

**DISSIPATION AND CARRYOVER OF IMIDAZOLINONE HERBICIDES
IN IMIDAZOLINONE-RESISTANT RICE (*Oryza sativa*)**

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Master of Science

by

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**DISSIPATION AND CARRYOVER OF IMIDAZOLINONE HERBICIDES
IN IMIDAZOLINONE-RESISTANT RICE**

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DISSIPATION AND CARRYOVER OF IMIDAZOLINONE HERBICIDES
IN IMIDAZOLINONE-RESISTANT RICE

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ABSTRACT

The development of Imidazolinone (IMI) Resistant (IR) rice now allows rice producers to selectively control red rice (*Oryza sativa*, *O. rufipogon*, and *O. nivara*), weedy relatives of commercial rice (*O. sativa*). Imazethapyr the primary herbicide used with this technology has been shown to be relatively persistent in the soil and may cause injury to rotational crops including non-IR rice.

Imazamox has less soil persistence in non-flooded environments; however, this herbicide has not been studied in rice environments including flooded soils.

Thirteen selected treatments of two and three sequential applications of imazethapyr and imazamox were applied to IR rice in 2004 and 2005. In 2005 and 2006, non-IR rice was planted into the previous years' plots to evaluate herbicide carryover. Studies were conducted on two soils commonly utilized for rice production: a DeWitt silt loam and a Sharkey clay soil. Treatments included several variations including common programs with imazamox added, double-rate treatments, and imazamox-only treatments and treatments where imazamox was substituted for imazethapyr. Non-IR rice was evaluated for carryover injury at pre-flood and 2-week post-flood timings. No injury was observed on the silt loam soil in 2005 or on the clay soil in 2006. The addition of imazamox at the pre-flood in 2005 on the clay soil to any treatment was the main factor increasing injury to significant levels. In 2006 on the silt loam soil, doubling the imazethapyr rate

was the main factor increasing injury. However, in all cases, injury was low and in some instances treatments that caused or did not cause injury did not correlate to the herbicide rates applied.

To further investigate imidazolinone dissipation, imazethapyr, imazamox and imazapyr were applied to flooded and non-flooded plots on silt loam and clay soils. Soil samples were taken periodically during the year following application. Samples were frozen to stop dissipation. Soil samples were tested using a bioassay and standard curve. From this information, dissipation rates and half lives were estimated. Visual injury was found to provide the best measurement of herbicide quantity in the soil. Half lives for imazamox were found to be 16 d on flooded silt loam, 8 d on flooded clay. Half lives were longer under non-flooded conditions with half lives of 270 and 13 d being calculated on silt loam and clay soils. Imazethapyr half lives ranged from 5 d on flooded clay to 128 d on non-flooded loam. Half lives calculated for imazapyr ranged from 8 to 78 d under flooded and non-flooded conditions on the clay soil, and from 50 to 539 d on the silt loam soil. The active herbicide concentrations declined more quickly under flooded conditions as compared to non-flooded conditions, regardless of soil type. However, dissipation occurred faster on the clay soil as compared to the silt loam.

Chapter I

Review of Literature

Introduction

Rice is one of the world's most important food grains. In the United States production is concentrated primarily in the states of Arkansas, Louisiana, Mississippi, Missouri, Texas, and California (Street and Bollich 2003). According to Coats (2003), the United States harvested 1.23 million hectares of rice in 1999, or approximately 0.81% of the world's rice hectareage, while milling 6.1 million metric tons or 1.5% of world production.

Rice production can be more challenging than production of other grain crops. This can be attributed to the cultural methods involved in production such as flooding. Flooding is performed primarily for weed control; however it is an efficient means of irrigating the crop. There are two general flooding methods used in the U.S.; delayed flood and continuous flood. Delayed flood is used mainly in drill seeded rice. This method allows for the use of ground equipment to apply fertilizer and pesticides before the permanent flood is established. Continuous flood systems are used to suppress populations of non-aquatic weeds, in particular red rice. Red rice (*Oryza sativa*, *O. nivara*, and *O. rufipogon*) are close relative of commercial rice. They all belong to the same genus and sometimes the same species, which means they are similar genetically. Consequently, no herbicides are available that selectively control red rice in commercial rice.

Recently, non-transgenic cultivated rice lines have been developed that express tolerance to imidazolinone herbicides (Ottis et al. 2003). This technology allows rice producers for the first time to herbicidally control red rice. This system makes use of the imidazolinone (IMI) herbicide imazethapyr (trade name *NewPath*¹) for preemergence (PRE) and postemergence (POST) weed control. However, imazethapyr has been shown to persist in the soil. Studies have shown that imazethapyr residues from a previous crop can affect field corn (*Zea mays*), sweet corn (*Z. mays* L. *saccharata*), cucumber (*Cucumis sativus* L.), and grain sorghum (*Sorghum bicolor* (L.) Moench) (Loux and Reese 1993; Vencill et al. 1990; Goetz et al. 1990) for 18 months or more following application. Imazamox (trade name *Beyond*¹) is another imidazolinone herbicide that has been shown to be less persistent. According to Silva et al. (1995), carryover injury to corn and grain sorghum from imazamox applied at 50 and 100 g ai ha⁻¹ (1x and 2x rates) to soybean (*Glycine max* (L.) Merr.) did not occur 90 days after application (DAT). Because of its short soil persistence, imazamox could be of value in a rotation of imi-resistant rice followed by imi-susceptible rice.

General Weed Problems in Rice

The majority of rice grown in the United States is flooded to help control weeds. Rice tolerates low oxygen (hypoxic) conditions better than most weeds; thus, flooding is an effective method of cultural control for many weed species (Masson et al. 2001). Some weeds have adapted to flooded environments as rice has. Flood tolerant *Echinochloa* sp. have been reported to exhibit higher photosynthetic activity than rice under flooded conditions (Masson et al. 2001;

¹ BASF Corporation, Florham Park, NJ 07932

Bouhache and Bayer 1993) resulting in greater competition for light and nutrients. Adequate water supplies are essential to provide weed control and a favorable environment for rice growth.

The use of water in the production of rice can have a strong influence on the weed spectrum that will be present as well as the program employed to manage or eliminate their growth (Bayer 1991). Common rice weeds include barnyardgrass (*Echinochloa crus-galli*), listed as one of the worlds 10 worst weeds (Kendig et al. 2003), hemp sesbania (*Sesbania exaltata*), nutsedge (*Cyperus* spp), amazon and bearded sprangletop (*Leptochloa panicoides* and *L. fascicularis*) and red rice (*Oryza sativa* L.) among others. These weeds are well adapted to wet and flooded environments, but they do not require such conditions (Kendig et al. 2003).

Weed problems may be expected to be more varied in species and intensity in dry-seeded rice than in water-seeded or transplanted rice because of differences in seedbed preparation, the presence or absence of moisture during germination, and early growth stages of the rice (Bayer 1991). Most of these weeds gain an early season advantage because of little competition from rice plants before the flood is established.

Weeds interfere with rice growth in different ways. Weeds a) compete for light, nutrients and water; b) living or decaying weeds may secrete toxic root exudates or leaf leachates which depress the normal growth of the rice plant; c) high weed densities create a habitat for growth of various pest organisms (insects, nematodes, pathogens) which adversely affect rice production; d) weeds demand

high labor inputs for control, and e) large crop losses may take place in rice fields with high weed infestations which prevent normal harvesting operations (Labrada 1996).

Yield losses due to weed competition can vary from field to field depending on the types of weeds present. Short weeds or those that germinate later in the growing season tend to compete less than tall weeds or those that germinate at the beginning of the growing season (Bayer 1991). As breeders have developed shorter statured varieties, weed populations in rice fields have adapted. The replacement of the older tall-statured rice cultivars with the modern short-statured cultivars has created a situation more favorable for tall-growing weeds (Bayer 1991).

Production Problems caused by Red Rice

Red rice is one of the most important weeds in almost all regions where rice is grown (Eleftherohorinos and Dhima 2002). Red rice possesses several characteristics that are unwanted in commercial rice production. These characteristics include easily shattering, competitive growth habit, seed dormancy and a tendency to lodge. One uncontrolled red rice plant can theoretically yield 1,500 seeds in one season, and this can result in 2,250,000 seeds during the next season (Eleftherohorinos and Dhima 2002).

Harvesting red rice with commercial rice is unwanted because the red color of the grains is considered undesirable. Milling costs of rice contaminated with red rice are higher because the duration of the milling process must be extended to destroy the pericarp of red rice. This results in a greater fraction of

broken white rice kernels and lower head rice yields (Gealy et al. 2000). Red rice is also of lower quality than white rice because of grain size and cooking characteristics.

Red rice is one of the most complex problems in rice production. Based on micromorphological studies, red rice is considered the same species as domestic rice (Gealy et al. 2000). Vaughn et al. (2001) indicated that this classification was inadequate and that at least three genetically distinct red rice varieties exist. These include *O. sativa* subspecies *indica*, *O. sativa* subspecies *japonica*, and *O. nivara* and *O. rufipigon*, two weedy relatives of *O. sativa*. The high level of diversity should be considered when developing and testing red rice management strategies and that a range of red rice types should be used in herbicide studies to prevent the loss of important herbicides (Vaughn et al. 2001). Steele et al. (2002) noted that because of the genetic similarity between commercial rice and red rice, red rice control with traditional herbicides has been mostly unsuccessful.

Flooding is a good means of control because red rice and cultivated forms can only germinate through soil or water but not through both. Water seeded rice is capable of germinating due to a thin layer of oxygen that exists at the water-soil interface. (Beck and Smith 2000; Kendig et al. 2003). Some escapes occur however, because some red rice seed may lay on the soil surface instead of being buried in the soil. Rotation to soybeans is another cultural practice used to control red rice. Rotation to corn and sorghum is used. A cotton and rice rotation is also utilized. However, the use of arsenical herbicides (monosodium methanearsonate

or MSMA) in cotton has been associated with a physiological condition termed ‘straighthead’ in rice. A rotation of soybeans and rice is probably the best solution as soybean herbicides provide growers with the best and widest range of red rice control options (Kendig et al. 2003).

Herbicide-Resistant Rice

The genetic similarities between red rice and cultivated forms significantly limit selective control of red rice. Through genetic engineering and conventional breeding, three herbicide resistant weed control systems are currently under development (or have been released in recent years). Rice lines have been developed which are resistant to glufosinate (trade names Liberty, Ignite²), imazethapyr, and glyphosate (trade name Roundup³ and others) (Kendig et al. 2003). Of these three, only resistance to imazethapyr, an imidazolinone herbicide has been released for public use. Glufosinate and glyphosate resistant crops are considered transgenic crops because the genes which induce tolerance to these respective chemicals were derived from foreign species. Imidazolinone resistant crops were developed using mutation breeding techniques and selecting for herbicide tolerance.

Glufosinate-resistant rice could soon become an option for selective control of red rice and other weeds in rice. LibertyLink² rice was developed by AgrEvo (now Bayer CropScience) through recombinant DNA technology to be tolerant to glufosinate-ammonium (4-[hydroxy(methyl)phosphinoyl]-DL-homoalanine) the active ingredient in Liberty² herbicide. Glufosinate resistance

² Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle, NJ 27709

³ Monsanto Company 800 Lindbergh Ave, St. Louis, MO 63167

was incorporated into plants by using the phosphinothricin acetyl transferase (*PAT*) gene of *Streptomyces viridochromogenes* (Coetzer et al. 2002) that encodes for phosphinothricin acetyl transferase. This enzyme has high substrate specificity for glufosinate and acetylates (introduces an acetyl group to) the free amino group of glufosinate, rendering it herbicidally inactive (Coetzer et al. 2002).

Glufosinate is a nonselective postemergence herbicide that inhibits the synthesis of glutamine from glutamate and ammonia by inhibiting the activity of glutamine synthetase. This causes accumulation of ammonium and inhibition of photosynthesis (Coetzer et al. 2002). The application of glufosinate essentially leads to reduced glutamine and increased ammonia levels in the plant tissues. This causes photosynthesis to stop and the plant dies within a few days. Jansen et al. (2000) summarizes the herbicidal action of glufosinate ammonium as a rapid accumulation of ammonia in the plant, a deficiency in several amino acids, an inhibition of photosynthetic processes, and finally in the death of the plant cell.

Glufosinate resistant rice is not yet commercially available. Although all United States governmental agencies have approved the glufosinate-resistant technology, it is not expected to be commercially released until sometime after 2007.

Another herbicide resistant rice system that has been developed is glyphosate resistant rice. This technology developed by Monsanto would allow the use of the company's Roundup herbicide with the active ingredient glyphosate (N-(phosphonomethyl) glycine) to be used for non-selective weed control in rice.

Glyphosate is a unique herbicide in the way that it causes plant death. Glyphosate is the only commercial herbicide that attacks Enolpyruvylshikimatephosphate (EPSP) synthase, an enzyme of the shikimate pathway (Sherman et al. 1996).

Glyphosate resistant technology was introduced in soybeans in 1996 and is now being used in corn, cotton, and canola among other crops. At this time it is unclear whether Monsanto will release this technology in rice because of concerns that outcrossing between modified rice cultivars and red rice could occur.

Glyphosate is a valuable red rice control in rotational crops and therefore if outcrossing were to convey glyphosate resistance to red rice, control options would be lost in both the rice crop and the rotational crop (Kendig et al. 2003).

Rice resistant to the imidazolinone herbicides was developed from a single rice plant that survived a chemically induced mutation trial in 1993 (Steele et al. 2002). Further breeding has increased these lines' tolerance to imazethapyr. Initially, imazethapyr was applied in split applications to total 140 g ai ha⁻¹ per growing season, and that 70 g ai ha⁻¹ pre plant incorporated (PPI) or PRE followed by (fb) 70 g ai ha⁻¹ POST would be the most effective program (Pellerin and Webster 2004). Injury to first generation imi-tolerant varieties was generally most severe when a POST application was applied as opposed to PPI or PRE (Ottis et al. 2003; Hackworth et al. 1998; Steele et al. 2000). Increased tolerance or resistance has been developed in recent years. Recommendations at the initiation of this study were to apply imazethapyr in sequential applications of 70g ai ha⁻¹ POST at the 1-leaf stage fb 70g ai ha⁻¹ POST and the 4-leaf stage. Imazethapyr can now be applied at a rate up to 105 g ai ha⁻¹ at both timings for

enhanced-tolerance varieties (Anonymous 2006). This improved technology allows the use of an imidazolinone herbicide with little or no effect upon the crop itself. In addition to rice, this technology has also been used to produce other IMI-resistant crops.

Imidazolinone-resistant canola (*Brassica napus L.*), wheat (*Triticum aestivum*), and corn production systems are now being used. These three, in addition to imidazolinone-resistant rice, are used in conjunction with imidazolinone family herbicides. Imazamox (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) is labeled for use in imidazolinone-resistant canola and wheat. A mixture of imazethapyr and imazapyr (2-(4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl)-3-pyridinecarboxylic acid) under the trade name *Lightning*⁴ is used in imidazolinone-resistant corn. Currently the herbicide imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) is labeled for exclusive use in imidazolinone-resistant rice production systems. All imidazolinone-resistant technologies were developed using mutation breeding techniques and are considered non-genetically modified organisms (non-GMO) and are sold under the *CLEARFIELD*⁵ name.

Imidazolinone-Resistant Rice

Masson et al. (2001) and Croughan et al. (1995) stated that the development of imidazolinone-resistant (IMI-resistant) rice will allow producers using this technology to apply a PRE imidazolinone herbicide. Research

⁴ BASF Corporation, Florham Park, NJ 07932

⁵ Horizon Ag. LLC, 8275 Tournament Drive, Memphis, TN 38125

conducted by Steele et al. (2002) indicated that PRE or PPI treatments of imazethapyr at 70 g ai ha⁻¹ and a POST treatment at the same rate are needed to adequately control red rice with first generation varieties. However, as mentioned above, the current recommendation on second generation varieties is a PPI or PRE application of 70 to 105 g ai ha⁻¹ fb a POST application of 70 to 105g ai ha⁻¹.

Complete control of red rice is desirable because of the potential of outcrossing with the cultivar (Steele et al. 2002). There are concerns that since both red rice and commercial rice belong to the same genus and species, interbreeding and the transfer of the imidazolinone-tolerance trait to red rice could occur. Growing herbicide-resistant varieties in proximity with sexually compatible *Oryza* relatives such as red rice provides an opportunity for the unintended transfer of these resistance traits by cross-pollination with these non-cultivated relatives. These modified genes could then become part of the red rice genetic base. If this occurs, any field where red rice occurs could be contaminated with the tolerance gene indefinitely (Vaughan et al. 2001; Ellstrand et al. 1999). Repeated applications of herbicides to which the rice variety is resistant can create a strong selection pressure that will tend to increase the populations of weeds possessing the herbicide-resistant trait (Gealy et al. 2003). This would render the technology ineffective for control of red rice.

Many crops, including rice, sunflower (*Helianthus annuus*), sugarbeet (*Beta vulgaris*), canola, barley (*Hordeum vulgare*), and wheat hybridize freely with their wild relatives (Massinga et al. 2003). Herbicide-resistance gene flow from herbicide resistant crops (HRCs) to wild relatives was reported for several crop-

weed systems (Massinga et al. 2003). Brown and Brown (1996) reported that glufosinate resistant canola can outcross with field mustard (*Brassica rapa*) producing glufosinate resistant hybrids (Massinga et al. 2003).

Studies have shown that herbicide tolerant plants are no more invasive of cultivated or natural habitats than their herbicide susceptible counterparts, unless the relevant herbicide is used exclusively to eliminate competing vegetation (Downey 1999). In a study conducted to evaluate the transfer of fitness-related genes into wild relatives, which would result in more invasive and more difficult to control weeds. Marshall et al. (2001) found no difference in photosynthesis, leaf area, height, and dry weight between imazethapyr-resistant and -susceptible common sunflower (Massinga et al. 2003).

Rice, in contrast to common sunflower, is a largely self-pollinated crop. Red rice is also primarily self-pollinated and natural hybridization at low rates has been documented (Baldwin 2003; Langevin et al. 1990; Beachell et al. 1938). Outcrossing rates between red rice and cultivated rice (herbicide-resistant or non-resistant) have been variable, but nearly always less than 0.5% (D. Gealy, personal communication). This is supported by data from a 1938 study (Beachell et al. 1938) which reported 0 to 3.39% natural crossing between red rice and rice with an average of 0.45% (Baldwin 2003). However, in late 2004, reports in the popular press indicated that imidazolinone-resistant red rice had been observed in grower fields.

An imidazolinone-resistant rice production system can be useful in controlling red rice and can prevent future infestations if used properly (Steele et

al. 2002). A program initiated by BASF and Horizon Ag termed the CLEARFIELD Stewardship Initiative, is being used to help ensure proper use and understanding of the technology so that it remains viable for many years. Some goals of the stewardship program include: 1) Ensure the long-term viability of the imidazolinone chemistry as a weed control option, 2) Encourage continued investment in new seed and chemical technology research, 3) Maximize the agronomic potential of the system through the use of certified seed, 4) Provide for education on responsible weed resistance management practices (Anonymous 2005).

Imazethapyr and Imazamox

The imidazolinone herbicides inhibit the enzyme acetolactate synthase, a key enzyme in the biosynthesis of branched-chain amino acids valine, leucine, and isoleucine (Masson and Webster 2001; Stidham 1991; Walsh 1991; Anderson and Hibberd 1985). Once in the phloem and translocated to the site of action, the imidazolinones inhibit ALS, causing death of meristematic cells resulting in plant death (Masson and Webster 2001). Imazethapyr is the first imidazolinone herbicide selected for use in imidazolinone resistant rice because of crop tolerance, weed control spectrum and effectiveness as a soil or foliar applied treatment (Zhang et al. 2001). Currently, the two herbicides being used in imidazolinone resistant rice are imazethapyr and imazamox. The imazamox formulation *Beyond* has received special local need (24(c)) registration “to remove late emerging or previously missed red rice only in imidazolinone-resistant rice varieties and hybrids possessing the second generation tolerance trait

following the two 4-oz./acre (70g ai ha⁻¹) applications of imazethapyr at labeled timings” (Anonymous 2004a). The replacement of the ethyl substituent on the pyridine carboxylic acid ring of imazethapyr with a methoxymethyl group is the only structural difference between imazamox and imazethapyr (Nelson et al. 1998).

Imazethapyr is readily absorbed through roots and foliage, making it ideal for PPI, PRE, and POST applications (Pellerin and Webster 2004; Cantwell et al. 1989a). Imazamox also is absorbed through the roots and foliage when applied POST, but should be applied when weeds are actively growing and before they exceed the maximum recommended size. It also provides activity on susceptible weeds that may emerge shortly after application (Anonymous 2003).

The use of ALS inhibitors became very popular soon after their introduction. This is due to the relatively low use rates, environmental safety, low mammalian toxicity, wide crop selectivity and high efficacy. Many of the qualities that make ALS inhibitors popular also favor the increase of resistant populations (Bader 1995; Holt and Lebaron 1990). The high efficacy of these chemicals quickly selects for the resistant phenotype (Saari et al. 1994). These resistant plants quickly become the dominating phenotype in an area. Several weed species have become tolerant or resistant to the ALS mode of action. In 1987, five years after their introduction, the first case of resistance to an ALS inhibitor was reported in prickly lettuce (*Lactuca serriola* L.) (Franssen et al. 2001, Mallory-Smith et al. 1990). Since then, 95 species have evolved resistance

to the ALS inhibitors, worldwide (Franssen et al. 2001), over 30 species confirmed resistant in the U.S.

Imidazolinone Herbicides in Non-Flooded Crops

Imazamox and imazethapyr herbicides are used for grass and broadleaf control in soybean (Nelson et al. 1998). Imazethapyr has also been evaluated for weed control in alfalfa (*Medicago sativa*), peanuts (*Arachis hypogae*), edible beans (*Phaseolus spp.*) and peas (*Pisum sativum*) (Walsh 1991; Carlson and Taylor 1988; Hartberg and Harvey 1988). Imazethapyr used in soybeans at rates greater than 70 g ai ha⁻¹ or under stressful conditions may cause some injury. Under favorable conditions, soybeans quickly outgrow these temporary symptoms and yields are not affected (Hart et al. 1991). No significant difference in soybean injury from imazethapyr and imazamox was reported by Nelson and Renner (1995). However, Gednalski et al. (1995) reported injury to be greater when imazamox was used along with methylated seed oil (Nelson and Renner 1998)

These two herbicides are very similar structurally and in performance. The differences while few, can have a large effect upon the situations in which these two herbicides are used. Imazamox offers an additional option for postemergence grass and broadleaf weed control for producers whose choices are limited by rotational crop restrictions (Nelson et al. 1998; Lueschen 1997). Corn is quite tolerant to soil residues of imazethapyr, however grain sorghum, cotton and rice are sensitive to residues. Planting of these crops are restricted to 18 months after application in order for the residues to degrade to safe levels (Hart et al. 1991). The rotational crop restrictions are shorter for imazamox. Carryover

injury to corn and sorghum from imazamox (50 and 100 g ai/ha) applied to soybean did not occur 90 days after application (Cobucci et al. 1998).

