampled as well. This may have led to higher concentrations of chlorophyll because newer leaves that emerged after glyphosate applications had more chlorophyll than older leaves.

Field Trial. As with the greenhouse experiment, there was no interaction between imazethapyr and glyphosate for relative plant height or relative yield when conducted under field conditions. Again, without this interaction there is no evidence to support that applications of imazethapyr or sub-lethal rates of glyphosate will predispose CL 161 to greater injury from either herbicide when applied sequentially.

Plant Height. When conducted under field conditions, an interaction between herbicide sequence, application interval, and glyphosate rate also occurred for plant height. Glyphosate at 45 and 90 g/ha applied before imazethapyr also reduced relative plant height of plants grown under field conditions (Table 6). Although initial applications of glyphosate were applied to plants at the four- to five-leaf growth stage in field and greenhouse experiments, rice plants in the field experiment were able to recover faster than those in the greenhouse. For example, glyphosate at 90 g/ha applied 3 d before imazethapyr reduced plant height more compared to other intervals 1 WAFA in the field trial (Table 6) compared to applications of glyphosate at 90 g/ha applied 14 d before imazethapyr in the greenhouse experiment (Table 1). Plants in the field were exposed to longer day-length periods than plants in the greenhouse which may be the reason for faster recovery. Glyphosate applied at 45 g/ha 14 to 0 d after imazethapyr reduced plant height 7 to 13 percentage points more than applications of glyphosate at 45 g/ha applied 14 to 1 d before imazethapyr 2 WAFA, which may be attributed more to the time between glyphosate applications and the evaluation interval (Table 6). Similar to the greenhouse experiment, glyphosate at 90 g/ha applied 7 d after imazethapyr reduced relative plant more 2 WAFA compared to applications made on the same day (0 d), which may be due to underlying reductions in relative plant height from imazethapyr (Table 1 and Table 6). At harvest, there were no differences in plant height among treatments (Appendix Tables 1 and 2).

Yield. Applications of glyphosate at 45 and 90 g/ha 14 d before imazethapyr had no effect on relative yield. Glyphosate applied 14 d before imazethapyr was the initial treatment at the four- to five-leaf growth stage. Glyphosate at 45 g/ha applied 1 d after imazethapyr reduced relative yield more compared to glyphosate at 45 g/ha applied on the same day (0 d) as imazethapyr (Table 7). Applications made 7 to 0 d after were applied close to, or during, panicle initiation and would have been more likely to reduce yield (Ellis et al. 2003; Kurtz and Street 2003). Reductions in relative yield were also observed when glyphosate at 90 g/ha was applied 7 to 0 d before, and 14 to 1 d after imazethapyr (Table 7).

Glyphosate applied before and after applications of imazethapyr reduced relative plant height, relative dry mass, and relative yield of CL 161. When conducted under greenhouse conditions, imazethapyr applied 7 d before glyphosate also reduced relative plant height, but did not affect plant height or yield reduction when applied after glyphosate. There was no interaction effect between imazethapyr and glyphosate on relative plant height, relative dry mass, relative leaf chlorophyll content, or relative yield; therefore, influence is independent. There is no evidence that sequential applications of imazethapyr or sub-lethal rates of glyphosate will predispose CL 161 to greater injury from either herbicide.

Sources of Materials

[1] Pro-Mix Soil, Premier Horticulture, Quakertown, PA 18951.

[2] Miracle-Gro® Water Soluble All Purpose Plant Food (24-8-16), Scotts Miracle-Gro Products Inc., Marysville, OH 43041.

[3] Glyphosate (Roundup Weathermax), 540 g ae L^{-1}, potassium salt of glyphosate, Monsanto Company, 800 North Linbergh Boulevard, St. Louis, MO 63167.

[4] Imazethapyr (NewPath), 240 g ai L^{-1}, BASF Corporation, P.O. Box 13528, Research Triangle Park, NC. 27709.

[5] Non-ionic surfactant (Preference), Winfield Solutions, P.O. Box 64281, St. Paul, MN 55164.

[6] Spray Chamber, O'Brien Industrial Equipment Co., San Francisco, CA.

[7] SPAD-502, Soil and plant analysis development (SPAD), Konica Minolta Sensing, Inc. Osaka, Japan.

[8] AirMix 110015 Nozzle, Greenleaf Technologies, P.O. Box 1767, Covington, LA 70434.

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Table 1. Height of rice in greenhouse experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and glyphosate rate, averaged over imazethapyr rate (P=<0.0001)[a].