Imidazolinone Dissipation

Herbicide dissipation is the loss of herbicide from the sampled soil zone (Johnson 1993). Certain soil factors including microbial population, moisture, organic matter, pH, temperature, and texture have been shown to influence the persistence of imazethapyr and related imidazolinones in the soil (Ayeni et al. 1998). Other factors such as photolysis, hydrolysis, and leaching can also contribute to the dissipation of these herbicides.

The routes and/or rates of microbial transformations of herbicides are influenced by environmental factors, agricultural techniques, and the properties of the herbicide (Torstensson 1980). Microbial degradation is the major soil dissipation pathway for the imidazolinones (Cantwell et al. 1989b; Flint and Witt 1997; Lehmann et al. 1993). Cantwell et al. (1989a) observed degradation of ^{14}C -imazaquin and ^{14}C -imazethapyr in irradiated and non-irradiated samples of two soils. An average of 95% of the radioactivity in the form of unaltered herbicide was recovered after 12 wk of incubation in irradiated soils. In contrast, only 22.8 to 69.8% of the ^{14}C -labeled herbicides was recovered in the non-irradiated soil. This indicates that microbial enzymes are the primary mechanism of imidazolinone degradation.

Factors that increase microbial activity, such as higher temperatures and adequate soil moisture, increase the rate of dissipation from the soil (Beyer et al. 1988; Cobucci et al. 1998; Flint and Witt 1997; Goetz et al. 1990; Lehmann et al.

1993; Loux and Reese 1993). Extreme heat, cold, and a lack or overabundance of soil moisture severely diminishes the activity of soil biomass (Cantwell et al. 1989a). Vischetti (2002) determined that the half life for imazamox was 27 (± 2) days at 25°C and moisture at 75% field capacity (FC) but that lowering the temperature to 10°C at 75% FC increased the half life to 83 (± 8) days. The temperature for optimum microbial activity varies according to average air temperature with microbes generally more active during the warmer seasons. Optimum temperatures for microorganism activity ranges from 20° to 30°C but in temperate regions may be 10° to 15°C (Torstensson 1980). Vischetti (1995) found in laboratory studies that the half lives of three concentrations of imazethapyr (0.1, 1.0, 10.0 ppm) increased as the temperature increased and that as temperatures varied between 10° and 20°C, the half lives increased by 55, 250, and 140% respectively.

The presence of water also affects how quickly imidazolinones degrade in the soil. According to Sciumbato et al. (2003) soil moisture was the most influential factor in determining the amount of imazethapyr available for plant uptake. Bauer and Calvet (1999) studied the range of 50-90% soil water capacity and found that for several herbicides, high soil moisture resulted in enhanced herbicide dissipation. Several factors complicate studies on the soil-water-plant-herbicide system. Among these are 1) changes in the herbicide concentration in the soil solution as soil water content changes, 2) a reduction of herbicide movement toward the root with decreasing water content, and 3) the reduced

herbicide translocation in a plant due to reduced respiration during periods of water stress (Green and Obien 1969).

Soil moisture affects the activity of soil applied herbicides by altering the herbicide concentration and mobility in the soil (Zhang et al. 2001; Moyer 1987). However, herbicides applied to foliage have shown reduced activity when applied to soils with low moisture content (Zhang et al. 2001). Absorption and translocation of ^{14}C -glyphosate by common milkweed was greater at 25% than 13% soil moisture (Zhang et al. 2001; Waldecker and Wyse 1985). This may be due to less overall movement of materials in the vascular system of the plant under moisture stress. Ball et al. (2003) and Cobucci et al. (1998) noted that insufficient soil moisture for microbial degradation may limit the decomposition of imazamox and increase the potential for injury to rotational crops.

While dry soils result in a higher herbicide concentration in the soil, phytotoxicity may in fact be decreased (Green and Obien 1969). This is because of the mechanisms which move the herbicide through the soil and into the plant. Herbicide transport through the soil to plant root or hypocotyl takes place by mass flow or molecular diffusion. Mass flow, the product of water movement rate and herbicide concentration, and diffusion both decrease with a reduction in water content (Green and Obien 1969). This limits the amount of herbicide available to the plant.

Under aerobic conditions, aerobic metabolism (of imidazolinones) is relatively slow, but extensive (Mangels 1991a). Under flooded conditions, when the soil becomes anaerobic, no significant degradation occurred over 2 months of

incubation, indicating that the imidazolinones are stable in an anaerobic environment.

Herbicide adsorption is lower in wet than dry soils and therefore the herbicide molecules remain in solution longer and are subjected to greater leaching, plant absorption, and microbial degradation (Cobucci et al. 1998). However the imidazolinones have been shown to leach very little. Little downward movement of C¹⁴ imazethapyr in the field under normal application conditions was observed (Mangels 1991a). In addition, 95% of the ¹⁴C-imazethapyr was recovered in the top 15 cm of soil. This indicates that imazethapyr did not leach significantly (Mangels 1991a). In studies conducted by American Cyanamid Company, imazethapyr showed no degradation over 1 year in aerobic or anaerobic sediment/water systems taken from a river in Canada (Gagne et al. 1991).

The pH and type of soil affects the activity of the organisms in the soil that contribute to dissipation. Soil pH did not affect imazamox persistence but affected the bioavailability according to Bresnahan et al. (2002). While 82% of imazamox had dissipated from a soil with three different pHs, the remaining residue (18%) was more bioavailable and greater injury resulted. Microbial activity is often higher in soils with high organic matter content, and has a higher density of microorganisms near organic and clay surfaces (Johnson 1993; Hance 1988). Loux et al. (1989b) observed that imazethapyr and imazaquin were both more persistent as soil organic matter and clay content increased.

The soil texture can also determine how well the herbicide is bound or adsorbed to soil particles. Loux et al. (1989a) found that imazethapyr adsorption was strongly correlated with clay content, while Johnson (1993) observed that dissipation was slower in soils with high adsorption than in soils with low adsorption (low organic matter, low clay soils) (Braverman et al. 1985; Cantwell et al. 1989a; Goetz et al. 1990; Loux et al. 1989a; Loux and Reese 1993; Rogers et al. 1986; Savage 1978; Schroeder and Banks 1986). It has been proposed by Cantwell et al. (1989a) that imidazolinones are protected from biodegradation when adsorbed to colloids. Likewise, application of imidazolinones to highly adsorbent soils and subsequent environmental conditions which are unfavorable to microbial growth will result in increased persistence. Injury to normally sensitive plants may not occur due to the herbicide being strongly bound to soil organic matter and clay particles and unavailable for plant uptake.

Photolysis and hydrolysis are physical forces which change the rate of herbicide degradation. Photolysis is a chemical process by which molecules are broken down into smaller units through the absorption of light. Photolysis of imidazolinones in water is fairly rapid while on soil the rate is somewhat slower. The generally accepted half-life due to photolysis on dry soil for the imidazolinones is 4 months (Mangels 1991b). Photolysis of imidazolinones in aqueous solution however may take only 48 hs (WSSA 1994).

Hydrolysis, a chemical decomposition process that uses water to split chemical bonds of substances, can be affected by the amount of water present. Hydrolysis of the acid imidazolinones has been found to occur at an extremely

slow rate at environmentally relevant pHs and temperatures (Mangels 1991b).

Summary

The introduction and release of imidazolinone-resistant rice has had a great impact on the rice growing areas of the southern United States by allowing for the first time the use of a simple selective herbicide system for control of red rice. The use of imazethapyr also allows for ease of application and control of a broad spectrum of problematic weeds in rice. However, there are restrictions on what crops can be planted following imidazolinone-resistant rice due to carryover. Imazamox is closely related to imazethapyr but has a shorter carryover restriction to rotational crops. Evaluation of imazamox as a replacement for imazethapyr in rice culture could allow farmers to use imidazolinone-resistant rice to help control red rice and plant imi-susceptible rice the following year without the risk of injuring the crop.

Many factors play a role in dissipation of these herbicides. Interactions among these factors can greatly affect the time it takes for herbicide residues in the soil to reach levels safe to a rotational crop. If the herbicide persists too long because of these factors, rotational crops may be injured and yield losses may occur. While many studies have focused on how these herbicides dissipate under normal, non-flooded cropping conditions, very little is known about their fate in a rice production environment. Imazapyr has been shown to persist for 2-3 days in shallow ponds compared to 25-142 days in a terrestrial setting depending upon soil type and environmental factors (WSSA 1994). Baldwin (2003) found that the risk of carryover injury from labeled rates of imazethapyr could be unpredictable

in a IR rice conventional oat or rice rotation. However, no data was found regarding imazamox persistence in similar settings.

Flooded conditions may affect the microbial and light activity in degrading these compounds. Soil types capable of holding water for rice growing may exhibit properties which affect the rate of degradation. Dissipation times need to be calculated for both imazethapyr and imazamox under these unique circumstances so that injury and yield reductions do not occur when a conventional crop, primarily imi-susceptible rice, is planted the year after utilization of imi-resistant rice technology.

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Chapter II

The Effects of Imazethapyr and Imazamox Residues to Imidazolinone-Susceptible Rice Following Treatment in Imazethapyr-Resistant Rice the Previous Year

Introduction

Red rice (*Oryza sativa*) is a serious production concern for rice (*O. sativa*) producers in the Mid-south region of the United States because it can reduce yield and milling quality of commercial white rice (Diarra et al. 1985, Smith Jr. et al. 1977, and Khodayari et al 1987). Yield reductions of 22 and 77% have been reported when 5 and 108 plants m⁻² are present, respectively (Diarra et al. 1985). Due to red rice and commercial rice being physiologically identical, herbicide selectivity is extremely limited. Control of red rice in production rice fields was, until recently, dependent mainly on cultural practices supplemented with herbicides that provided selectivity based upon spatial separation and pre-germination of the rice cultivar. A permanent flood will prevent germination of red rice seeds below the soil surface; while aurally sown rice can grow in the thin layer of oxygen that exists at the soil-water interface (Scott et al. 2003). Crop rotation is another traditional red rice control method. In the crop grown in rotation with rice, complete control of red rice is necessary to limit infestations in the next rice crop, as 5% escapes can replenish the red rice seed bank in the soil (Askew et al. 1998).

Imidazolinone-resistant¹ (IR) rice provides rice growers with a herbicidal option to control red rice with little effect on crop safety (Steele et al. 2002). The herbicide currently labeled for red rice control in IR rice is imazethapyr² (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), from the imidazolinone family of herbicides. This class of herbicides inhibits the acetolactate synthase (ALS) enzyme in susceptible plant species (Ahrens 1994, Stidham and Singh 1991), and is essential for the biosynthesis of the branched chain amino acids leucine, isoleucine, and valine (Steele et al. 2002) which are required for DNA synthesis and growth.

Imazethapyr was selected for use with this technology because of the broad spectrum of weeds controlled, effectiveness as either a soil- or foliar-applied herbicide (Stougaard 1990), relative crop safety, and red rice efficacy (White and Hackworth 1999). Two applications of 70 to 105 g ai ha⁻¹ at pre-plant incorporated (PPI), preemergence (PRE), or early postemergence (EPOST) followed by (fb) mid postemergence (MPOST), are required to completely control red rice, and preclude IR rice from cross pollinating with red rice and producing IR red rice.

While crop rotations may provide rice producers an effective cultural control option for red rice, economics can influence this decision. A popular rotation consists of rice followed by two years of soybeans (*Glycine max*). This rotation allows for herbicidal control of red rice for two years. However, in recent

¹ Marketed as CLEARFIELD™ Rice. CLEARFIELD™ and the CLEARFIELD™ product system are trademarks of BASF Corporation Box 13528, Research Triangle Park, NC 27709. Orygen and Orygen seed marketing system are trademarks of Horizon Ag, LLC. 1611 International Drive, Suite 400 Memphis, TN 38125

² NewPath® 2AS Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

years, profits gained from soybean crops have been less than profits earned from rice crops (Baldwin 2003). For this reason, many rice producers may opt to produce rice continuously in the same fields. However, if imazethapyr were to be used for the control of red rice, a rotation back to imi-susceptible rice would not be an option due to an 18 month rotational crop restriction listed in the herbicide label³. Imazamox⁴ (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) is an imidazolinone herbicide similar to imazethapyr which has been used for grass and broadleaf weed control in soybeans (Nelson et al. 1998), and has been shown to have less persistence than imazethapyr (Blackshaw 1998, Aichelle and Penner 2005). Currently, imazamox only has a 9 month rotation restriction for imi-susceptible rice⁵.

While imazamox has been shown to persist for a shorter period than imazethapyr (Nelson et al. 1998), it is not currently labeled for use in IR rice as a primary herbicide. However, imazamox can be applied once for the control of and/or seed head suppression of red rice which escaped the required two applications of imazethapyr. The primary objective of these studies was to determine if imazamox red rice control programs offered enhanced rotational crop safety when compared to imazethapyr in an imi-resistant / imi-susceptible rice rotation.

³ NewPath® Herbicide label, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

⁴ Beyond 1AS Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

⁵ Beyond® Herbicide label, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

Materials and Methods

Field experiments were conducted in 2004 through 2005 and 2005 through 2006 to determine imi-susceptible rice tolerance to residues of imazamox and imazethapyr applied to IR rice the previous year. Tests were located at the University of Missouri Delta Research and Extension Center (DREC) located near Portageville, MO, (36° 23' N 89° 36' W) and at the Missouri Rice Research Farm (MRRF) located near Glennonville, MO (36° 34' N 90° 07' W). The soil at DREC was a Sharkey clay, (fine, montmorillonitic, nonacid, thermic, Typic Haplaquolls) and the soil at MRRF was a DeWitt silt loam (fine, smectitic, thermic, Typic Albaqualfs). Characteristics for each soil are presented in Table 2.1. These soils were selected because they represent a major portion of the rice producing soils in the Mid-South. Rice was grown at both sites previously.

Plot size was 3 m (2.25 treated) by 4 m. Tillage for the original plot areas was accomplished with a tandem disk followed by a soil conditioner equipped with 'S' tines in the front and rolling baskets in the rear. No tillage was performed during the second year of the studies to prevent movement of treated soil into adjacent plots. Site preparation for the second year consisted of burning crop residues from the previous year in early spring, and applying glyphosate⁶ at a rate of 0.84 kg ae ha⁻¹ one week prior to planting.

In the first year of the studies, CLEARFIELD® 161⁷ (CL161) rice was planted in 22 cm rows with a no-till drill into the plot areas at approximately 100 kg ha⁻¹ to a depth of 1 cm. Plot preparation, maintenance and data collection dates

⁶ Roundup WeatherMax® 4.5AE Herbicide, Monsanto Company, 800 Lindbergh Ave. St. Louis Mo. 63167

⁷ BASF Corporation, Box 13528, Research Triangle Park, NC 27709

are shown in Tables 2.3 and 2.4 for Sharkey clay and DeWitt silt loam sites, respectively. Treatments included in this test (Table 2.2) reflect imazethapyr labeling at the time of study initiation ($0.07 \text{ kg ai ha}^{-1}$), and expected labeling if imazamox were to be used as a primary option with this system based upon the application rate on the supplemental labeling ($0.044 \text{ kg ai ha}^{-1}$). Also included were treatments to evaluate whether double the labeled rates, additional applications or substitution of imazamox for one of the current application timings of imazethapyr increased carryover, decreased carryover or had no effect upon the imi-susceptible rice crop the following year. Treatments were applied with a CO_2 pressurized backpack sprayer with a hand held boom consisting of 6 nozzles on 38 cm spacings. The sprayer was calibrated to apply 187 L ha^{-1} application volume at 160 kPa using TeeJet⁸ 8002 VS flat fan nozzles. Crop oil concentrate was added to all treatments at 1.0% v/v.

In the second year of the studies, the imi-susceptible rice cultivar ‘Cocodrie’ was no-till drilled into the plot areas at 100 kg ha^{-1} at a depth of 1 cm to evaluate imi-susceptible rice injury caused by residues of the imidazolinone herbicides applied the previous year. Dates of operations performed are shown in Tables 2.3 and 2.4 for Sharkey clay at DREC and DeWitt silt loam at MRRF, respectively. Weed control in plot areas where imi-susceptible rice was grown was accomplished by applying $4.48 \text{ kg ai ha}^{-1}$ propanil⁹ and $0.56 \text{ kg ai ha}^{-1}$ quinclorac¹⁰ when grassy weeds had 2-3 leaves. During all years of the studies,

⁸ TeeJet Spraying Systems Co., PO Box 7900, Wheaton, IL 60189

⁹ Stam™ M4 Herbicide, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268

¹⁰ Facet® 75DF Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

plot areas were fertilized with 130 kg actual N ha⁻¹ applied in the form of urea within 3 d of permanent flood establishment.

Plots were evaluated by percent visual injury at two timings: pre flood and post flood. Pre flood ratings were taken within 3 days prior to initiation of the permanent flood, while post flood ratings were taken within 3 wk after the permanent flood was established. Visual injury ratings were based upon a 0 to 100 scale. A rating of 0 is equal to no plant injury while 100 reflected to complete crop destruction.

Data were subjected to the appropriate factorial analysis of variance. Main plots for the individual tests were year and soil combinations. Herbicide treatments were subplots with the timing of injury ratings being sub-subplots. There were a large number of 0 ratings which had no variance. These data were removed to make the variance more homogeneous. Treatment means were separated using Fishers LSD at the $\alpha=0.05$ significance level.

Results and Discussion

Injury in both years consisted of low (< 15%) injury as compared to the untreated check plots. This reduction was generally in the form of height reduction and plant density. These injury symptoms were reported by Grymes et al. (1995) when assessing the risk of AC 263,222 (imazapic), another imidazolinone, when applied to soybeans in rotation with rice. Some chlorosis was also observed in our studies. The analysis of variance (Table 2.5) showed that rating times could be combined; however there were interactions between herbicide treatments, soils and years. This interaction is reflected in that no injury

was observed in 2005 on the DeWitt silt loam and in 2006 on the Sharkey clay (Table 2.6).

On the Sharkey clay in 2005, injury was often associated with treatments that included two imazethapyr applications at early postemergence and mid postemergence followed by (fb) a pre-flood imazamox application, or when imazamox was applied three times at (early and mid postemergence, pre-flood) at a rate of 0.044 kg ai ha⁻¹. Injury was also observed from two applications of 0.07 kg ai ha⁻¹ imazethapyr fb a pre-flood application of imazamox at either 0.044 kg ai ha⁻¹ or 0.088 kg ai ha⁻¹. However, three applications of a double rate of imazamox (0.088 kg ai ha⁻¹), and a double rate of imazethapyr (0.140 kg ai ha⁻¹) fb a double rate of imazamox did not result in significant injury. While equivalent treatments at the normal rates did cause injury, in all cases injury was low (<8%). Crop recovery would be expected from injury of this magnitude based upon rating guidelines presented by Camper (1986).

In 2006, injury on the silt loam soil was generally associated with double (0.140 kg ai ha⁻¹) rates of imazethapyr, as opposed to being associated with pre-flood imazamox applications (Table 2.6) as was observed on the clay soil in 2005. Injury was actually lower (9% versus 16%) when pre-flood imazamox was added to imazethapyr applied twice at 0.140 kg ai ha⁻¹. As in 2005 on clay, injury was low (<15%) and at levels not expected to result in yield loss. Because the injury was inconsistent in some instances, the injury we observed may be due to random variability in crop height and biomass.

Imidazolinone herbicides are very dependent upon environmental factors for dissipation. Therefore, factors such as an extremely cold or dry winter (when rice fields may not be flooded) may prolong persistence, causing more injury than illustrated in these studies. Precipitation data for the periods in which these tests were conducted are shown in Table 2.7 and Table 2.8. The amount of precipitation from the time of herbicide application in the first year of the studies until planting of the rotational crop the following year were similar at both sites and years. Precipitation amounts received during the summer months when rice was flooded should not be viewed as a contributing factor to affecting dissipation rates. The amount of precipitation in the autumn following application was somewhat different between years, and may have influenced the dissipation rates in these studies. Cultural practices such as keeping fields flooded during winter months for recreational purposes may also affect dissipation rates.

In fall 2004 (September 1 through November 30), 29 cm of precipitation was recorded at the Sharkey clay site compared to only 11 cm in the fall of 2005. Similarly, precipitation was higher on the DeWitt soil in the fall of 2004 with 42 cm precipitation compared to 16 cm the following fall. These differences could explain some of the accelerated dissipation, namely the silt loam results from 2006, where no injury was seen from any treatment. Monks and Banks (1993) suggested that differences in precipitation from one year to the next were partly responsible for differences in the dissipation rates for imazethapyr. Baldwin (2003) proposed that more soil moisture contributed to more microbial activity and thus greater breakdown of soil residues of imazethapyr.

Temperatures can also affect the microbial breakdown of imidazolinone herbicides. The average daily air and soil temperatures during the period beginning with the early postemergence application date in 2004 until planting in 2005 was found to be approximately 2 °C cooler than the same interval in 2005 through 2006 on the clay soil (Table 2.9 and 2.10). Average daily temperatures from the period from early postemergence application until harvest in 2004 were approximately 1°C cooler than the same period in 2005 at the Sharkey site. Temperatures at the silt loam site were also slightly warmer during the period from early postemergence treatments until harvest in 2005 compared to the same period in 2004. Average daily temperatures from a period beginning with crop harvest until rotational crop planting the following spring were similar at the silt loam site, but a 1 to 2° C difference was noted at the clay site with 2005-2006 being warmer (Table 2.9 and 2.10). Because these temperature differences were small, temperature may have slightly affected the dissipation rates of the herbicides in these studies. Walker (1987) suggests that herbicide half life is increased 2 to 3 times with a 10°C decrease in temperature. Longer half lives would result in more herbicide residue remaining the following year and therefore more rotational crop injury

The time period from herbicide application one year until planting of the rotational crop the following year can also be a factor in the amount of injury detected. In 2006, no injury was detected on the clay soil, whereas in 2005 injury was above 0 and in some cases significantly higher than the untreated check plots. This may be due to an interval of only 332 d from the last application in 2004

until planting in 2005 and 398 d between the final application in 2005 and planting in 2006. Grymes et al. (1995) acknowledged that differences in elapsed days from herbicide application to rotational crop planting could result in increased degradation of the compounds, and suggests a later planting date for increased herbicide degradation. Thus, the concentrations causing the low levels of injury observed in 2005 could have decreased to be non-effective toward imidazolinone-susceptible rice if elapsed days from application until planting were equal between years.

Conclusions

While treatment differences exist in these data, the extent of all injury observed during both years and at both locations was less than that typically causing yield reduction (Camper 1986). Biomass reduction, and to some extent chlorosis, were the only injury symptoms observed during these studies, and may be caused by any number of random events. Most of what was rated as injury is probably due to random differences in rice growth.

Based upon these results, imi-susceptible rice could be grown without concern for crop safety the season following either imazethapyr or imazamox used for the control of red rice in IR rice. Some injury may be observed but at very low levels. It should be noted however that Baldwin (2003) stated that the risk of this rotation can be unpredictable even with the recommended rates of imazethapyr. The addition of a pre flood salvage application of either imazethapyr or imazamox, could only add to the unpredictability associated with this rotation and may result in some injury and yield reduction.

Table 2.1. Characteristics of soils used in imidazolinone carryover studies at the Delta Research and Extension Center (DREC) at Portageville, Mo. and The Missouri Rice Research Farm (MRRF) at Glennonville, Mo.

Location	Series	pH	Organic	Sand	Silt	Clay	Texture
			matter				
			-----%-----				
DREC	Sharkey	5.4	2.6	9.8	31.1	59.1	Clay
MRRF	DeWitt	6.3	1.8	21.3	67.2	11.5	Silt Loam

Table 2.2. Listing of treatments and application timings for imidazolinone carryover studies.

TREATMENT	RATE (kg ai ha ⁻¹)	APPLICATION ^a TIMING
Imazethapyr	0.070	EPOST
Imazethapyr	0.070	MPOST
Imazethapyr	0.070	EPOST
Imazethapyr	0.070	MPOST
Imazamox	0.044	PRE FLD
Imazethapyr	0.070	EPOST
Imazethapyr	0.070	MPOST
Imazamox	0.088	PRE FLD
Imazethapyr	0.070	EPOST
Imazamox	0.044	MPOST
Imazethapyr	0.140	EPOST
Imazethapyr	0.140	MPOST
Imazethapyr	0.140	EPOST
Imazethapyr	0.140	MPOST
Imazamox	0.044	PRE FLD
Imazethapyr	0.140	EPOST
Imazethapyr	0.140	MPOST
Imazamox	0.088	PRE FLD
Imazethapyr	0.140	EPOST
Imazamox	0.088	MPOST
Imazamox	0.044	EPOST
Imazamox	0.044	MPOST
Imazamox	0.088	EPOST
Imazamox	0.088	MPOST
Imazamox	0.044	EPOST
Imazamox	0.044	MPOST
Imazamox	0.044	PRE FLD
Imazamox	0.088	EPOST
Imazamox	0.088	MPOST
Imazamox	0.088	PRE FLD
Untreated	-----	-----

^aEarly postemergence (EPOST) on 1-2 leaf rice, mid postemergence (MPOST) on 4-5 leaf rice, and pre-flood (PRE FLD) approximately 3 d prior to permanent flood establishment.

Table 2.3. Dates of operations performed during studies on Sharkey clay at Portageville, Mo. to evaluate imi-susceptible rice tolerance to residues of imazethapyr and imazamox applied in a previous Imidazolinone Resistant rice crop.

	DATE			
	2004	2005	2005	2006
Variety Planted	CL161	Cocodrie	CL161	Cocodrie
<u>Maintenance</u>				
Plant	May 25	May 11	May 12	June 5
Replant		May 25		June 26
Flush ^a				
First		May 19	May 19	June 13
Second		May 27	May 27	
Permanent Flood	June 29	June 30	June 22	July 21
<u>Herbicide Applications</u>				
EPOST ^b	June 9		May 25	
MPOST ^c	June 24		June 8	
PRE FLD ^d	June 28		June 17	
<u>Rice Injury Ratings</u>				
PRE FLD		June 30		July 21
POST FLD ^e		July 18		August 3
Harvest	September 28		October 10	

^aThis term is used to describe a quick application of flood water followed by immediate draining.

^b Early postemergence, 1 to 2 leaf rice.

^c Mid postemergence, 4 to 5 leaf rice.

^d Pre Flood, prior to initiation of the permanent flood.

^e Post Flood, after establishment of the permanent flood.

Table 2.4. Dates of operations performed during studies on DeWitt silt loam at Glennonville, Mo. to evaluate imi-susceptible rice tolerance to residues of imazethapyr and imazamox applied in a previous Imidazolinone Resistant rice crop.

	DATE			
	2004	2005	2005	2006
Variety Planted	CL161	Cocodrie	CL161	Cocodrie
<u>Maintenance</u>				
Plant	May 12	May 23	May 23	May 23
Replant				
1		June 17		June 7
2		June 23		June 29
Flush ^f				
First		May 26	May 26	
Second		June 17	June 17	
Permanent flood	June 16	July 19	June 24	August 4
<u>Herbicide Applications</u>				
EPOST ^g	May 24		June 7	
MPOST ^h	June 2		June 17	
PRE FLD ⁱ	June 10		June 23	
<u>Rice Injury Ratings</u>				
PRE FLD		July 19		July 25
POST FLD		August 17	July 18	August 4
Harvest	October 1	October 19	October 19	

^fThis term is used to describe a quick application of flood water followed by immediate draining.

^g Early postemergence, 1 to 2 leaf rice.

^h Mid postemergence, 4 to 5 leaf rice.

ⁱ Pre Flood, prior to initiation of the permanent flood.

^j Post Flood, after establishment of the permanent flood.

Table 2.5. Analysis of variance table for imidazolinone carryover injury ratings^k from studies performed during 2004 through 2006 on a Sharkey clay and DeWitt silt loam.

Source	DF ^l	Type I SS ^m	Mean Square	F Value	Pr > F ⁿ
soil	1	460.928342	460.928342	27.59	<.0001
year	0	0.000000	.	.	.
year*soil	0	0.000000	-	-	-
rep(year*soil)	6	375.805349	62.634225	3.75	0.0070
treat	12	1836.020947	153.001746	9.16	<0.0001
soil*treat	12	524.001464	43.666789	2.61	0.0170
year*treat	0	0.000000	.	.	.
year*soil*treat	0	0.000000	.	.	.
treat*rep(year*soil)	46	1348.813127	29.322025	1.75	0.0552
time	1	23.086538	23.086538	1.38	0.2494
year*time	1	1915.461438	1915.461438	114.64	<0.0001
soil*time	0	0.000000	.	.	.
treat*time	11	460.131035	41.830094	2.50	0.0236
year*treat*time	10	211.279323	21.127932	1.26	0.2949
soil*treat*time	0	0.000000	.	.	.
year*soil*time	0	0.000000	.	.	.
year*soil*treat*time	0	0.000000	.	.	.

^kMissing data due to the removal of all injury ratings of 0. Removal of these data resulted in loss of degrees of freedom for several interactions (gross measure of variability). With 0 degrees of freedom, no statistical data could be calculated.

^lDegrees of Freedom.

^mType I Sum of Squares.

ⁿProbability > F value.

Table 2.6. Average injury by treatment observed on Cocodrie rice planted in 2005 and 2006 on a Sharkey clay and DeWitt silt loam the year after herbicide treatments were applied to Imidazolinone-Resistant Rice. Injury assessed at pre-flood (~3d before permanent flood) and postflood (within 2 weeks after permanent flood establishment). (Injury scale: 0=no injury, 100=total crop death)

Treatment	Rate ^o	Timing ^p	Injury			
			Clay		Silt Loam	
			2005	2006	2005	2006
Imazethapyr	0.070	EPOST				
Imazethapyr	0.070	MPOST	5	0	0	1
Imazethapyr	0.070	EPOST				
Imazethapyr	0.070	MPOST				
Imazamox	0.044	PRE FLD	6	0	0	2
Imazethapyr	0.070	EPOST				
Imazethapyr	0.070	MPOST				
Imazamox	0.088	PRE FLD	8	0	0	1
Imazethapyr	0.070	EPOST				
Imazamox	0.044	MPOST	5	0	0	3
Imazethapyr	0.140	EPOST				
Imazethapyr	0.140	MPOST	4	0	0	14
Imazethapyr	0.140	EPOST				
Imazethapyr	0.140	MPOST				
Imazamox	0.044	PRE FLD	5	0	0	9
Imazethapyr	0.140	EPOST				
Imazethapyr	0.140	MPOST				
Imazamox	0.088	PRE FLD	7	0	0	9
Imazethapyr	0.140	EPOST				
Imazamox	0.088	MPOST	3	0	0	6
Imazamox	0.044	EPOST				
Imazamox	0.044	MPOST	3	0	0	4
Imazamox	0.088	EPOST				
Imazamox	0.088	MPOST	4	0	0	3
Imazamox	0.044	EPOST				
Imazamox	0.044	MPOST				
Imazamox	0.044	PRE FLD	6	0	0	1
Imazamox	0.088	EPOST				
Imazamox	0.088	MPOST				
Imazamox	0.088	PRE FLD	0	0	0	2
Untreated	-----	-----	0	0	0	0

LSD (0.05) within site years = 5

LSD (0.05) across site years = 6

^oHerbicide rates are listed as kg ai ha⁻¹.

^pTimings are early postemergence (EPOST) on 1-2 leaf rice, mid postemergence (MPOST) on 4-5 leaf rice, and pre flood (PRE FLD), approximately 3d before initiation of the permanent flood.

Table 2.7 Precipitation received at the Sharkey clay site by month for the duration of imidazolinone carryover studies. Precipitation measured in cm.

Month	Precipitation (cm)			
	2004	2005	2006	
Jan		13.92	13.51	
Feb		7.04	6.43	
Mar		8.56	12.78	
Apr		10.64	6.48	
May		1.52	12.37	
Jun	0.01 ^q	5.74 ^r	5.16	
Jul	15.14	11.38	13.18	
Aug	8.18	9.96	4.39	
Sep	0.00	5.82		
Oct	14.05	0.00		
Nov	21.26	4.01		
Dec	8.51	1.70		
Total	67.15	80.29	74.30	221.74

^qPrecipitation received after the PRE FLD herbicide application until the end of the month

^rPrecipitation before the PRE FLD application accounted for 5.08 cm of the total.

Table 2.8. Precipitation received at the DeWitt silt loam site by month for the duration of imidazolinone carryover studies. Precipitation measured in cm.

Month	Precipitation (cm)			
	2004	2005	2006	
Jan		13.79	9.09	
Feb		6.45	6.07	
Mar		6.65	11.46	
Apr		9.63	5.03	
May	3.10 ^s	1.14	8.66	
Jun	4.01	3.18 ^t	4.06	
Jul	10.21	13.11	12.29	
Aug	9.27	9.17	12.62	
Sep	0.28	9.73	38.56	
Oct	10.21	0.00		
Nov	23.60	7.92		
Dec	6.68	1.30		
Total	67.36	82.07	107.84	257.27

^sPrecipitation received after the PRE FLD herbicide application until the end of the month.

^tPrecipitation before the PRE FLD application accounted for 2.51 cm of the total.

Table 2.9. Average daily air temperature during imidazolinone carryover studies conducted on a Sharkey clay and a DeWitt silt loam in 2004-2005 and 2005-2006.

Soil ^u	Year	Average Daily Air Temperature (°C)		
		Treat-Harvest ^v	Harvest-Planting ^w	Avg. ^x
Clay	2004-2005	23.8	10.6	14.9
	2005-2006	25.0	12.7	17.0
Silt Loam	2004-2005	23.2	12.1	15.7
	2005-2006	24.2	12.2	16.3

^uSharkey clay and DeWitt silt loam.

^vPeriod from early postemergence application on Imidazolinone-Resistant rice until fall harvest.

^wPeriod from fall harvest until planting of the rotational crop the following spring.

^xAverage daily temperature from early postemergence applications on Imidazolinone-Resistant rice until planting of Imidazolinone –susceptible rice the following spring.

Table 2.10. Average daily bare soil temperature at a depth of 5.1 cm during imidazolinone carryover studies conducted on a Sharkey clay and a DeWitt silt loam in 2004-2005 and 2005-2006.

Soil ^y	Year	Average Daily Soil Temperature (°C)		
		Treat-Harvest ^z	Harvest-Planting ^{aa}	Avg. ^{ab}
Clay	2004-2005	26.8	11.2	16.4
	2005-2006	28.1	12.4	18.2
Silt Loam	2004-2005	25.6	10.9	16.2
	2005-2006	26.9	10.3	17.1

^ySharkey clay and DeWitt silt loam.

^zPeriod from early postemergence application on Imidazolinone-Resistant rice until fall harvest.

^{aa}Period from fall harvest until planting of the rotational crop the following spring.

^{ab}Average daily temperature from early postemergence applications on Imidazolinone-Resistant rice until planting of Imidazolinone –susceptible rice the following spring.

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Chapter III

Imidazolinone Dissipation in Flooded Environments as Compared to Non-Flooded Environments

Introduction

Many rice fields in the Mid-South and Delta regions of the U.S. are in continuous rice production due to poor drainage, zero-grade leveling of fields, or the desire of producers to grow rice due to favorable crop prices. Because of continuous rice production, many fields are now infested with red rice (*Oryza sativa*) a weedy relative of commercially grown white rice (*O. sativa*). Due to the physiological similarity between weedy red rice and commercially grown varieties, there are no herbicides with bio-chemical selectivity with the exception of imidazolinone-resistant (IR) rice¹. This system utilizes the herbicides imazethapyr² (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) and imazamox³ (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid) for control of red rice and most grass and broadleaf weeds (Nelson et al. 1998) found in rice with the exception of legume weeds.

Many studies have been conducted to determine the rotational cropping intervals for imazethapyr and imazamox. Loux et al. (1989b) found that imazethapyr and imazaquin applied to soybeans could persist and injure wheat

¹ Marketed as CLEARFIELD® Rice. CLEARFIELD® and the CLEARFIELD® product system are trademarks of BASF Corporation Box 13528, Research Triangle Park, NC 27709. Orygen and Orygen seed marketing system are trademarks of Horizon Ag, LLC. 1611 International Drive, Suite 400 Memphis, TN 38125

² NewPath® 2AS Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

³ Beyond® 1AS Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

and corn planted after soybeans. Loux (1989b) also found that injury would most likely occur on soils with a high adsorptive capacity for these herbicides. Ball et al. (2003) found that low soil moisture and low pH can contribute to injury of rotational crops such as barley and canola, following imazamox application. The main focus of these and other studies was conventional, non-flooded cropping environments such as corn, cotton, and soybeans for imazamox and imazethapyr, and forestry and right-of-way areas for imazapyr⁴ (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid). Therefore, little is known regarding the dissipation rates and mechanisms that occur in a flooded rice production environment.

Imazethapyr has been shown to be more persistent than imazamox (Ahrens 1994). Producers who apply imazethapyr in imi-resistant rice for red rice control may not be able to rotate to imi-susceptible rice for up to eighteen months based on rotational crop restrictions currently in place⁵. Therefore, the use of imazamox in IR rice may allow for an imi-resistant / imi-susceptible rice rotation to be practiced due to the more rapid dissipation of imazamox.

Degradation of imidazolinones occurs primarily by microbial degradation (Johnson 1993; Basham and Lavy 1987; Cantwell et al. 1989). Microbial populations tend to function more efficiently in the presence of oxygen. However, a poorly drained and oxygen poor soil is not detrimental to all soil microorganisms as some members of the microbial consortia can function in environments with little or no oxygen (Hartel 2005). However, under anaerobic

⁴ Arsenal® 2IS Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

⁵ NewPath® Herbicide label, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

conditions, imidazolinones have been shown to have no significant degradation during a two month period (Mangels 1991). Rice fields are not completely anaerobic. A thin layer of oxygen exists at the soil water interface (Scott et al. 2003). This oxidized zone may allow for some aerobic soil microbial activity to degrade imidazolinones to concentrations safer for rotational crops. Due to the lack of information on degradation in flooded rice fields versus dryland crop fields, it is necessary to determine if imidazolinone persistence is altered when used in IR rice to ensure rotational crop safety.

The objectives of this study were 1) determine if the dissipation rates of imazamox, imazethapyr and imazapyr are altered under flooded rice production conditions when compared to dissipation rates under non-flooded conditions, and 2) to determine and compare the half lives of the selected compounds applied to flooded and non-flooded clay and silt loam rice soils. Knowing the persistence of these compounds in rice production conditions will aid producers in selecting safe and appropriate crop rotations.

Materials and Methods

Field experiments were conducted in 2005 through 2006 at the Delta Research and Extension Center (DREC) at Portageville and at the Missouri Rice Research Farm (MRRF) near Glennonville, MO to determine and compare the duration of soil activity, and the dissipation rates of imazamox, imazethapyr, and imazapyr under flooded and non-flooded conditions.

The soil at DREC was a Sharkey clay, (fine, montmorillonitic, nonacid, thermic, typic Haplaquolls), and the soil at MRRF was a DeWitt silt loam (fine,

smectitic, thermic, Typic Albaqualfs). Characteristics for each soil are presented in Table 3.1. These soils were selected because they represent a major portion of the rice producing soils in the Mid-South.

Plot areas were disked and rolled prior to initiation of the tests, and kept free of vegetation during the sampling period by applying glyphosate⁶ (N-(phosphonomethyl) glycine) at a rate of 0.84 kg ai ha⁻¹ as needed. Plots were kept vegetation-free to keep the selected herbicides from being absorbed and metabolized by plants, and to allow soil samples to be collected and processed more efficiently.

Two tests were conducted at each location. One area was subjected to a flood similar to conditions found in commercial drill-seeded rice production fields. Another area was not flooded. Both tests at each site received applications of imazamox, imazethapyr and imazapyr at three rates each, shown in Table 3.2. The rates selected corresponded to a two, a one, and a one-half of the labeled rate for one application. It should be noted that two applications of imazethapyr are required per growing. Imazamox was applied similarly to imazethapyr, assuming two applications required per growing season. The imazapyr label presented a range of use rates from 0.035 to 0.841 kg ha⁻¹. A rate toward the middle of this range of 0.35 kg ha⁻¹ was selected as a '1x' rate.

Treatments were applied on June 20 and June 23, 2005 on the clay and silt loam soils, respectively. Herbicides were applied using a CO₂-pressurized backpack sprayer calibrated with a handheld boom consisting of six nozzles on 38

⁶ Roundup WeatherMax® 4.5AE Herbicide, Monsanto Company, 800 Lindbergh Ave. St. Louis, MO. 63167

cm spacing. The sprayer was calibrated to deliver 187 L ha⁻¹ application volume at approximately 160 kPa using TeeJet⁷ 8002VS flat fan nozzles. Plots were arranged in a randomized complete block with four replications. Plot size was 3 by 4.6 m. An untreated check was also included in each replication. Following herbicide application, non-flooded tests were flushed⁸ to activate the herbicides on June 22 and 28, 2005 for the clay and silt loam soils respectively. The permanent flood was established on the Sharkey clay on June 23 and on the DeWitt silt loam on June 29. The permanent flood was maintained until September 14 on the clay soil and until September 17, 2005 on the silt loam soil. The duration of the flood for these tests corresponded with the duration of the flood maintained in adjacent rice experiments.

At both sites, 0 wk samples were collected within 12 hs after herbicide application. Soil samples were also taken from the plots at 3, 6, 12, 24, and 52 WAA⁹. Soil samples were taken from the top 5 cm at 4 to 8 random areas within each plot, and placed into plastic storage bags. Samples from the flooded test areas were taken with the flood in place. As these samples were taken, excess water was removed from the bag in which the soil was placed. After all samples had been collected at each site, they were transported immediately to the DREC Weed Science chemical lab and placed into freezers. The amount of time from sample collection until placement in storage freezers ranged from approximately 15 minutes for samples from the Sharkey clay test site up to 2 hours for silt loam

⁷ TeeJet Spraying Systems Co., PO Box 7900, Wheaton, IL 60189

⁸ This term is used to describe a quick application of flood water followed by immediate draining

⁹ WAA = wk after application

samples. All samples were frozen within 12 hrs of removal from the field to stop degradation of the herbicides.

Soil samples from the first five sampling times were transported to Columbia, MO. for processing, while 52 wk samples were processed in Portageville, MO. Samples were dried for approximately 5 d on greenhouse benches and ground with a hand-operated soil grinder. Excess debris was removed from samples. Samples were then re-frozen.

A bioassay was used to estimate the quantity of herbicide residue remaining in the soil samples. Untreated soil was collected in bulk from both sites and dried and ground in the same manner as the soil collected from the plot areas. To generate standard curves, this soil was placed into 10.2 cm plastic pots and received applications of imazamox, imazethapyr, and imazapyr at rates ranging from 2 to 1/32 times the label rate (Table 3.3). Treated soil in each pot was placed into a plastic storage bag and shaken for one minute to simulate incorporation, and returned to the appropriate pot. These treatments were replicated three times and treatments were placed randomly within replications. Standard curve and field treated soil pots were filled to within 2 cm from the top (approximately 350 g of the clay soil and 290 g of the silt loam).

Due to the large number of soil samples taken, it was necessary to conduct three bioassays. One standard curve was developed for each soil type. The bioassay of the soil collected at the 0 through 24 wk sampling times from the Sharkey clay began on February 10, 2006, and the DeWitt silt loam assay began on March 18, 2006. The bioassay for the one year samples from both locations

began on December 12, 2006. The standard curves were developed during the bioassay of the 0 through 24 wk samples.

Within 24 h of the standard curve treatments being initiated, field samples were removed from frozen storage and placed into individual 10.2 cm pots. Approximately 10 seeds of imi-susceptible rice 'Cheniere' (80% germination) were planted at this time into the field-sample and standard-curve pots. An imi-susceptible rice cultivar was chosen as the assay species because rice has been shown to be adequately sensitive to soil residues of imazethapyr (Zhang et al. 2002, Johnson 1993) as well as imi-susceptible rice tolerance to imidazolinone residues being a primary objective of the studies. All pots were sub-irrigated using individual plastic saucers (15.2 cm by 3.5 cm) placed beneath the pots, which were refilled as needed.

After plant emergence, each pot was randomly thinned to three plants per pot to reduce any variance in growth due to uneven populations. All 0 through 24 wk samples received an application of propanil¹⁰ (3', 4'-dichloropropionanilide) and quinclorac¹¹ (3,7-dichloro-8-quinolinecarboxylic acid) at 4.48 and 0.56 kg ha⁻¹, respectively, for control of weedy plants emerging during the study. These treatments occurred on February 22 for the clay assay and on April 1 for the silt loam assay. Weed control in 52 wk samples was accomplished by hand weeding each pot as needed.

Rice plants in all pots were allowed to grow for 4 wk under natural light supplemented with artificial light on a 14 h day / 10 h night cycle. After 4 wk,

¹⁰ Stam™ M4 Herbicide, Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268

¹¹ Facet® 75DF Herbicide, BASF Corporation, Box 13528, Research Triangle Park, NC 27709

visual crop injury ratings were taken, the height of the plants recorded, and above-ground biomass was removed and weighed. In addition, the biomass from each pot was dried for 1 wk and re-weighed to obtain dry weight measurements.