		Relative height								
		1 WAFA			2 WAFA			3 WAFA		
Sequence	Interval	0 g	45 g	90 g	0 g	45 g	90 g	0 g	45 g	90 g
		%								
G fb I	14 d	100	80	62	101	85	67	102	90	72
G fb I	7 d	95	74	70	96	78	68	97	84	68
G fb I	3 d	98	84	80	98	79	75	99	80	69
G fb I	1 d	98	84	83	97	80	78	96	75	68
G & I	0 d	98	91	86	98	87	81	99	79	74
I fb G	14 d	99	91	88	99	84	83	101	77	75
I fb G	7 d	88	81	80	94	77	76	94	74	73
I fb G	3 d	94	89	84	99	84	81	100	78	75
I fb G	1 d	102	94	90	102	87	86	101	77	77

LSD (0.05) to compare means at the same sequence, interval, and rate over time = 3.
LSD (0.05) to compare means between sequence, interval, rate, and time = 4.
[a] Abbreviations: WAFA, wk after final application; g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 2. Height of rice in greenhouse experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and imazethapyr rate, averaged over glyphosate rate (P=0.0021)[a].

		Relative height								
		1 WAFA			2 WAFA			3 WAFA		
Sequence	Interval	0 g	105 g	210 g	0 g	105 g	210 g	0 g	105 g	210 g
		%								
G fb I	14 d	73	80	79	77	84	82	81	88	88
G fb I	7 d	73	79	80	75	80	81	78	82	82
G fb I	3 d	81	87	89	77	83	84	75	83	83
G fb I	1 d	82	89	89	77	86	85	71	80	80
G & I	0 d	88	91	92	84	89	89	76	83	85
I fb G	14 d	87	93	93	82	90	89	80	82	83
I fb G	7 d	85	84	79	81	84	79	75	81	78
I fb G	3 d	89	88	88	84	87	87	77	83	84
I fb G	1 d	91	96	95	86	93	90	77	88	82

LSD (0.05) to compare means at the same sequence, interval, and rate over time = 3.
LSD (0.05) to compare means between sequence, interval, rate, and time = 4.
[a] Abbreviations: WAFA, wk after final application; g, g ai/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 3. Dry mass of rice in greenhouse experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and glyphosate rate, averaged over imazethapyr rate (P=<0.0001)[a].

		Relative dry mass		
Sequence	Interval	0 g	45 g	90 g
			%	
G fb I	14 d	103	53	19
G fb I	7 d	108	66	32
G fb I	3 d	86	43	29
G fb I	1 d	96	49	33
G & I	0 d	98	54	40
I fb G	14 d	85	53	41
I fb G	7 d	89	49	38
I fb G	3 d	108	62	43
I fb G	1 d	94	52	39
LSD (0.05)			24	

[a] Abbreviations: g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 4. Dry mass of rice in greenhouse experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, imazethapyr rate, and glyphosate rate, averaged over application interval (P=0.0434)[a].

		Relative dry mass		
Sequence	Imazethapyr rate	0 g	45 g	90 g
	g ai/ha		%	
G fb I	0	100	54	31
G fb I	105	98	51	33
G fb I	210	98	54	29
I fb G	0	100	54	41
I fb G	105	95	58	40
I fb G	210	94	50	39
LSD (0.05)			18	

[a] Abbreviations: g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 5. Leaf chlorophyll content of rice in greenhouse experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and glyphosate rate, averaged over imazethapyr rate (P=0.0235)[a].

		Relative leaf chlorophyll content								
		1 WAFA			2 WAFA			3 WAFA		
Sequence	Interval	0 g	45 g	90 g	0 g	45 g	90 g	0 g	45 g	90 g
		%								
G fb I	14 d	104	83	81	101	100	94	104	105	102
G fb I	7 d	97	104	112	98	112	114	103	118	122
G fb I	3 d	94	106	116	103	117	114	105	116	120
G fb I	1 d	98	106	108	99	109	114	107	125	120
G & I	0 d	97	99	103	99	101	105	101	115	112
I fb G	14 d	93	99	102	98	101	105	96	106	106
I fb G	7 d	97	106	102	99	106	106	100	115	112
I fb G	3 d	96	100	105	98	103	106	100	112	106
I fb G	1 d	97	100	103	101	110	114	99	116	118

LSD (0.05) to compare means at the same sequence, interval, and rate over time = 6.
LSD (0.05) to compare means between sequence, interval, rate, and time = 7.
[a] Abbreviations: WAFA, wk after final application; g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 6. Plant height of rice in field experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and glyphosate rate, averaged over imazethapyr rate (P=0.0002)[a].

		Relative height					
		1 WAFA			2 WAFA		
Sequence	Interval	0 g	45 g	90 g	0 g	45 g	90 g
		%					
G fb I	14 d	96	93	87	99	98	93
G fb I	7 d	99	92	77	102	98	88
G fb I	3 d	97	86	68	101	97	69
G fb I	1 d	100	91	85	99	95	70
G & I	0 d	101	92	87	98	88	76
I fb G	14 d	99	93	82	100	88	70
I fb G	7 d	95	91	84	97	85	68
I fb G	3 d	96	89	81	100	85	67
I fb G	1 d	96	89	85	97	85	68

LSD (0.05) to compare means at the same sequence, interval, and rate over time = 4.
LSD (0.05) to compare means between sequence, interval, rate, or time = 6.
[a] Abbreviations: WAFA, wk after final application; g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Table 7. Yield of rice in field experiment relative to no herbicide treatment as affected by interaction of herbicide sequence, application interval, and glyphosate rate, averaged over imazethapyr rate (P=0.0331)[a].