Data from the pots with known herbicide rates were used to construct standard curves using appropriate non-linear regression. The statistical and graphing program SigmaPlot¹² was used for these procedures. The data for weight and height from standard curve treatments fit a first order exponential model:

$$Y = Y_0 e^{-bx}$$

In the equation, Y is the plant height or weight, Y₀ is the weight or height of plants with no herbicide present, b is a rate constant, x is the herbicide concentration, and e is the natural logarithm base number (~2.718). Regression results are shown in tables 3.5 through 3.7

For visual injury ratings, the data fit the form of exponential rise to max as follows:

$$\text{Injury} = I_{\max}(1 - e^{-bx})$$

where I_{max} is the upper asymptote of the curve which was set 100.1 to account for the 0 to 100% injury rating scale, e is the natural logarithm base, b is the rate constant, and x is the herbicide concentration. These regression results are shown in Table 3.8.

After constructing standard curves for each soil, herbicide, and variable, R-square values (Table 3.4) were used to determine which parameter provided the best estimate of herbicide concentration. Visual injury ratings provided the

¹² SigmaPlot®, Systat, Inc., San Jose, CA 95110

closest fit with R- square values being higher than the R-square values from the other regression analysis.

Injury ratings from the soil samples were entered into inverse versions of the visual injury regression equations to estimate the herbicide residue remaining in the soil collected from plots through the sampling period. The concentrations remaining in samples versus time were then plotted and fit to a first order decay model:

$$C_t = C_0(e^{-bt})$$

In this equation, C_t is the herbicide concentration at a given time, the concentration at time-zero (C_0) is derived from the regression analysis, b is the first order rate constant also derived from the regression analysis, e is the natural logarithm base number, and t is time measured in days. The rate constants (b) from the resulting regression equations were used to calculate half lives for the three herbicides under the four soil/moisture regimes by the equation:

$$T_{1/2} = \ln(2)/b$$

First-order equations approximately describe herbicide loss from the soil. However, this assumes a constant decay rate. Dissipation rates can vary with factors including temperature and moisture.

Results and Discussion

Imi-susceptible rice grown in soil samples previously treated with the selected herbicides displayed a variety of injury symptoms. Symptoms included twisting, stunting, stacking of nodes, chlorosis, and in some cases death following emergence. For the sake of continuity, data and results will only be shown for

visual injury ratings taken from the high application rates. Information pertaining to the low and medium rates, and the height and weight measurements for each treatment are included in the appendix.

In most circumstances, the concentration found in 0 wk samples did not equate with the rate applied. Data presented by Lehman et al. (1993) show 106.2% of the applied dose of flumetsulam being collected and assayed by high performance liquid chromatography (HPLC) at 0 WAA. This suggests that some error may exist in sampling, collection and storage techniques. It is also possible that residues detected at lower than the applied rate may be due to the assay not being able to detect concentrations above that lethal to the assay species. For example, at 0 WAA with imazapyr on the clay soil, we recovered only 70 and 45% (0.49 and 0.32 kg ai ha⁻¹) of the original herbicide concentration applied in flooded and non-flooded plots, respectively. However, visual injury ratings for the flooded clay test showed 85 to 100% injury while non-flooded clay visual injury ratings ranged from 80 to 90% injury.

Half lives for imazapyr were found to be shorter under flooded conditions as compared to non-flooded conditions on both soils (Table 3.9). A higher concentration of imazapyr was detected at 0 WAA under non-flooded conditions than flooded conditions on clay (Figure 3.1). However, the herbicide under flooded conditions dissipated rapidly. At 0 WAA, 75% of the herbicide applied was detected. By the six wk sampling, the detectable concentration was below 10%. The detected concentration at 0 WAA in non-flooded clay was at 50% of the applied rate, and was still detectable at 10% at 52 WAA.

The difference in imazapyr dissipation on the silt loam soil (Table 3.9) was more pronounced. Under both conditions, only 40% of the applied herbicide rate was detected at 0 WAA (Figure 3.2). At 24 WAA, the detectable concentration under flooded conditions was 5% and non-flooded was 30%. Between 24 and 52 WAA the concentration of imazapyr reached zero under flooded conditions while the imazapyr concentration in the non-flooded test remained above 20% of the original dose at 52 WAA.

Imazethapyr dissipation was also more rapid under flooded conditions as compared to non-flooded conditions on both soils. Half lives ranged from 5 d in flooded conditions to 28 d in non-flooded conditions on a Sharkey clay soil (Table 3.9). Based upon visual injury ratings, imazethapyr dissipated to a non-detectable at 3 WAA on the Sharkey clay under flooded conditions (Figure 3.3), while approximately 90% of the original dosage was detected at 3 WAA under non-flooded conditions. However, by 24 WAA, this concentration was found to be less than 10%.

Imazethapyr dissipation on the silt loam soil occurred at a slower rate when compared to imazethapyr on the clay soil based upon the half lives calculated (Table 3.9). Imazethapyr was detected at approximately 10% of the initial concentration by 6 WAA under flooded conditions (Figure 3.4) on the silt loam soil. In contrast, non-flooded imazethapyr concentrations were 55% of the applied rate at 6 WAA. The plant available concentration remained above zero during the 52 wk sampling period on the non-flooded DeWitt soil. This would

appear to agree with Johnson et al. (1993) who reported injury to corn, sorghum, rice, and cotton 52 WAA of imazethapyr.

Based upon the estimated half lives (Table 3.9), imazamox dissipated at a similar rate on Sharkey clay when subjected to a flooded or non-flooded environment. It should be noted however, that a higher herbicide concentration was detected at the 0 WAA under flooded conditions than under non-flooded conditions (Figure 3.5). At 3 WAA, the concentration for this herbicide-soil combination under both moisture conditions was at approximately 20% of the original concentration. Between the 6 and 12 wk samplings, the plant available concentration reached zero for both flooded and non-flooded conditions.

Unlike the clay soil, where half lives were similar for both moisture conditions, imazamox under flooded conditions was found to have a much shorter half life as compared to the half life of imazamox under non-flooded conditions on the silt loam soil (Table 3.9). Imazamox plant-available concentrations on the silt loam soil reached zero between 12 and 24 WAA (Figure 3.6) in flooded conditions, while imazamox subjected to non-flooded conditions on the loam soil dissipated more slowly, with residues detected at 25% of the original application rate of $0.087 \text{ kg ai ha}^{-1}$ at 52 WAA.

Both imazethapyr and imazapyr were found to have shorter half lives on the clay soil as compared to the silt loam soil (Table 3.9). Half lives of imazapyr on the silt loam soil were six to seven times longer than the half lives under the same conditions on the clay soil. As with imazapyr, the rate of imazethapyr dissipation was influenced by soil type. Half lives under flooded and non-flooded

conditions on the clay soil were found to be 18 and 100 d shorter than the comparable half lives on the silt loam soil. Imazamox half lives were similar between the clay and silt loam soils under flooded conditions at 8 and 16 d respectively. In contrast, the non-flooded half lives were found to be 270 d on the silt loam and only 13 d on the clay soil.

As with previous literature, imazamox generally dissipated more rapidly than imazethapyr (Aichelle and Penner 2005), and based on the results of these studies there is little risk of carryover injury to imi-susceptible rice following imazamox applied the previous year. Imazamox was found to persist for much longer than anticipated when applied to a silt loam soil and kept under non-flooded conditions (Table 3.9). Under flooded conditions, the other herbicides exhibited shorter or similar half lives when compared to the non-flooded half lives calculated for the same soil. Johnson et al. (1992, 1993) reported imazethapyr injury to rice at 52 WAA in the second year of a two year study. However, rice quickly outgrew injury symptoms and no yield reductions were reported.

Bioassays only detect plant available herbicide residues, whereas analytical methods can detect plant available and sorbed residues in soil. High organic matter and clay content soils, e.g. Sharkey clay, may cause a herbicide to be adsorbed and unavailable to microbial degradation while also making it unavailable to be taken up by plants. Therefore, lower injury levels may be observed. This does not necessarily mean that herbicide dissipation occurs more rapidly in a soil with high clay and organic matter content, but that injury may be reduced because the plants are not exposed to the herbicide if it is sorbed to clay

and organic matter. Dissipation may in fact be slower in these situations as compared to a soil with a low clay and organic matter content such as a DeWitt silt loam.

Mangles (1991) demonstrated that the sorption of imidazolinones decreased with an increase in soil pH toward 7. Imidazolinones have five different charged species, which are present at various pHs. As a soil's pH is lowered from 6 to 3, the species predominating goes from a largely unsorbed, anionic form, to a form which is mostly uncharged. The amount of sorption is also determined by the amount of organic matter present. With the pH of our two soils being between 6.0 and 6.5, relatively little sorption differences should have occurred.

The half lives derived from these data (Table 3.9) generally do not agree with half lives found in previous literature regarding imidazolinone dissipation under non-flooded conditions. According to the New York State Department of Environmental Conservation (2003) imazamox has a half life in the range of 35 to 118 d derived from five field dissipation studies. Vischetti (2004) found imazamox half lives under non-flooded conditions to occur from 17.1 to 92.4 d compared to 8 to 270 d found in these studies depending upon soil type and moisture regime. Imazethapyr half lives have been recorded by Goetz et al. (1990) between 2.6 and 10.6 months (78-318d) compared with 5 to 128 d found in these studies. Our results showed imazapyr to have a half life between 8 and 539 d whereas Tu et al. (2001) showed half lives ranging from one to five months (30-150 d) and Wang et al. (2005) reported 22 to 36 d. The half lives produced by

Vischetti and Goetz were derived from laboratory techniques. Aichelle and Penner (2005) state that the field dissipation rates of imazaquin and imazethapyr were greater than the dissipation rates in laboratory studies. This may explain some of the disparity between previously presented half lives and those presented here.

Conclusions

High injury ratings were expected and observed with applications of imazapyr. Imazapyr was included not for screening for use in imi-resistant rice production system, but to provide a benchmark for detection of imidazolinone persistence as well as dissipation.

While Shaner (1991) reported virtually no dissipation of imazapyr and imazethapyr in anaerobic conditions in laboratory situations, we found that flooded conditions increased the dissipation rates for the selected imidazolinones. While a typical flooded rice field soil is oxygen deficient, or hypoxic, it technically is not anaerobic. A thin oxidized layer is present at the soil surface due to floodwaters generally having relatively high concentrations of O₂, low densities of O₂-consuming organisms present in fields, photosynthetic O₂ production by algae and the mixing of air and water by wind movement (Scott et al. 2003).

The herbicides were applied without physical incorporation in these studies, and would have been at or near the soil / water interface or oxidized region. This region could provide the oxygen for normal aerobic microbial degradation, and may actually stimulate the breakdown of these chemicals.

Sciumbato et al (2003) found that imazethapyr became more plant available as soil moisture increased. It would therefore be likely that the herbicide would also be more available to microbes and increased dissipation would occur. Streck (2005) stated that soil moisture is a more critical factor than soil temperature for microbial degradation of herbicides that require microbes for degradation. During the summer months when temperatures are high and flooded rice fields are adequately moist, microbial degradation would occur at a rapid rate. The three factors stated above may explain why dissipation in flooded environments may occur more quickly than in non-flooded situations.

Leaching is another pathway for herbicide dissipation. However, Johnson et al (2000) reported that imidazolinone leaching in field studies was minor and that concentrations were below detectable levels. In addition, soils used for rice production generally have an impermeable layer which not only stops the leaching of flood waters, but also the leaching and loss of herbicides. For these reasons, it is thought that leaching was responsible for little or no dissipation in these studies.

Imazamox and imazethapyr dissipated quite rapidly under flooded conditions at both locations when compared to the non-flooded tests. Based upon the half lives calculated under these conditions (Table 3.9), it would be expected that little or no injury would occur to an imi-susceptible rice crop planted the year following an imi-resistant rice crop. However, this rotation is not recommended due to the risk of outcrossing of imi resistant rice with red rice, and due to the risk of exerting a high selection pressure on ALS-resistant biotypes already present.

Table 3.1. Characteristics of soils used in imidazolinone dissipation studies^a

<u>Location</u>	<u>Series</u>	<u>pH</u>	<u>Organic matter</u>	<u>Sand</u>	<u>Silt</u>	<u>Clay</u>	<u>Texture class</u>
			-----%				
DREC ^b	Sharkey	6.5	2.0	9.8	31.1	59.1	Clay
MRRF ^c	DeWitt	6.0	1.0	21.3	67.2	11.5	Silt loam

^aSampled from upper five to eight centimeters

^bDelta Research and Extension Center at Portageville, Mo

^cMissouri Rice Research Farm located near Glennonville, Mo

Table 3.2. Herbicide rates applied to flooded and non-flooded bare ground imidazolinone dissipation tests. Rates are shown in kg ai ha⁻¹.

<u>Rate^d</u>	<u>Herbicide Rate (kg ai ha⁻¹)</u>		
	<u>Imazamox</u>	<u>Imazethapyr</u>	<u>Imazapyr^e</u>
1/2	0.022	0.035	0.175
1	0.045	0.070	0.350
2	0.087	0.140	0.700

^dBased upon rates for a single application. Imazethapyr requires two applications per year of the 1x rate. For this study, imazamox was assumed to have split applications if it were to be labeled as a primary herbicide in imi-resistant rice.

^eDue to imazapyr not being labeled for crop use, a '1x' rate was selected from a range of rates listed on the label.

Table 3.3. Listing of treatments applied to previously untreated soil to develop standard curves for comparison to soil samples collected from field tests. Rates are shown in kg ai ha⁻¹

<u>Rate</u>	<u>Herbicide Rate (kg ai ha⁻¹)</u>		
	<u>Imazamox</u>	<u>Imazethapyr</u>	<u>Imazapyr</u>
2x	0.0450	0.0700	0.3504
1x	0.0220	0.0350	0.1752
1/2x	0.0110	0.0176	0.0876
1/4x	0.0056	0.0088	0.0438
1/8x	0.0028	0.0044	0.0219
1/16x	0.0014	0.0022	0.0110
1/32x	0.0007	0.0011	0.0055

Table 3.4. R-square values from standard curves of herbicide rate versus rice response.

<u>Herbicide</u>	<u>Soil</u>	<u>Visual</u>	<u>Dry Wt.</u>	<u>Fresh Wt.</u>	<u>Height</u>
Imazamox	Clay	0.8998	0.8079	0.8496	0.8279
	Loam	0.9126	0.5555	0.5759	0.5266
Imazethapyr	Clay	0.7204	0.3933	0.4276	0.5327
	Loam	0.5278	0.4254	0.4527	0.3893
Imazapyr	Clay	0.9313	0.8615	0.8897	0.9025
	Loam	0.9145	0.6375	0.6523	0.6276

Table 3.5. Regression parameters for fresh weight standard curves for imazamox, imazethapyr, and imazapyr on Sharkey clay (Clay) and DeWitt silt loam (Loam) soils. Parameters were used to construct standard curves following the equation^f: $F = F_0(e^{-bx})$

Herbicide	Soil	F ₀ term	B term	P-value	R-square
Imazamox	Clay	1.5126	1.5280	<0.0001	0.8079
	Loam	0.9892	1.9779	0.0050	0.5555
Imazethapyr	Clay	1.4509	0.8507	0.0061	0.3933
	Loam	0.8197	4.1083	0.0479	0.4254
Imazapyr	Clay	1.4183	7.8091	<0.0001	0.8615
	Loam	0.7214	9.0675	0.0080	0.6375

^fF represents plant fresh weight, F₀ refers to height of plants grown with no herbicide present, e is the algebraic number e, b is the b term from the regression analysis or decay constant and x is the herbicide concentration.

Table 3.6. Regression parameters from dry weight standard curves for imazamox, imazethapyr, and imazapyr on Sharkey clay (Clay) and DeWitt silt loam (Loam) soils. Parameters were used to construct standard curves following the equation^g: $D = D_0(e^{-bx})$

Herbicide	Soil	D ₀ term	B term	P-value	R-square
Imazamox	Clay	0.3679	1.7276	<0.0001	0.8496
	Loam	0.2308	2.1587	0.0042	0.5759
Imazethapyr	Clay	0.3412	0.9114	0.0039	0.7204
	Loam	0.1891	5.8772	0.0418	0.5278
Imazapyr	Clay	0.3385	6.6411	<0.0001	0.9313
	Loam	0.1703	13.0871	0.0063	0.9145

^gD represents plant dry weight, D₀ refers to height of plants grown with no herbicide present, e is the algebraic number e, b is the b term from the regression analysis or decay constant and x is the herbicide concentration.

Table 3.7. Regression parameters from plant height standard curves for imazamox, imazethapyr, and imazapyr on Sharkey clay (Clay) and DeWitt silt loam (Loam) soils. Parameters were used to construct standard curves following the equation^h: $H = H_0(e^{-bx})$

Herbicide	Soil	H ₀ term	B term	P-value	R-square
Imazamox	Clay	30.2597	1.1001	<0.0001	0.8279
	Loam	19.9287	1.3798	0.0026	0.5266
Imazethapyr	Clay	16.8363	0.7144	0.0004	0.5327
	Loam	28.9772	2.5146	0.0371	0.3893
Imazapyr	Clay	15.9136	5.9463	<0.0001	0.9025
	Loam	28.5312	5.0158	0.0036	0.6276

^h H represents plant height, H₀ refers to height of plants grown with no herbicide present, e is the algebraic number e, b is the b term from the regression analysis or decay constant and x is the herbicide concentration.

Table 3.8. Regression parameters for visual injury standard curves for imazamox, imazethapyr, and imazapyr on Sharkey clay (Clay) and DeWitt silt loam (Loam) soils. Parameters were used to construct standard curves following the equationⁱ: $Injury = 100.1(1 - e^{-bx})$

Herbicide	Soil	Decay constant (b)	P-value	R-square
Imazamox	Clay	1.4443	0.0127	0.8998
	Loam	2.5072	0.0002	0.9126
Imazethapyr	Clay	1.0663	0.2301	0.7204
	Loam	5.8772	0.0153	0.5278
Imazapyr	Clay	6.6411	<0.0001	0.9313
	Loam	13.0871	<0.0001	0.9145

ⁱ100.1 is the upper asymptote, set to account for the visual injury rating scale of 0 to 100, e is the algebraic number e, b is the b term from the regression analysis or decay constant and x is the herbicide concentration.

Table 3.9. Half lives \pm standard errors for imazamox, imazethapyr, and imazapyr under flooded and non-flooded conditions on two soils. Half lives rounded to the nearest day.

	Herbicide Half Life		
	<u>Imazamox</u>	<u>Imazethapyr</u>	<u>Imazapyr</u>
<u>DeWitt silt loam</u>			
Flooded	16 \pm 9	23 \pm 9	50 \pm 20
Non-Flooded	270 \pm 27	128 \pm 28	539 \pm 48
<u>Sharkey clay</u>			
Flooded	8 \pm 3	5 \pm 2	8 \pm 4
Non-Flooded	13 \pm 10	28 \pm 11	78 \pm 26

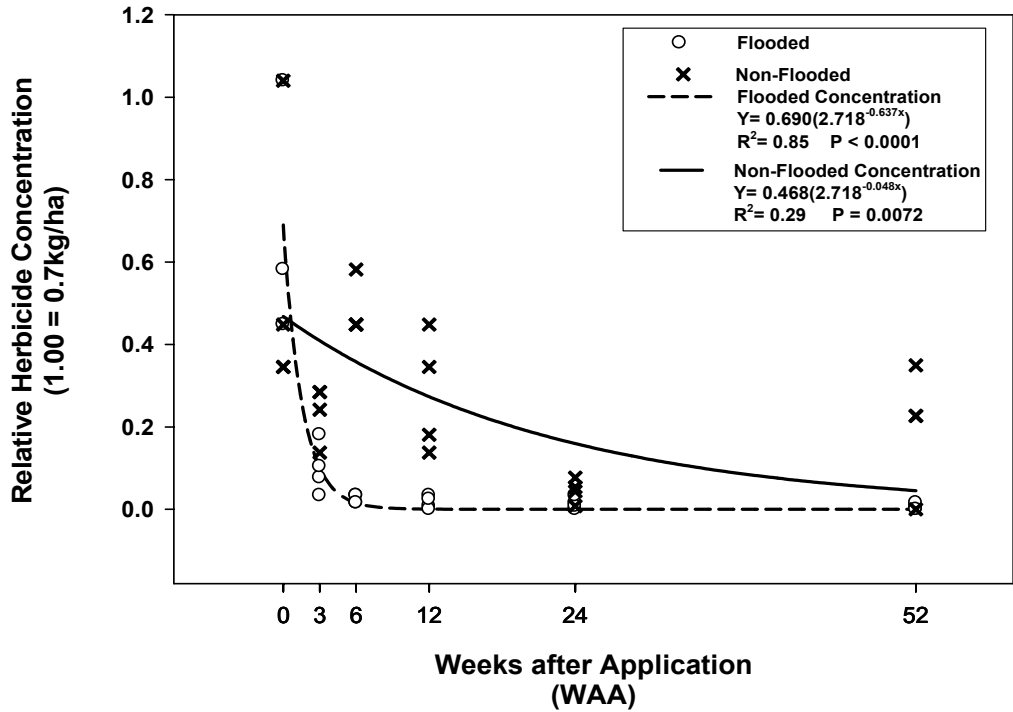


Figure 3.1. Relative imazapyr concentration remaining in a Sharkey clay soil at 0 through 52 wks after application of 0.7 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

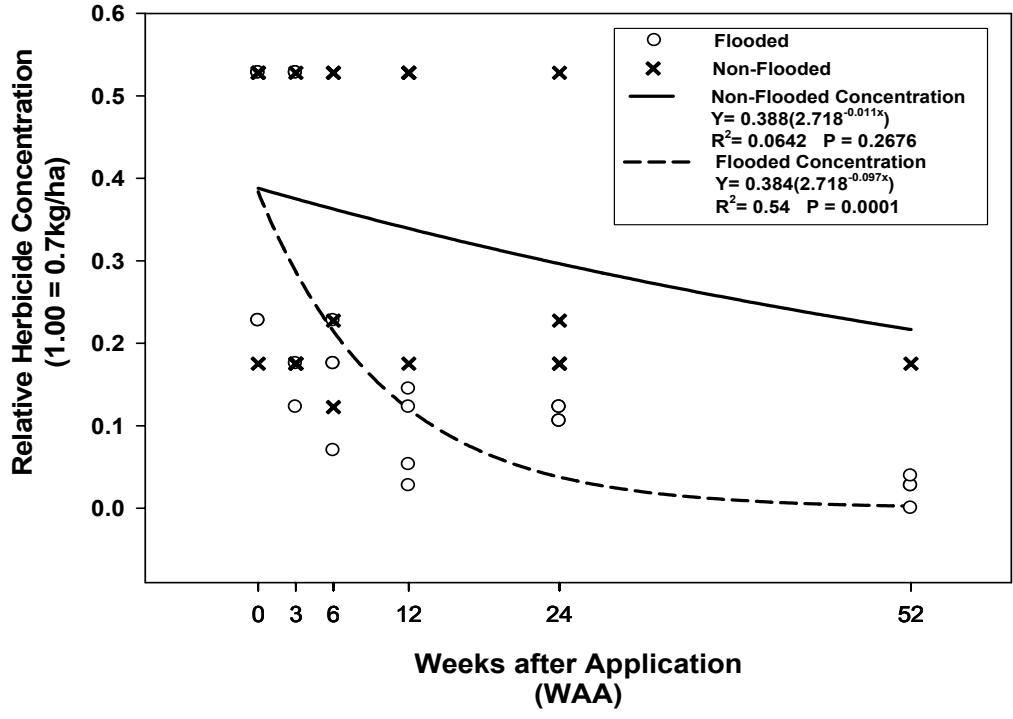


Figure 3.2. Relative imazapyr concentration remaining in a DeWitt silt loam soil at 0 through 52 wks after application of 0.7 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

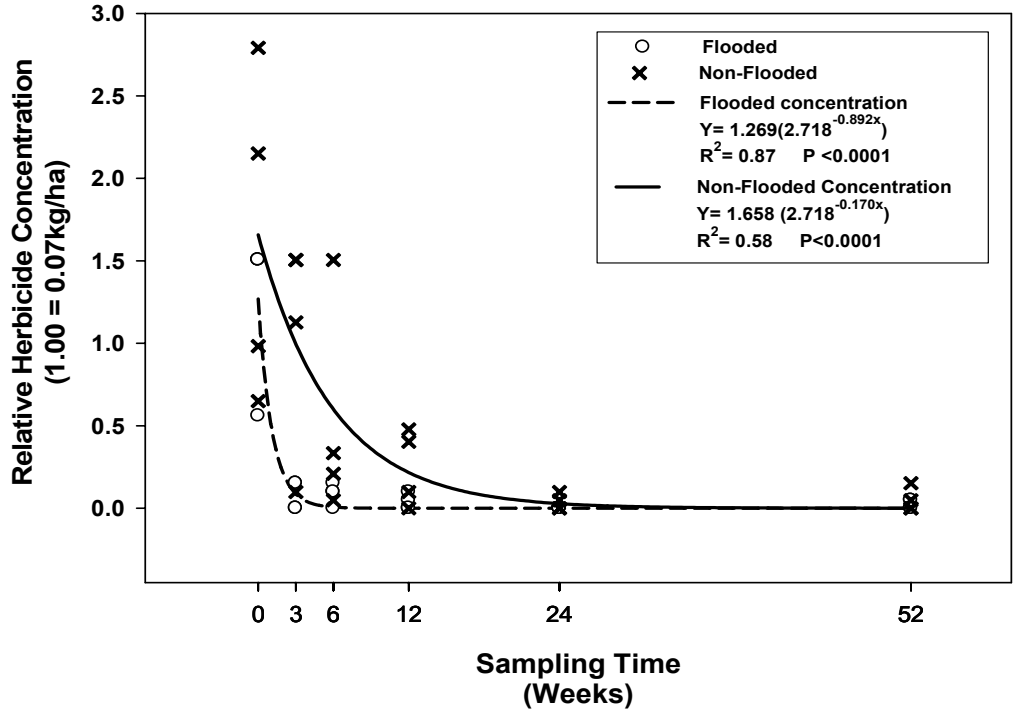


Figure 3.3. Relative imazethapyr concentration remaining in a Sharkey clay soil at 0 through 52 wks after application of 0.07 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

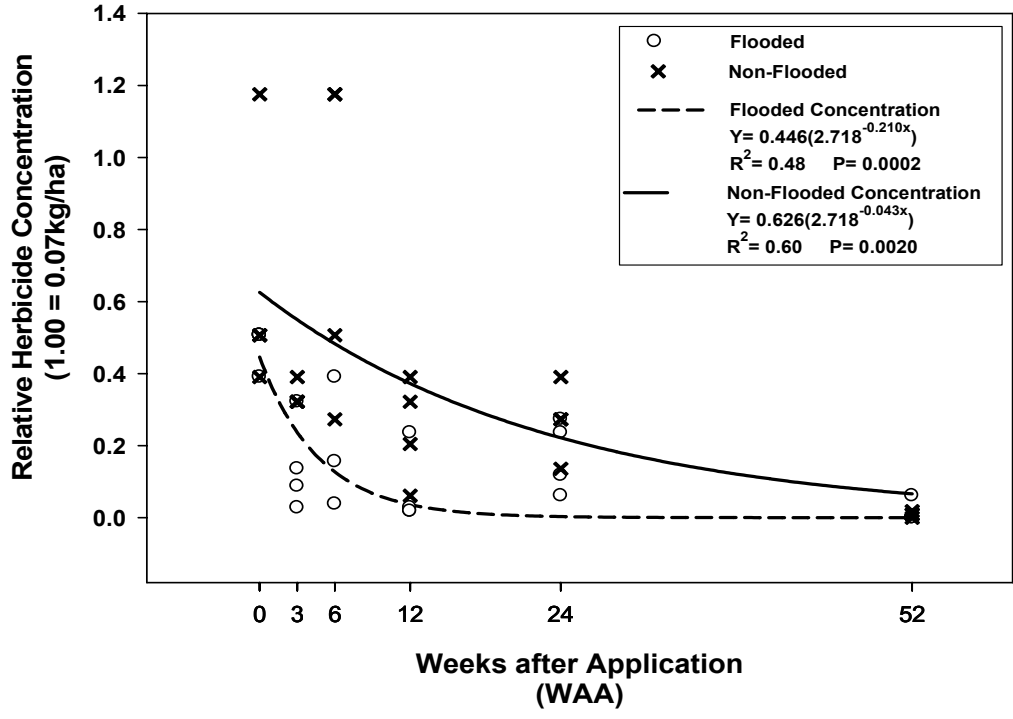


Figure 3.4. Relative imazethapyr concentration remaining in a DeWitt silt loam soil at 0 through 52 wks after application of 0.07 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

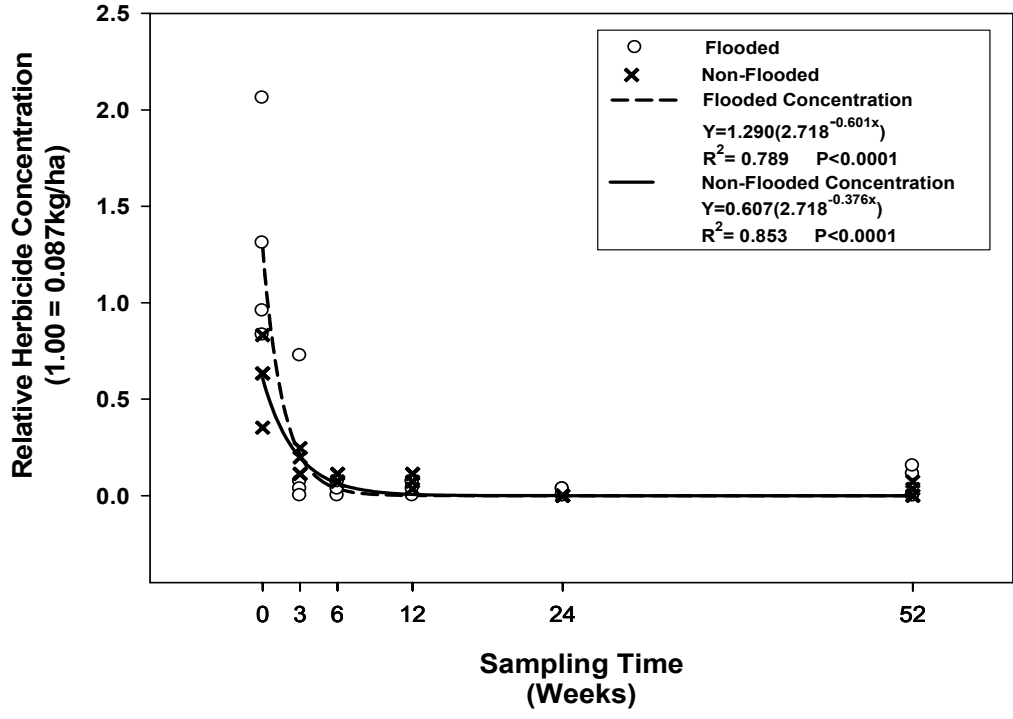


Figure 3.5. Relative imazamox concentration remaining in a Sharkey clay soil at 0 through 52 wks after application of 0.087 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

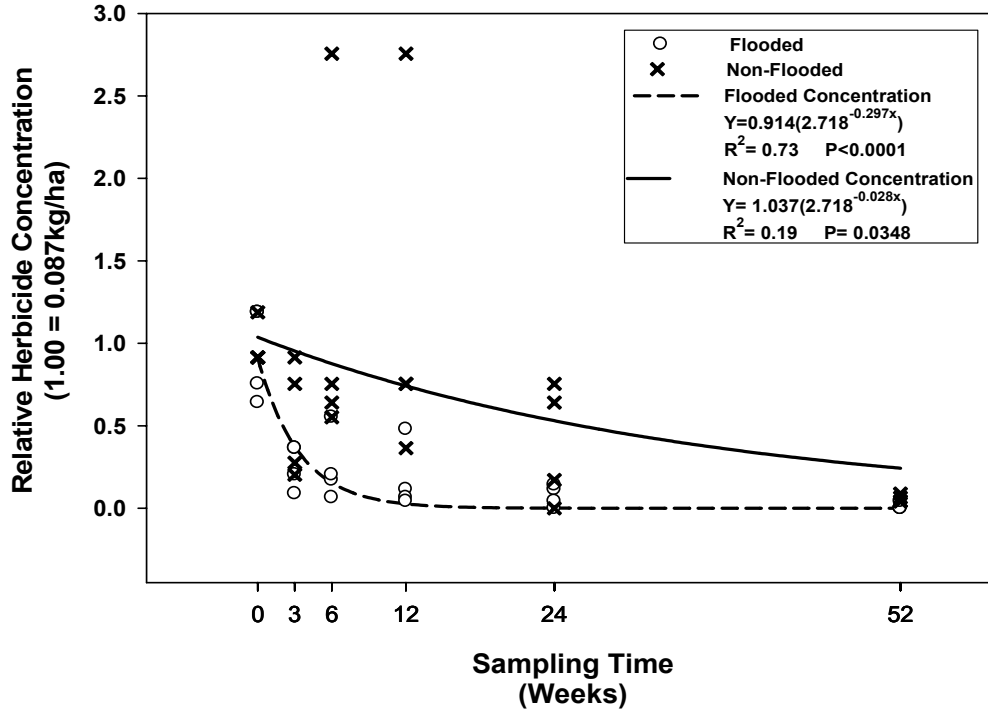


Figure 3.6. Relative imazamox concentration remaining in a DeWitt silt loam soil at 0 through 52 wks after application of 0.087 kg ai ha⁻¹. Relative concentrations are based upon visual injury ratings taken and compared to a standard curve with known herbicide concentrations. The flood was removed from flooded tests at approximately 22 weeks.

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Chapter IV

Summary and Conclusions

Two studies were conducted to determine the risks associated with the use of imidazolinone (IMI) herbicides used for red rice (*Oryza sativa*) control in imi-resistant (IR) rice (*Oryza sativa*). The objective of the first study was to determine if imazamox could be used safely as a shorter carryover option compared to imazethapyr in IR rice. Secondary objectives were to determine application rates, application timings and combinations of imazethapyr and imazamox which would cause the least injury to a non-IR rice crop planted the year following application. The second study focused on determining the dissipation rates and half lives of imazamox, imazethapyr and imazapyr in flooded conditions and comparing to the dissipation rates and half lives under non-flooded conditions.

Slight (<15%) injury was noted in a number of treatments; however, the injury level was well below levels that are usually associated with yield reduction. In addition in a few instances, injury was sometimes associated with lower herbicide rates, while higher herbicide rates did not cause injury. Some injury may have been due to random variability in rice growth and not necessarily damage from herbicide carryover.

When the dissipation of IMI herbicides were monitored over a one-year period, the plant-active levels of imazethapyr and imazamox generally declined rapidly with and generally caused little rice response after 12 weeks. Imazapyr,

which is known for being very persistent, did last longer than imazethapyr; however imazamox was less persistent than imazethapyr. All dissipation was more rapid on clay soils. Also, flooding increased dissipation of imidazolinone herbicides which would increase the chance of successfully growing non-IMI rice following imazethapyr or imazamox applied the previous year.

Based upon the results from both studies, imi-susceptible rice could be grown with little concern for crop safety the season following either imazethapyr or imazamox used for the control of red rice in IR rice. Some injury was observed during the carryover studies, but at very low levels. Likewise, the dissipation studies indicated that a rice flood reduced the persistence of the herbicides. Research conducted prior to these studies indicated that while imidazolinone carryover injury is typically low, it is not absent. Johnson et al. (1993) reported injury to corn, sorghum, rice, and cotton 52 WAA of imazethapyr. Johnson and Talbert (1996) reported imazethapyr residues in the soil injuring rotational crops some years but not others. Research by Baldwin (2003) also indicates that there may be an unpredictable injury potential to conventional rice (and fall planted oat) with the recommended rates of imazethapyr when applied to IR rice.

Appendix of Tables and Figures

Table A.1. Average injury ratings by treatment for Imidazolinone dissipation bioassay.

Average Visual Injury Ratings by Treatment									
(0=No injury, 100=Plant Death)				Sampling Time (Weeks After Application)					
Herbicide	Soil	Rate	Moisture	0	3	6	12	24	52
Imazamox	Sharkey Clay	0.045 kg ha ⁻¹	Flooded	81.25	20.00	6.25	7.50	2.50	8.75
		0.045 kg ha ⁻¹	Non-Flooded	57.50	21.25	12.50	11.25	0	12.50
		0.023 kg ha ⁻¹	Flooded	46.25	5.00	6.25	2.50	3.75	0.00
		0.023 kg ha ⁻¹	Non-Flooded	25.00	21.25	16.25	13.75	0.00	2.50
		0.011 kg ha ⁻¹	Flooded	17.50	15.00	16.25	2.50	1.25	3.75
		0.011 kg ha ⁻¹	Non-Flooded	15.00	43.75	11.25	12.50	6.25	2.50
	DeWitt Silt Loam	0.045 kg ha ⁻¹	Flooded	88.75	45.00	41.25	30.00	16.25	11.25
		0.045 kg ha ⁻¹	Non-Flooded	91.25	66.25	85.00	82.50	50.00	33.75
		0.023 kg ha ⁻¹	Flooded	61.25	23.75	13.75	5.00	6.25	2.50
		0.023 kg ha ⁻¹	Non-Flooded	85.00	62.50	53.75	72.50	52.50	8.75
		0.011 kg ha ⁻¹	Flooded	28.75	12.50	20.00	26.25	20.00	2.50
		0.011 kg ha ⁻¹	Non-Flooded	52.50	38.75	13.75	67.50	47.50	2.50
Imazethapyr	Sharkey Clay	0.07 kg ha ⁻¹	Flooded	71.25	7.50	8.75	3.75	1.00	2.50
		0.07 kg ha ⁻¹	Non-Flooded	75.00	60.00	33.75	21.25	3.75	3.75
		0.035 kg ha ⁻¹	Flooded	67.50	8.75	8.75	3.75	2.50	2.50
		0.035 kg ha ⁻¹	Non-Flooded	53.75	38.75	21.25	18.75	0.00	10.00
		0.018 kg ha ⁻¹	Flooded	25.00	8.75	6.25	2.50	1.25	11.25
		0.018 kg ha ⁻¹	Non-Flooded	36.25	59.50	17.50	15.00	1.25	2.50
	DeWitt Silt Loam	0.07 kg ha ⁻¹	Flooded	93.75	48.75	56.67	27.50	58.75	0.00
		0.07 kg ha ⁻¹	Non-Flooded	95.00	86.25	93.75	68.75	76.25	1.67
		0.035 kg ha ⁻¹	Flooded	87.50	27.50	26.25	15.00	20.00	2.50
		0.035 kg ha ⁻¹	Non-Flooded	88.75	37.50	73.75	75.00	56.25	7.50
		0.018 kg ha ⁻¹	Flooded	52.50	16.25	12.50	32.50	23.75	0.00
		0.018 kg ha ⁻¹	Non-Flooded	77.50	21.25	55.00	71.25	48.75	3.75
Imazapyr	Sharkey Clay	0.7 kg ha ⁻¹	Flooded	98.25	45.00	15.00	10.00	8.75	3.75
		0.7 kg ha ⁻¹	Non-Flooded	93.75	77.50	95.75	78.75	25.00	5.00
		0.35 kg ha ⁻¹	Flooded	92.5	11.25	18.75	5.00	0.00	0.00
		0.35 kg ha ⁻¹	Non-Flooded	90.00	81.25	78.75	72.50	10.00	0.00
		0.18 kg ha ⁻¹	Flooded	87.50	11.25	7.50	8.75	0.00	15.00
		0.18 kg ha ⁻¹	Non-Flooded	86.25	37.50	60.00	38.75	5.00	6.25
	DeWitt Silt Loam	0.7 kg ha ⁻¹	Flooded	97.50	92.50	83.75	61.25	77.50	8.75
		0.7 kg ha ⁻¹	Non-Flooded	97.50	92.50	93.75	97.50	93.75	6.67
		0.35 kg ha ⁻¹	Flooded	93.75	71.25	51.25	37.50	38.75	11.25
		0.35 kg ha ⁻¹	Non-Flooded	93.75	92.50	97.50	90.00	86.25	40.00
		0.18 kg ha ⁻¹	Flooded	82.5	48.75	26.25	17.50	33.75	6.25
		0.18 kg ha ⁻¹	Non-Flooded	92.50	75.00	78.75	88.75	78.75	11.67

Table A.2. Average plant fresh weight by treatment for Imidazolinone dissipation bioassay.

Average Fresh Weight (g) per Plant by Treatment									
Herbicide	Soil	Rate	Moisture	Sampling Time (Weeks After Application)					
				0	3	6	12	24	52
Imazamox	Sharkey Clay	0.045 kg ha ⁻¹	Flooded	0.057	0.413	0.536	0.299	0.303	0.123
		0.045 kg ha ⁻¹	Non-Flooded	0.103	0.178	0.221	0.203	0.175	0.127
		0.023 kg ha ⁻¹	Flooded	0.172	0.357	0.454	0.283	0.334	0.173
		0.023 kg ha ⁻¹	Non-Flooded	0.229	0.213	0.214	0.186	0.188	0.132
		0.011 kg ha ⁻¹	Flooded	0.299	0.313	0.343	0.332	0.340	0.123
		0.011 kg ha ⁻¹	Non-Flooded	0.235	0.233	0.285	0.190	0.213	0.106
	DeWitt Silt Loam	0.045 kg ha ⁻¹	Flooded	0.034	0.230	0.167	0.134	0.271	0.153
		0.045 kg ha ⁻¹	Non-Flooded	0.029	0.092	0.060	0.099	0.198	0.059
		0.023 kg ha ⁻¹	Flooded	0.124	0.442	0.305	0.186	0.340	0.154
		0.023 kg ha ⁻¹	Non-Flooded	0.089	0.204	0.210	0.141	0.165	0.070
		0.011 kg ha ⁻¹	Flooded	0.344	0.467	0.238	0.156	0.284	0.129
		0.011 kg ha ⁻¹	Non-Flooded	0.150	0.179	0.336	0.188	0.296	0.102
Imazethapyr	Sharkey Clay	0.07 kg ha ⁻¹	Flooded	0.068	0.306	0.503	0.294	0.318	0.159
		0.07 kg ha ⁻¹	Non-Flooded	0.061	0.144	0.176	0.174	0.218	0.081
		0.035 kg ha ⁻¹	Flooded	0.150	0.373	0.444	0.298	0.341	0.183
		0.035 kg ha ⁻¹	Non-Flooded	0.107	0.215	0.191	0.188	0.234	0.101
		0.018 kg ha ⁻¹	Flooded	0.253	0.369	0.485	0.292	0.353	0.091
		0.018 kg ha ⁻¹	Non-Flooded	0.182	0.130	0.321	0.181	0.178	0.112
	DeWitt Silt Loam	0.07 kg ha ⁻¹	Flooded	0.029	0.186	0.107	0.152	0.137	0.153
		0.07 kg ha ⁻¹	Non-Flooded	0.013	0.044	0.093	0.109	0.111	0.113
		0.035 kg ha ⁻¹	Flooded	0.036	0.280	0.188	0.163	0.254	0.146
		0.035 kg ha ⁻¹	Non-Flooded	0.033	0.212	0.133	0.086	0.183	0.080
		0.018 kg ha ⁻¹	Flooded	0.213	0.463	0.334	0.131	0.264	0.153
		0.018 kg ha ⁻¹	Non-Flooded	0.119	0.295	0.194	0.112	0.150	0.084
Imazapyr	Sharkey Clay	0.7 kg ha ⁻¹	Flooded	0.018	0.176	0.331	0.272	0.238	0.123
		0.7 kg ha ⁻¹	Non-Flooded	0.022	0.079	0.030	0.059	0.124	0.102
		0.35 kg ha ⁻¹	Flooded	0.027	0.319	0.393	0.293	0.338	0.181
		0.35 kg ha ⁻¹	Non-Flooded	0.036	0.079	0.083	0.100	0.156	0.091
		0.18 kg ha ⁻¹	Flooded	0.051	0.349	0.417	0.263	0.343	0.128
		0.18 kg ha ⁻¹	Non-Flooded	0.034	0.171	0.171	0.139	0.201	0.112
	DeWitt Silt Loam	0.7 kg ha ⁻¹	Flooded	0.010	0.027	0.045	0.050	0.060	0.060
		0.7 kg ha ⁻¹	Non-Flooded	0.026	0.015	0.024	0.015	0.027	0.036
		0.35 kg ha ⁻¹	Flooded	0.020	0.068	0.111	0.108	0.166	0.081
		0.35 kg ha ⁻¹	Non-Flooded	0.011	0.020	0.027	0.025	0.045	0.033
		0.18 kg ha ⁻¹	Flooded	0.058	0.158	0.209	0.136	0.143	0.112
		0.18 kg ha ⁻¹	Non-Flooded	0.020	0.074	0.082	0.045	0.084	0.052

Table A.3. Average plant height by treatment for Imidazolinone dissipation bioassay.