		Relative yield		
Sequence	Interval	0 g	45 g	90 g
			%	
G fb I	14 d	106	101	100
G fb I	7 d	98	93	88
G fb I	3 d	104	99	89
G fb I	1 d	98	95	86
G & I	0 d	97	98	87
I fb G	14 d	102	97	87
I fb G	7 d	98	93	85
I fb G	3 d	98	95	80
I fb G	1 d	93	88	82
LSD (0.05)			9	

[a] Abbreviations: g, g ae/ha; G, glyphosate; fb, followed by; I, imazethapyr.

Appendix

App. 7. Height at harvest in field experiment relative to no herbicide treatment as affected by application sequence and interval between applications, averaged over imazethapyr rate and glyphosate rate (P=<0.0001)[a].

Sequence	Interval	Relative plant height
		%
G fb I	14 d	99
G fb I	7 d	102
G fb I	3 d	99
G fb I	1 d	94
G & I	0 d	98
I fb G	14 d	97
I fb G	7 d	95
I fb G	3 d	92
I fb G	1 d	96
LSD (0.05)		3

[a] Abbreviations: G, glyphosate; fb, followed by; I, imazethapyr.

App. 8. Height at harvest in field experiment relative to no herbicide treatment as affected by application sequence and glyphosate rate, average over application interval and imazethapyr rate (P=0.0157)[a].

Sequence	Glyphosate rate	Relative plant height
	g ae/ha	%
G fb I	0	101
G fb I	45	99
G fb I	90	96
I fb G	0	100
I fb G	45	96
I fb G	90	90
LSD (0.05)		3

[a] Abbreviations: G, glyphosate; fb, followed by; I, imazethapyr.

Rice (*Oryza sativa*) Response to Low Glyphosate Rates as Influenced by Cultivar, Growth Stage, and Imazethapyr Applications

Jason R. Meier

Summary

Since the introduction and acceptance of glyphosate-resistant crops, the use of glyphosate for weed control in agriculture has increased tremendously as have complaints of glyphosate drift. The problem of drift does not occur only in Arkansas, but in all rice-producing states. In Arkansas, the State Plant Board has developed regulations for glyphosate applications attempting to reduce the occurrence of glyphosate drift. Until glyphosate-resistant rice is accepted and can be grown commercially, applicators must be more conscious of pesticide drift and the potential effects on non-target crops.

This research was established to identify rice cultivars that may be more tolerant to sub-lethal rates of glyphosate associated with glyphosate drift and if applications of imazethapyr on imidazolinone-tolerant rice would affect injury from these sub-lethal rates of glyphosate. Reductions in plant height, flag leaf length, and yield are possible in rice from exposure to reduced rates of glyphosate, and these reductions are influenced greatly by the growth stage of rice at the time of exposure. Although differences in plant height, flag leaf length and yield among cultivars in response to sub-lethal rates of glyphosate can be used to identify more tolerant cultivars, the most concerning of these effects to a producer is yield. Relative rough rice yield of some cultivars was reduced by sub-lethal rates of glyphosate more than others, but when yield potential is factored in, higher-yielding cultivars may still be more feasible even though they are more susceptible to injury. For example, Katy had the lowest yield potential of all cultivars, excluding CL 161, and was reduced only 10% from glyphosate at 45 g/ha applied at the boot stage of growth. Even though Cocodrie and Drew had higher yield potential than Katy,

relative yield was reduced more from glyphosate at 45 g/ha applied at boot resulting in a lower relative yield than Katy. However, relative yield of other cultivars such as CL XL8, Francis, LaGrue, and Wells was reduced by 16 to 20% but was still higher than relative yield of Katy. By examining rice cultivar tolerance to reduced rates of glyphosate, cultivars with greater tolerance to reduced rates of glyphosate can be identified and potentially used in breeding programs to develop higher-yielding cultivars with more tolerance to glyphosate.

Glyphosate applied before and after applications of imazethapyr reduced relative plant height, relative dry mass, and relative yield of CL 161. When conducted under greenhouse conditions, imazethapyr applied 7 d before glyphosate also reduced relative plant height, but did not affect plant height or yield reduction when applied after glyphosate. Applications of imazethapyr can injure CL 161, but there is no evidence from these experiments that either herbicide will predispose CL 161 to greater injury when applied sequentially.

Pesticide drift has been an issue since the invention of pesticides and although improvements in application technology have been made, incidences of drift still occur. The off-target movement of herbicides onto sensitive crops can have adverse effects on growth and yield, and glyphosate drift onto rice is especially detrimental and should always be avoided.