Average Plant Height (cm) by Treatment									
Herbicide	Soil	Rate	Moisture	Sampling Time (Weeks After Application)					
				0	3	6	12	24	52
Imazamox	Sharkey Clay	0.045 kg ha ⁻¹	Flooded	6.57	24.20	31.54	22.68	25.78	17.33
		0.045 kg ha ⁻¹	Non-Flooded	10.72	20.53	18.64	20.25	20.92	18.23
		0.023 kg ha ⁻¹	Flooded	14.45	26.17	29.45	22.78	26.13	21.23
		0.023 kg ha ⁻¹	Non-Flooded	20.06	22.12	19.94	20.01	20.71	17.17
		0.011 kg ha ⁻¹	Flooded	23.63	23.39	25.02	24.35	26.17	17.63
		0.011 kg ha ⁻¹	Non-Flooded	21.45	18.84	21.39	20.88	20.39	17.52
	DeWitt Silt Loam	0.045 kg ha ⁻¹	Flooded	3.99	13.51	12.93	13.82	19.97	17.63
		0.045 kg ha ⁻¹	Non-Flooded	3.62	8.22	10.35	11.33	15.69	9.92
		0.023 kg ha ⁻¹	Flooded	8.15	20.39	17.85	17.06	22.33	18.35
		0.023 kg ha ⁻¹	Non-Flooded	8.24	15.03	17.30	11.68	15.62	10.38
		0.011 kg ha ⁻¹	Flooded	16.31	21.38	17.13	15.66	21.53	15.38
		0.011 kg ha ⁻¹	Non-Flooded	12.25	14.78	21.24	16.12	20.08	12.07
Imazethapyr	Sharkey Clay	0.07 kg ha ⁻¹	Flooded	6.58	25.38	29.08	23.45	25.73	19.28
		0.07 kg ha ⁻¹	Non-Flooded	6.46	15.98	14.53	20.38	20.59	14.45
		0.035 kg ha ⁻¹	Flooded	10.20	26.75	27.42	23.12	25.43	20.43
		0.035 kg ha ⁻¹	Non-Flooded	12.17	19.10	17.93	20.38	22.13	15.36
		0.018 kg ha ⁻¹	Flooded	19.49	27.53	30.10	25.16	28.16	15.52
		0.018 kg ha ⁻¹	Non-Flooded	17.39	13.08	23.11	20.93	19.29	16.94
	DeWitt Silt Loam	0.07 kg ha ⁻¹	Flooded	2.22	12.84	8.91	13.73	13.36	16.01
		0.07 kg ha ⁻¹	Non-Flooded	2.87	5.49	8.93	10.02	11.55	11.51
		0.035 kg ha ⁻¹	Flooded	3.67	17.92	15.26	15.81	20.56	17.23
		0.035 kg ha ⁻¹	Non-Flooded	4.87	13.13	13.03	10.13	16.34	11.85
		0.018 kg ha ⁻¹	Flooded	12.18	23.17	21.11	14.03	19.76	17.58
		0.018 kg ha ⁻¹	Non-Flooded	10.78	18.26	13.62	13.23	14.88	11.52
Imazapyr	Sharkey Clay	0.7 kg ha ⁻¹	Flooded	1.65	11.78	21.28	21.23	20.06	16.99
		0.7 kg ha ⁻¹	Non-Flooded	2.97	8.60	4.59	6.45	12.13	13.01
		0.35 kg ha ⁻¹	Flooded	3.68	23.01	23.82	22.43	26.18	21.35
		0.35 kg ha ⁻¹	Non-Flooded	4.29	6.68	6.86	11.29	14.58	15.05
		0.18 kg ha ⁻¹	Flooded	5.72	25.71	26.76	22.13	27.63	17.69
		0.18 kg ha ⁻¹	Non-Flooded	5.22	16.88	12.59	15.23	18.52	17.61
	DeWitt Silt Loam	0.7 kg ha ⁻¹	Flooded	2.10	4.37	4.03	5.67	7.16	9.48
		0.7 kg ha ⁻¹	Non-Flooded	3.23	3.00	3.40	2.35	4.18	0.75
		0.35 kg ha ⁻¹	Flooded	2.15	6.68	11.97	11.02	13.51	11.61
		0.35 kg ha ⁻¹	Non-Flooded	1.98	2.73	5.43	5.08	7.01	5.63
		0.18 kg ha ⁻¹	Flooded	5.71	11.18	14.34	13.60	12.79	13.38
		0.18 kg ha ⁻¹	Non-Flooded	2.83	7.22	9.32	6.93	11.28	8.37

Table A.4. Average plant dry weight by treatment for Imidazolinone dissipation bioassay.

Average Plant Dry Weight (g) by Treatment									
Herbicide	Soil	Rate	Moisture	Sampling Time (Weeks After Application)					
				0	3	6	12	24	52
Imazamox	Sharkey Clay	0.045 kg ha ⁻¹	Flooded	0.012	0.094	0.117	0.070	0.074	0.035
		0.045 kg ha ⁻¹	Non-Flooded	0.022	0.049	0.053	0.050	0.042	0.037
		0.023 kg ha ⁻¹	Flooded	0.100	0.089	0.103	0.077	0.081	0.047
		0.023 kg ha ⁻¹	Non-Flooded	0.052	0.052	0.056	0.045	0.045	0.036
		0.011 kg ha ⁻¹	Flooded	0.045	0.071	0.088	0.085	0.084	0.030
		0.011 kg ha ⁻¹	Non-Flooded	0.042	0.060	0.073	0.068	0.051	0.030
	DeWitt Silt Loam	0.045 kg ha ⁻¹	Flooded	0.006	0.052	0.034	0.035	0.064	0.033
		0.045 kg ha ⁻¹	Non-Flooded	0.007	0.026	0.020	0.024	0.047	0.017
		0.023 kg ha ⁻¹	Flooded	0.024	0.097	0.073	0.051	0.081	0.030
		0.023 kg ha ⁻¹	Non-Flooded	0.020	0.047	0.050	0.037	0.043	0.020
		0.011 kg ha ⁻¹	Flooded	0.072	0.115	0.051	0.043	0.070	0.027
		0.011 kg ha ⁻¹	Non-Flooded	0.052	0.040	0.083	0.047	0.072	0.029
Imazethapyr	Sharkey Clay	0.07 kg ha ⁻¹	Flooded	0.017	0.070	0.113	0.073	0.077	0.041
		0.07 kg ha ⁻¹	Non-Flooded	0.013	0.033	0.042	0.041	0.051	0.025
		0.035 kg ha ⁻¹	Flooded	0.024	0.088	0.108	0.075	0.083	0.046
		0.035 kg ha ⁻¹	Non-Flooded	0.023	0.051	0.048	0.046	0.060	0.030
		0.018 kg ha ⁻¹	Flooded	0.050	0.086	0.111	0.073	0.086	0.026
		0.018 kg ha ⁻¹	Non-Flooded	0.042	0.041	0.079	0.045	0.043	0.031
	DeWitt Silt Loam	0.07 kg ha ⁻¹	Flooded	0.004	0.009	0.024	0.027	0.025	0.030
		0.07 kg ha ⁻¹	Non-Flooded	0.004	0.038	0.023	0.039	0.033	0.031
		0.035 kg ha ⁻¹	Flooded	0.006	0.068	0.045	0.043	0.065	0.031
		0.035 kg ha ⁻¹	Non-Flooded	0.008	0.049	0.032	0.024	0.043	0.021
		0.018 kg ha ⁻¹	Flooded	0.042	0.107	0.078	0.038	0.065	0.032
		0.018 kg ha ⁻¹	Non-Flooded	0.027	0.069	0.049	0.028	0.040	0.017
Imazapyr	Sharkey Clay	0.7 kg ha ⁻¹	Flooded	0.002	0.039	0.073	0.068	0.057	0.030
		0.7 kg ha ⁻¹	Non-Flooded	0.006	0.017	0.007	0.014	0.028	0.030
		0.35 kg ha ⁻¹	Flooded	0.005	0.074	0.091	0.074	0.081	0.016
		0.35 kg ha ⁻¹	Non-Flooded	0.010	0.016	0.018	0.023	0.036	0.029
		0.18 kg ha ⁻¹	Flooded	0.008	0.078	0.097	0.066	0.082	0.034
		0.18 kg ha ⁻¹	Non-Flooded	0.008	0.040	0.040	0.033	0.046	0.031
	DeWitt Silt Loam	0.7 kg ha ⁻¹	Flooded	0.002	0.006	0.008	0.012	0.015	0.011
		0.7 kg ha ⁻¹	Non-Flooded	0.005	0.005	0.008	0.004	0.008	0.000
		0.35 kg ha ⁻¹	Flooded	0.004	0.014	0.023	0.030	0.039	0.018
		0.35 kg ha ⁻¹	Non-Flooded	0.003	0.005	0.007	0.006	0.014	0.007
		0.18 kg ha ⁻¹	Flooded	0.010	0.035	0.049	0.036	0.034	0.017
		0.18 kg ha ⁻¹	Non-Flooded	0.005	0.017	0.019	0.013	0.022	0.016

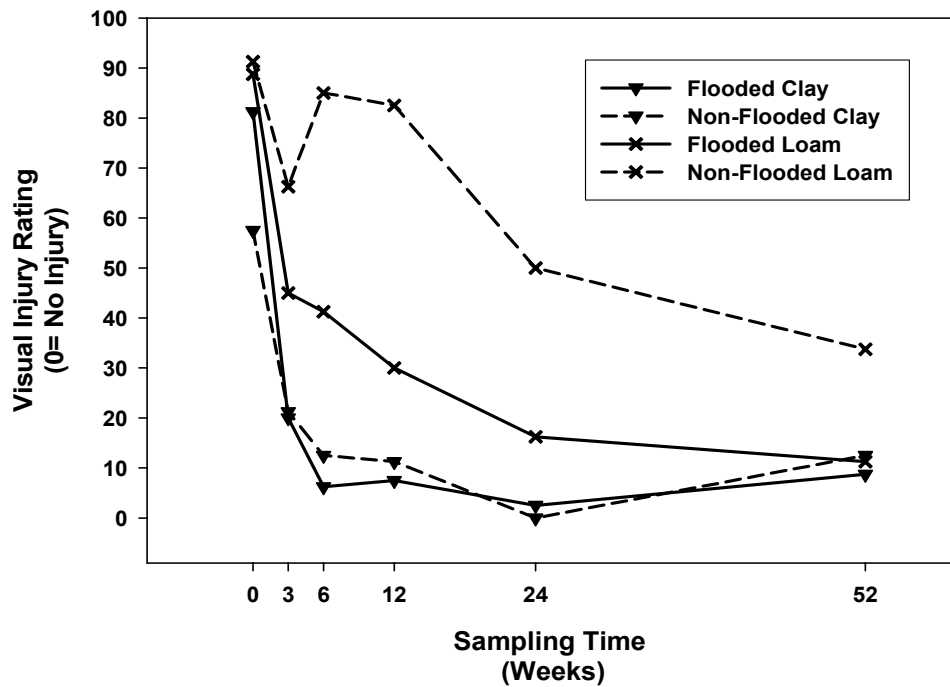


Figure A.1. Average visual injury ratings for 0.045 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

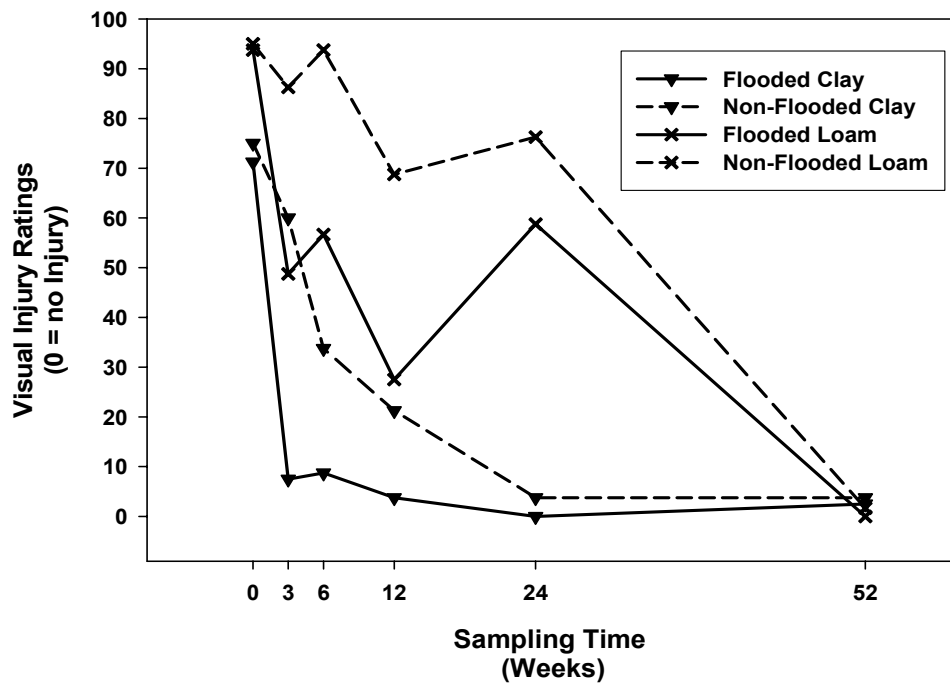


Figure A.2. Average visual injury ratings for 0.07 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

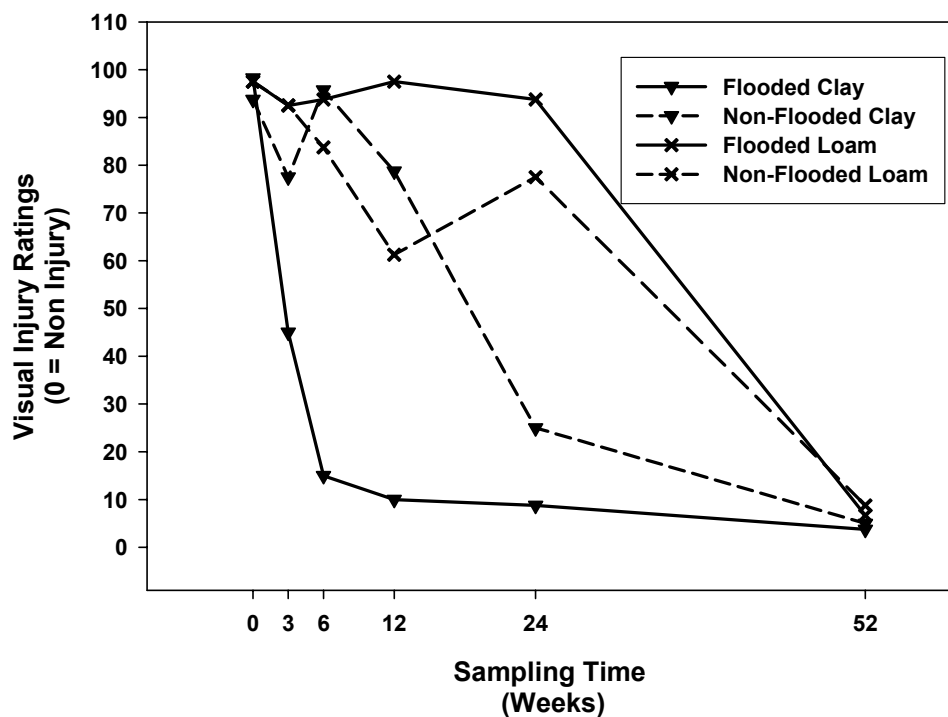


Figure A.3. Average visual injury ratings for 0.7 kg ai ha⁻¹ imazapyr taken from 0 to 52 weeks after application on two flooded and non-flooded soils.

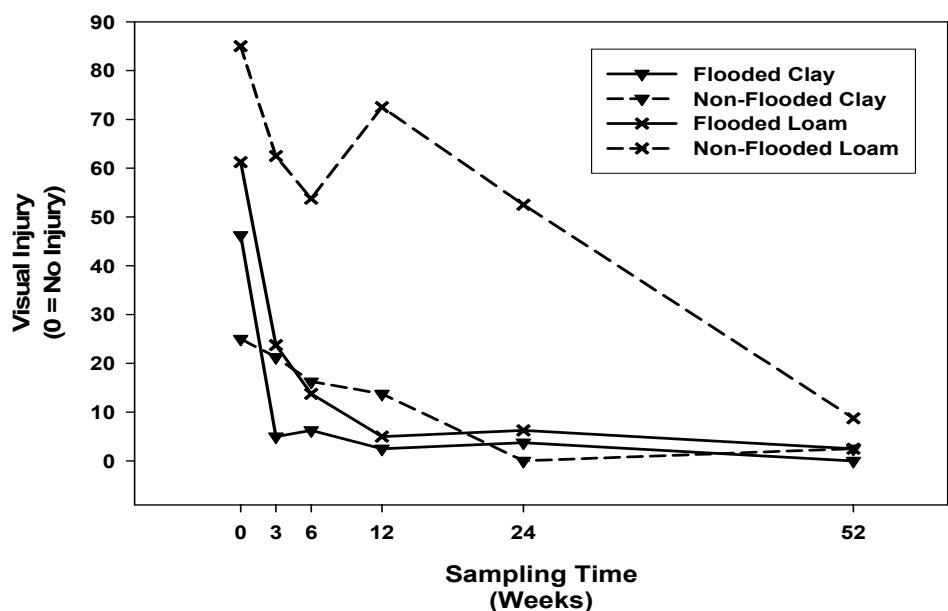


Figure A.4. Average visual injury ratings for 0.023 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

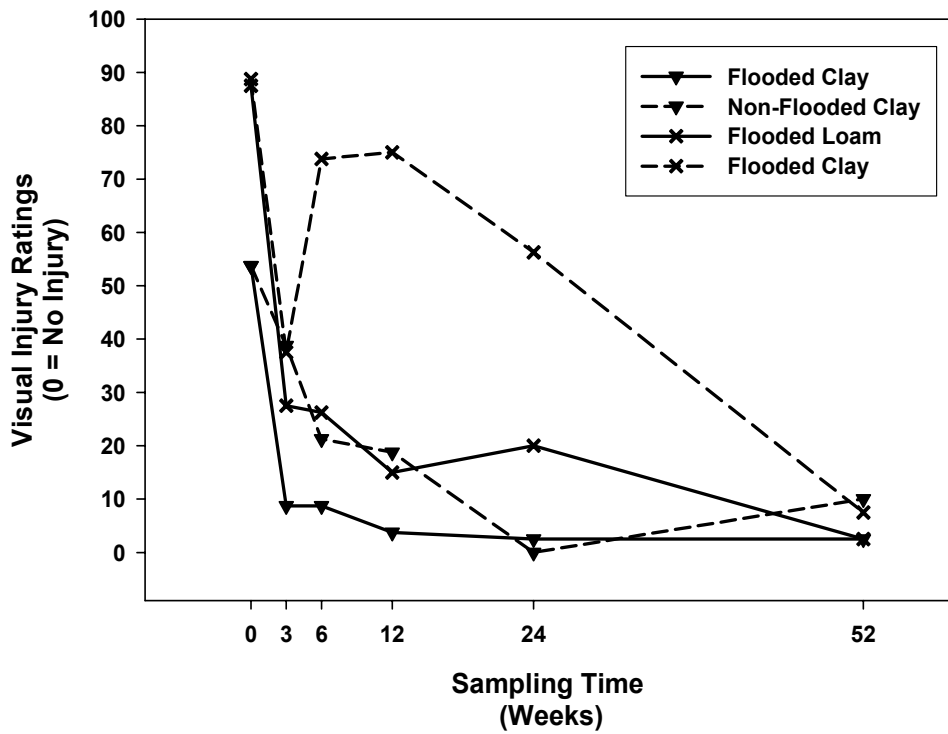


Figure A.5. Average visual injury ratings for 0.035 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

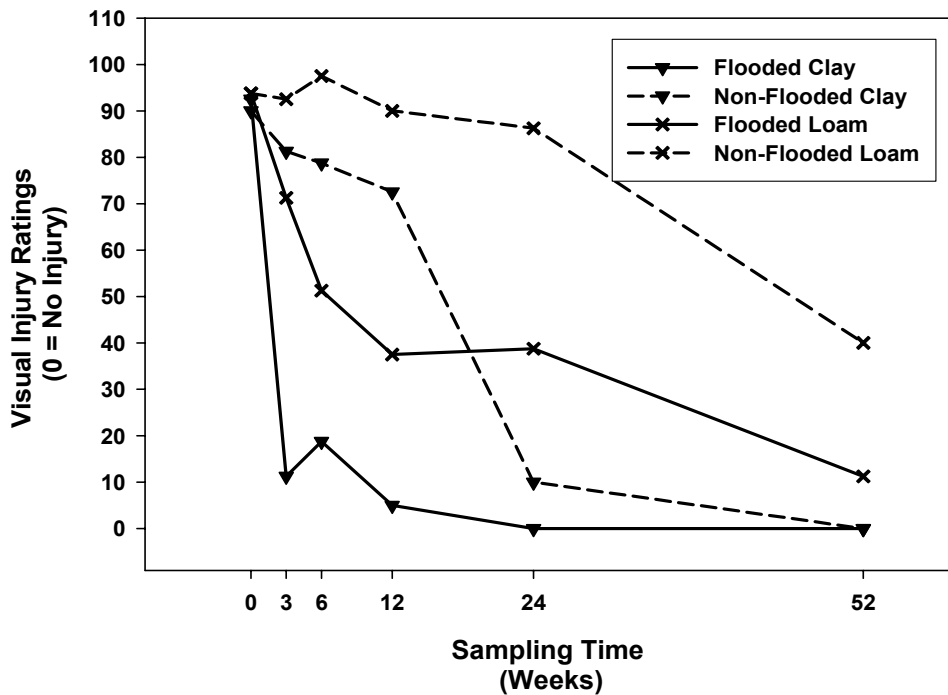


Figure A.6. Average visual injury ratings for 0.35 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

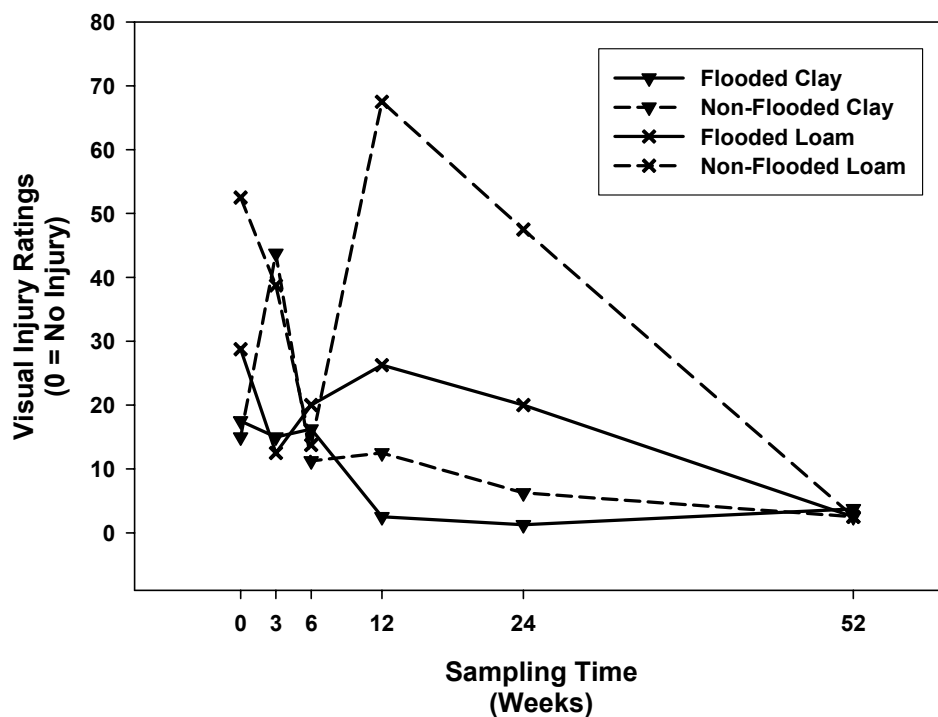


Figure A.7. Average visual injury ratings for 0.11 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

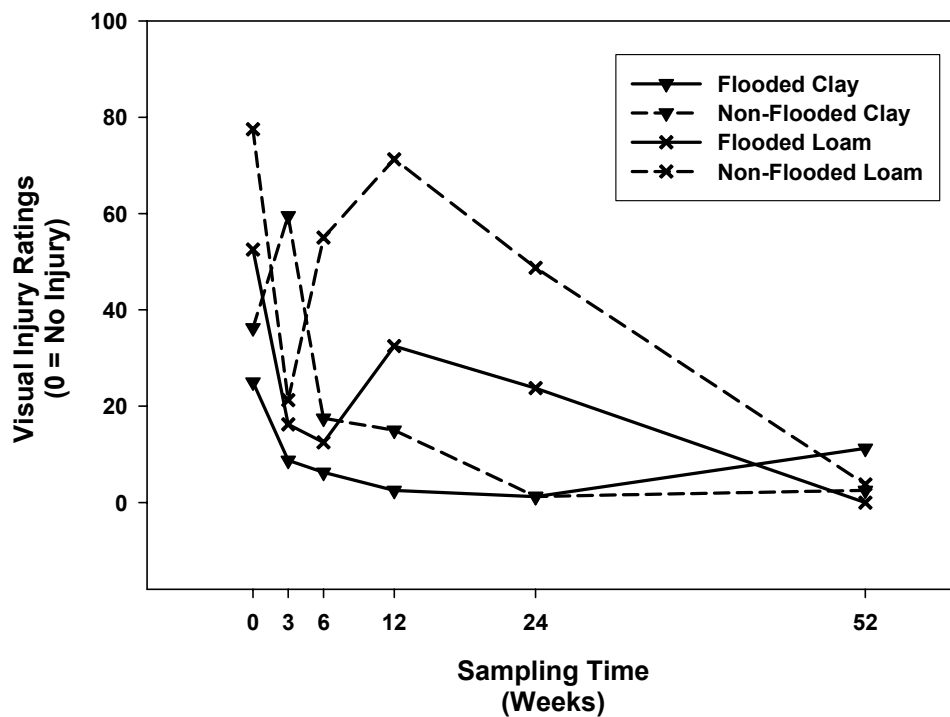


Figure A.8. Average visual injury ratings for 0.018 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

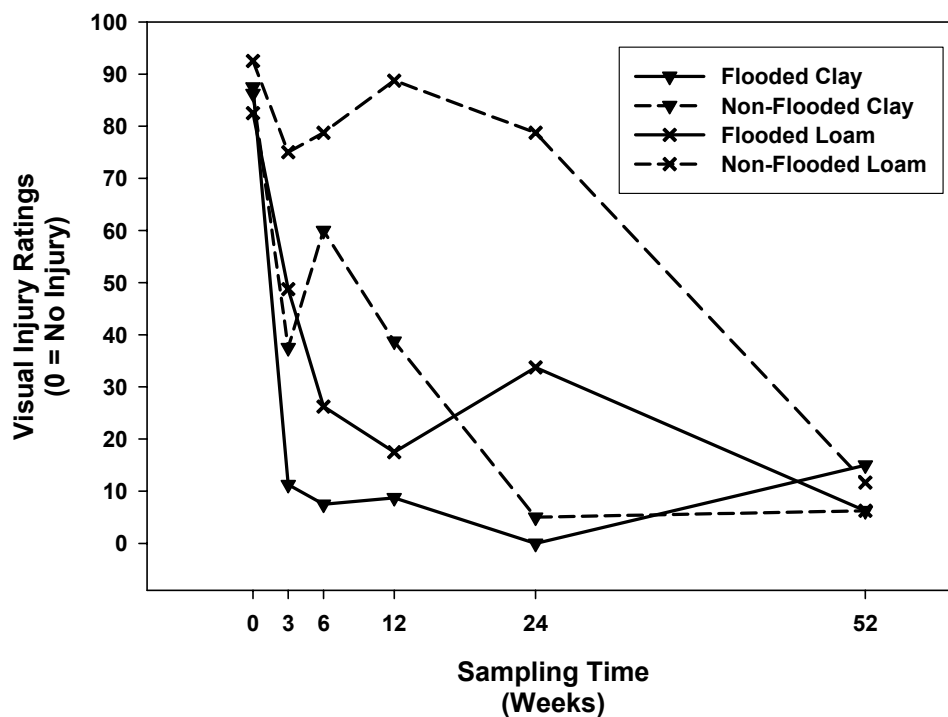


Figure A.9. Average visual injury ratings for 0.18 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

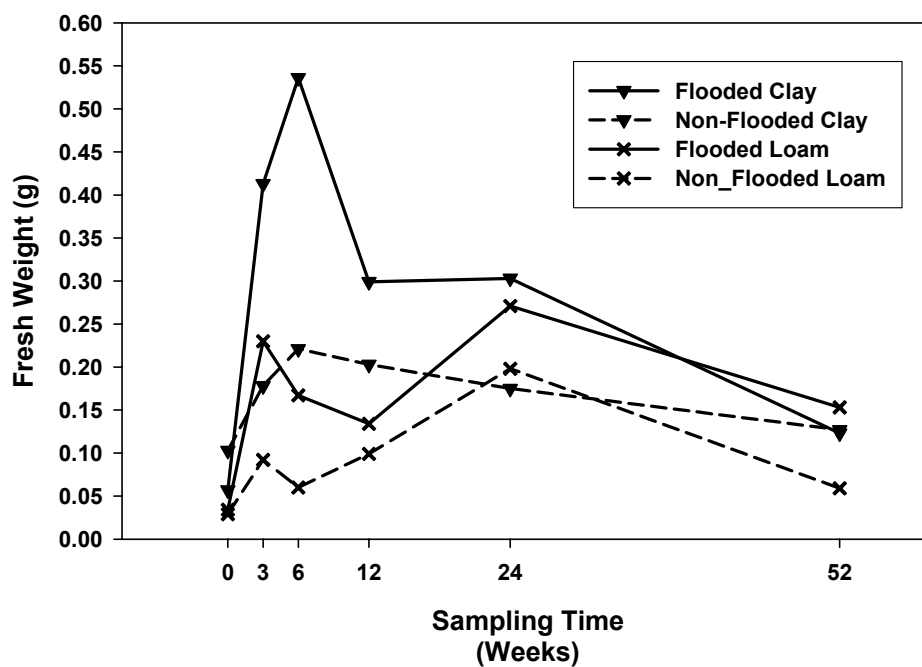


Figure A.10. Average fresh weight per rice plant from pots receiving 0.045 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

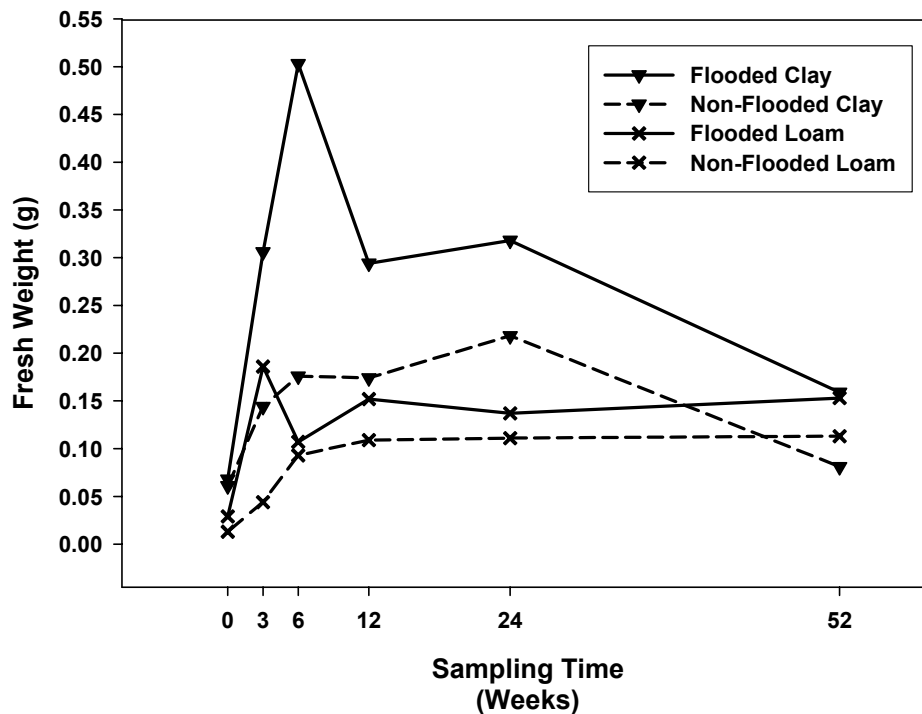


Figure A.11. Average fresh weight per rice plant from pots receiving $0.07 \text{ kg ai ha}^{-1}$ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

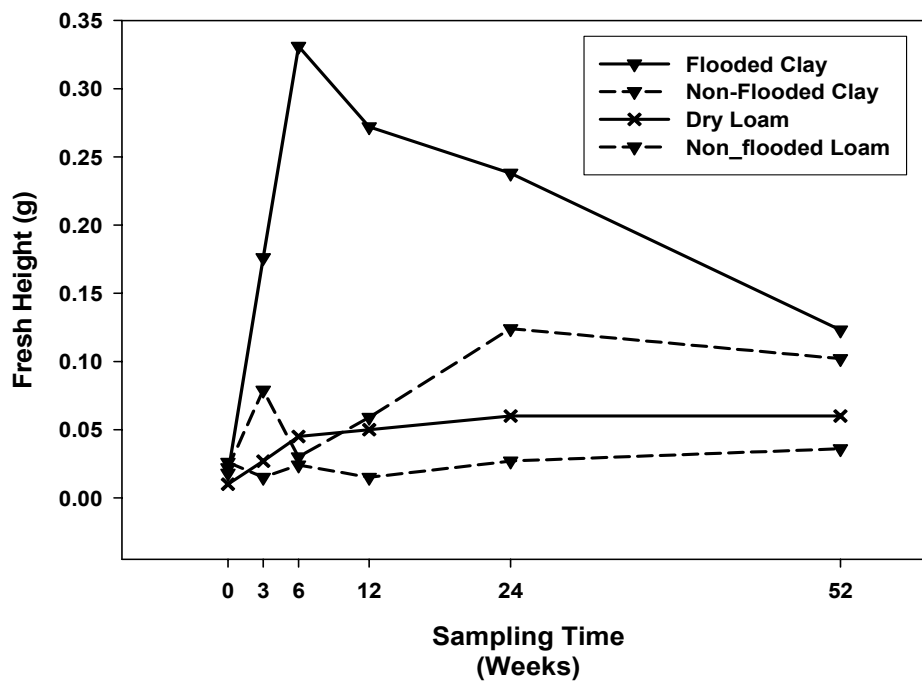


Figure A.12. Average fresh weight per rice plant from pots receiving $0.7 \text{ kg ai ha}^{-1}$ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

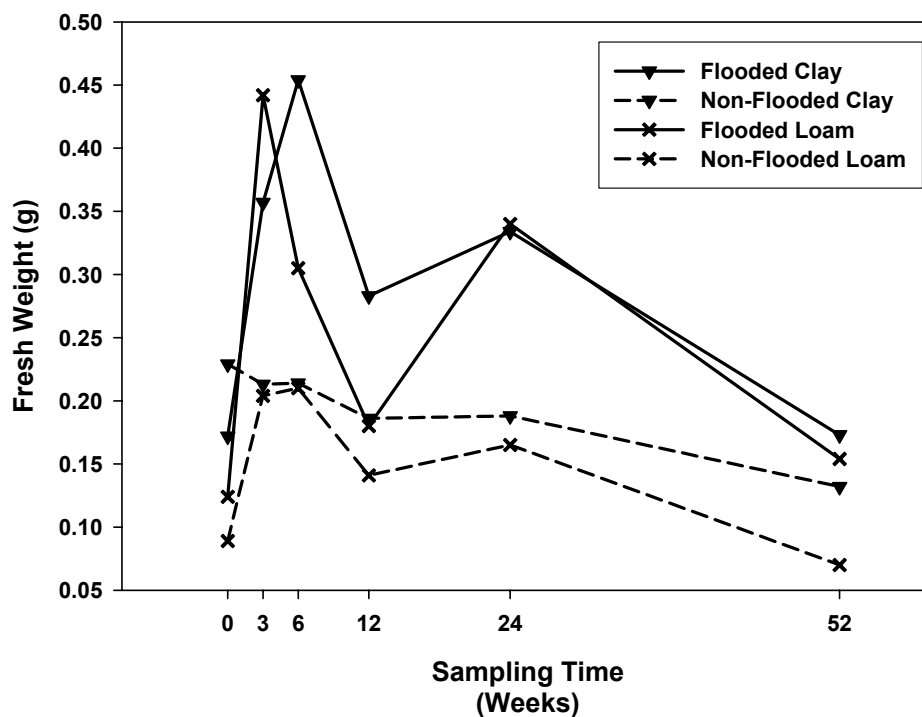


Figure A.13. Average fresh weight per rice plant from pots receiving 0.023 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

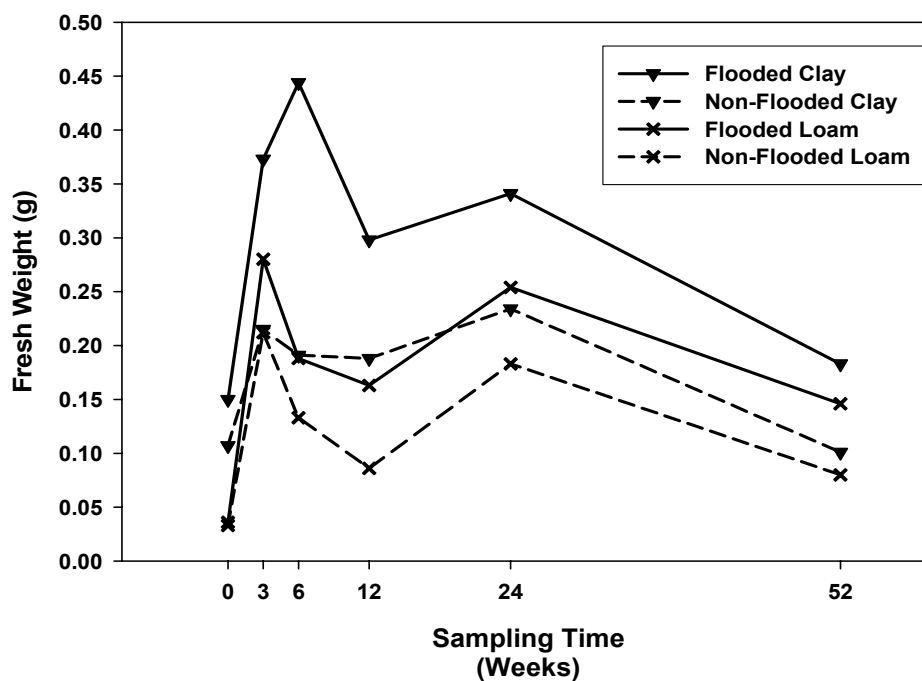


Figure A.14. Average fresh weight per rice plant from pots receiving 0.035 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

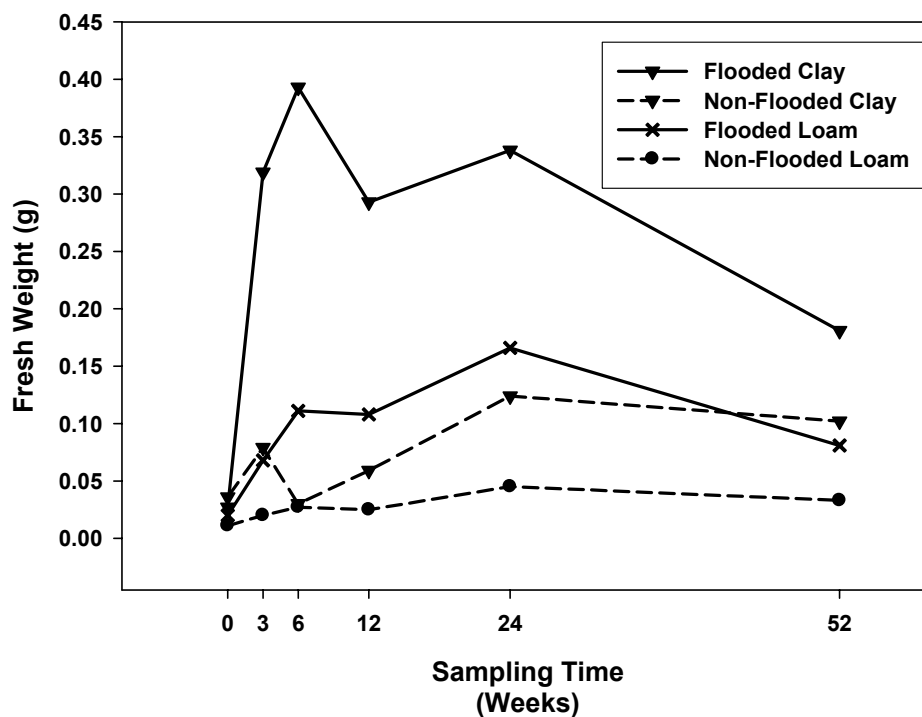


Figure A.15. Average fresh weight per rice plant from pots receiving 0.35 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

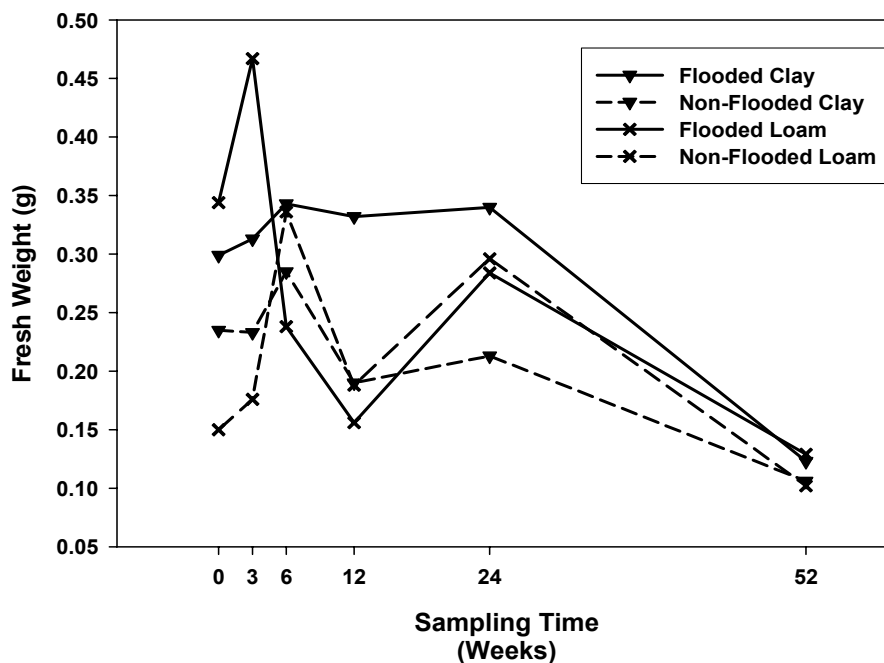


Figure A.16. Average fresh weight per rice plant from pots receiving 0.011 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

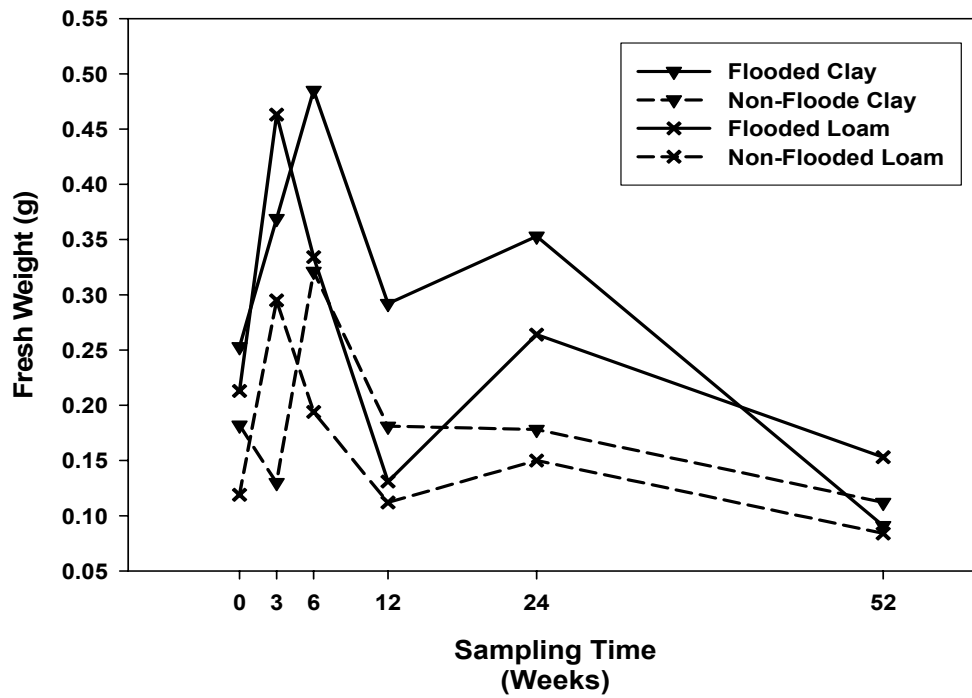


Figure A.17. Average fresh weight per rice plant from pots receiving 0.018 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

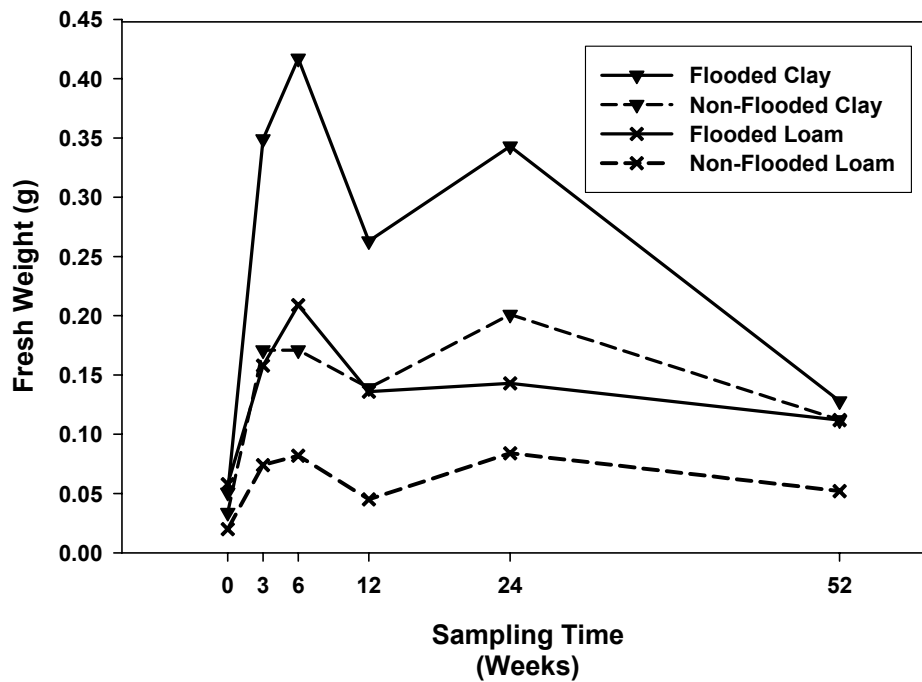


Figure A.18. Average fresh weight per rice plant from pots receiving 0.18 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

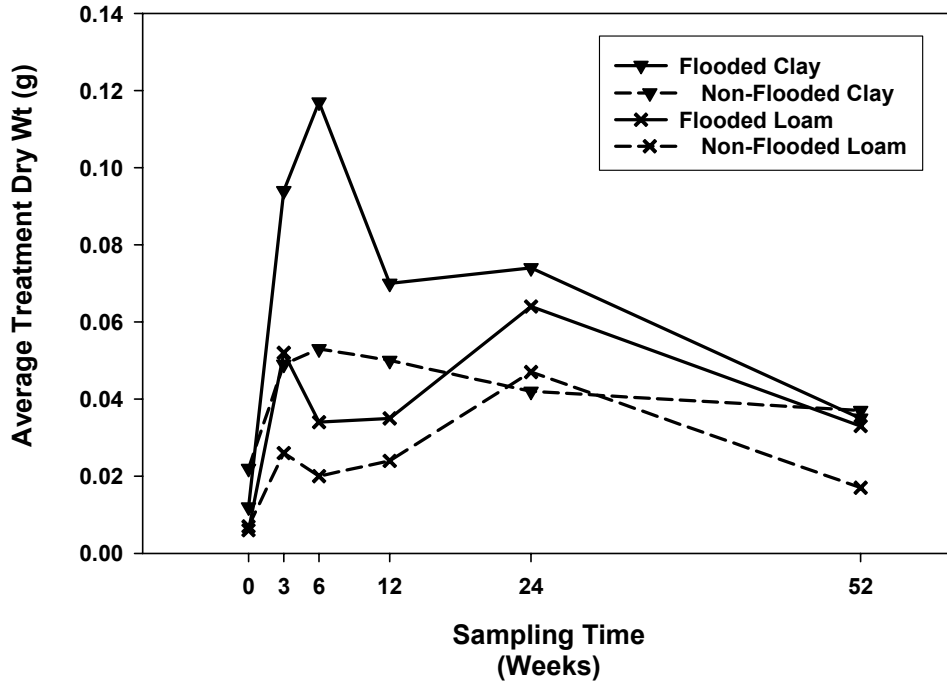


Figure A.19. Average dry weight per rice plant from pots receiving $0.045 \text{ kg ai ha}^{-1}$ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

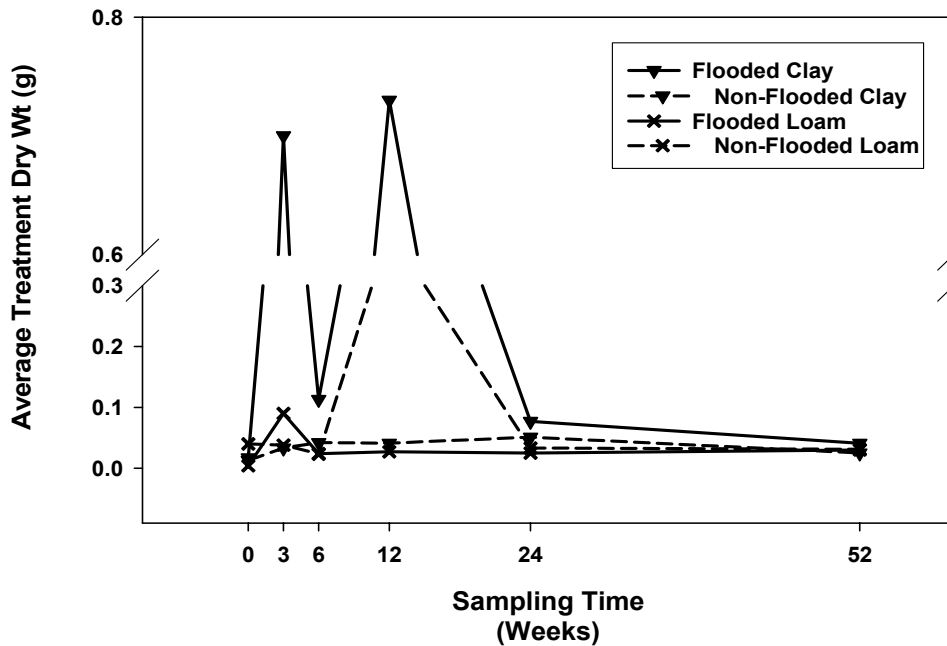


Figure A.20. Average dry weight per rice plant from pots receiving $0.07 \text{ kg ai ha}^{-1}$ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

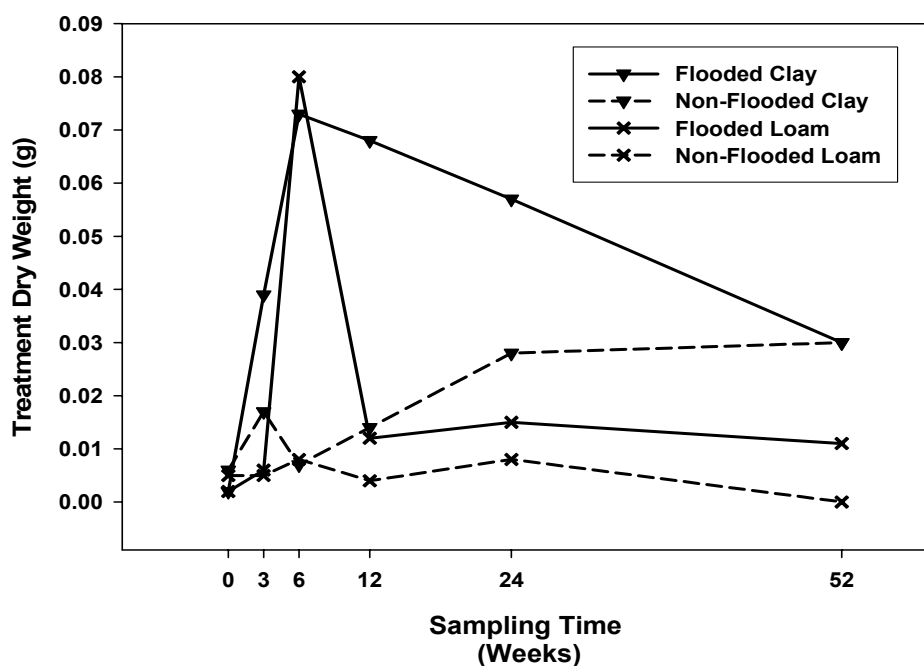


Figure A.21. Average dry weight per rice plant from pots receiving 0.7 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

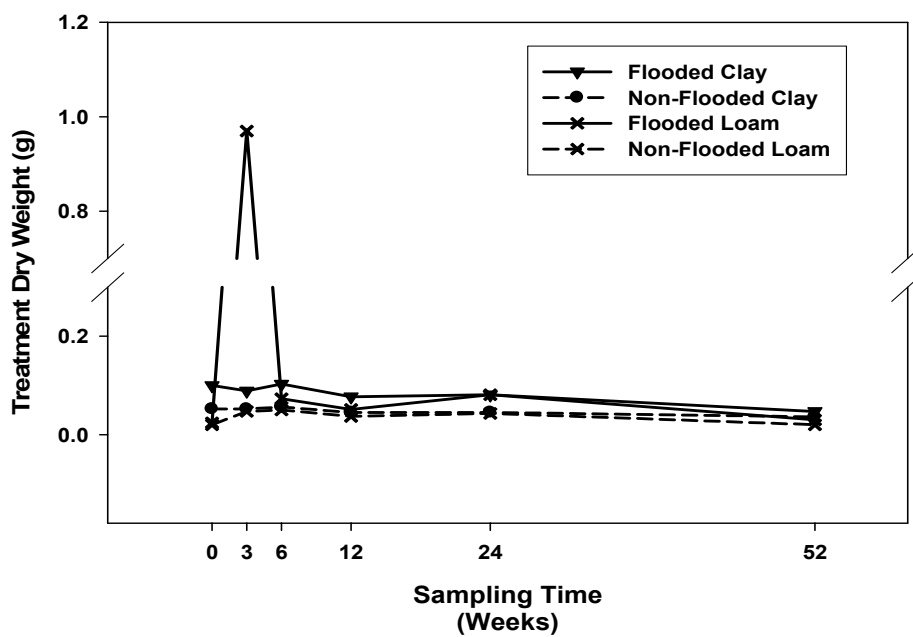


Figure A.22. Average dry weight per rice plant from pots receiving 0.023 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

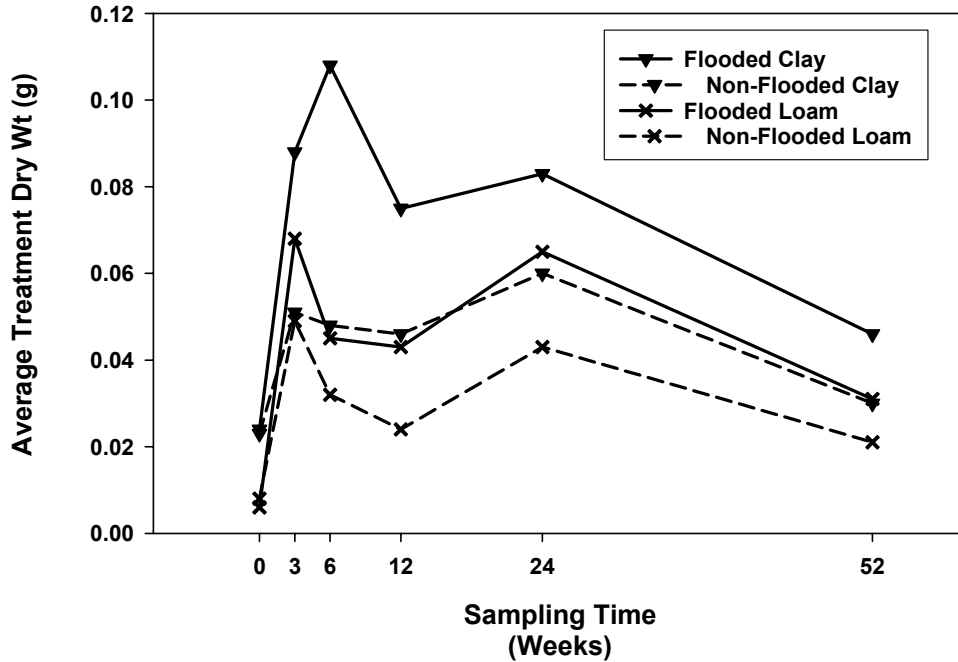


Figure A.23. Average dry weight per rice plant from pots receiving 0.035 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

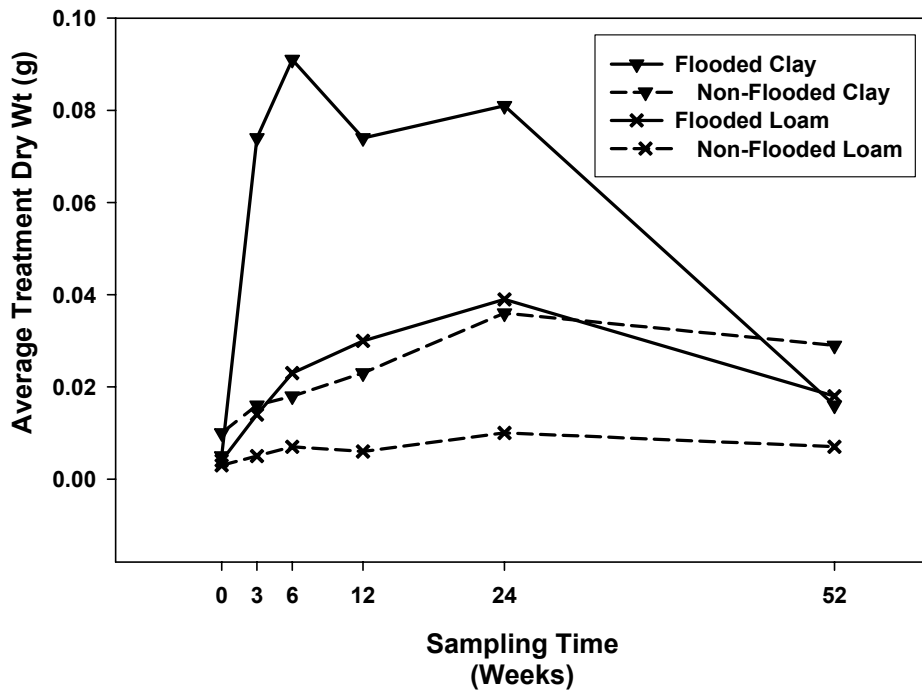


Figure A.24. Average dry weight per rice plant from pots receiving 0.35 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

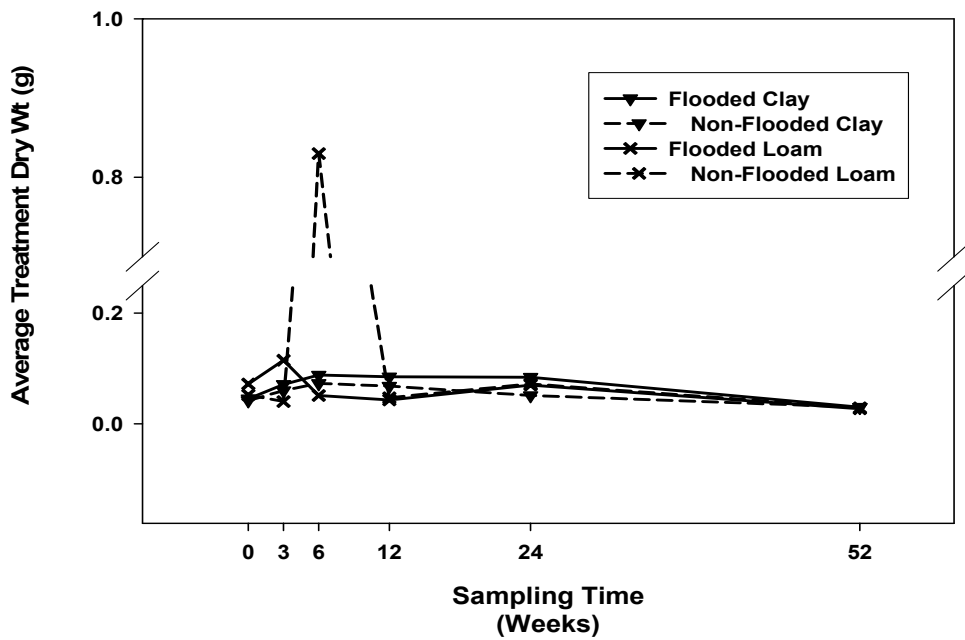


Figure A.25. Average dry weight per rice plant from pots receiving 0.011 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

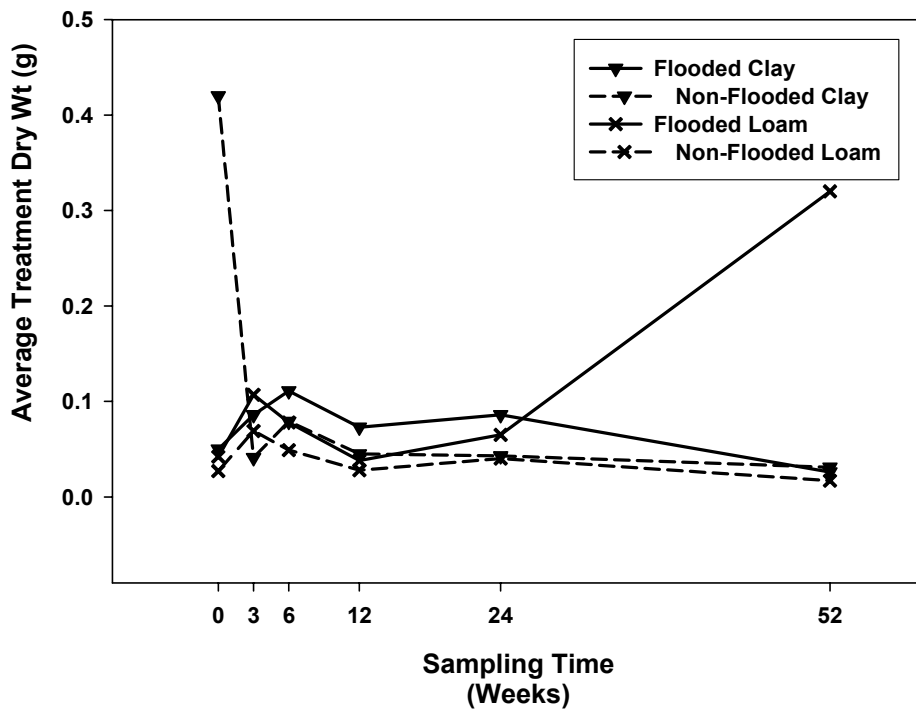


Figure A.26. Average dry weight per rice plant from pots receiving 0.018 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

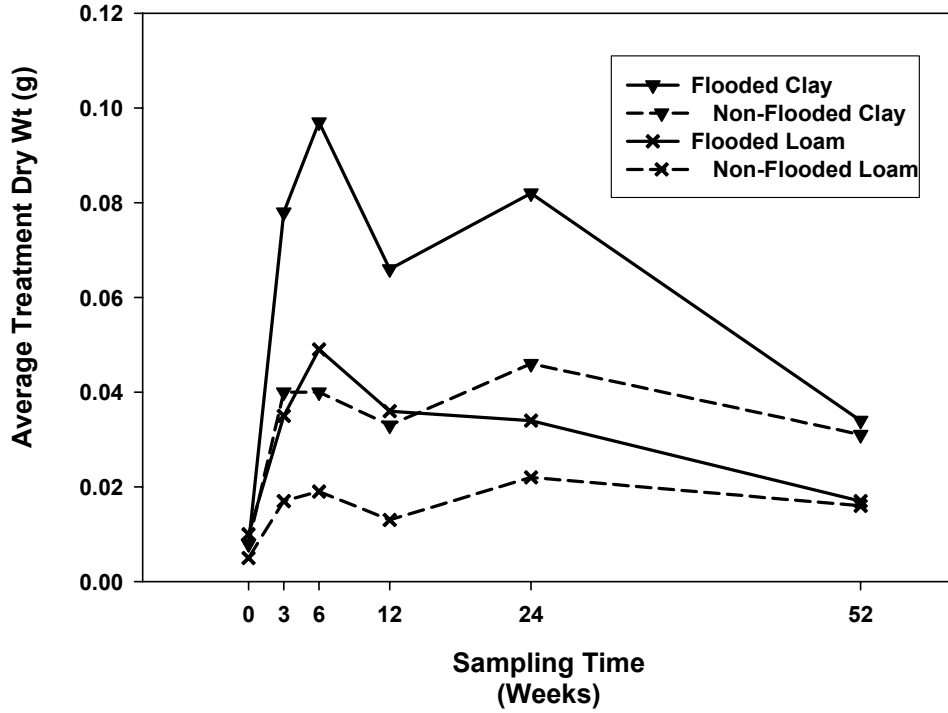


Figure A.27. Average dry weight per rice plant from pots receiving 0.18 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

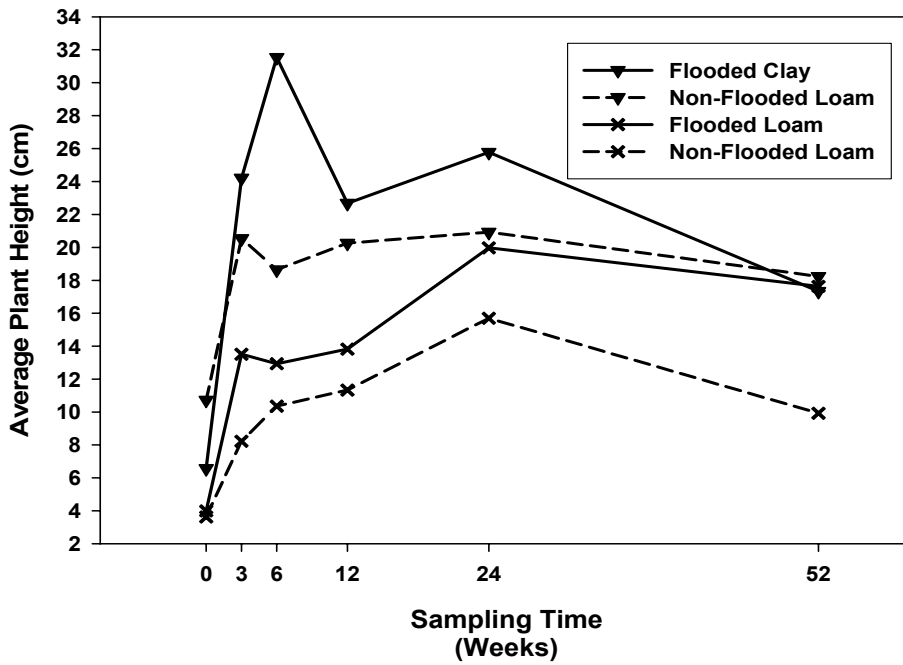


Figure A.28. Average height per rice plant from pots receiving 0.045 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

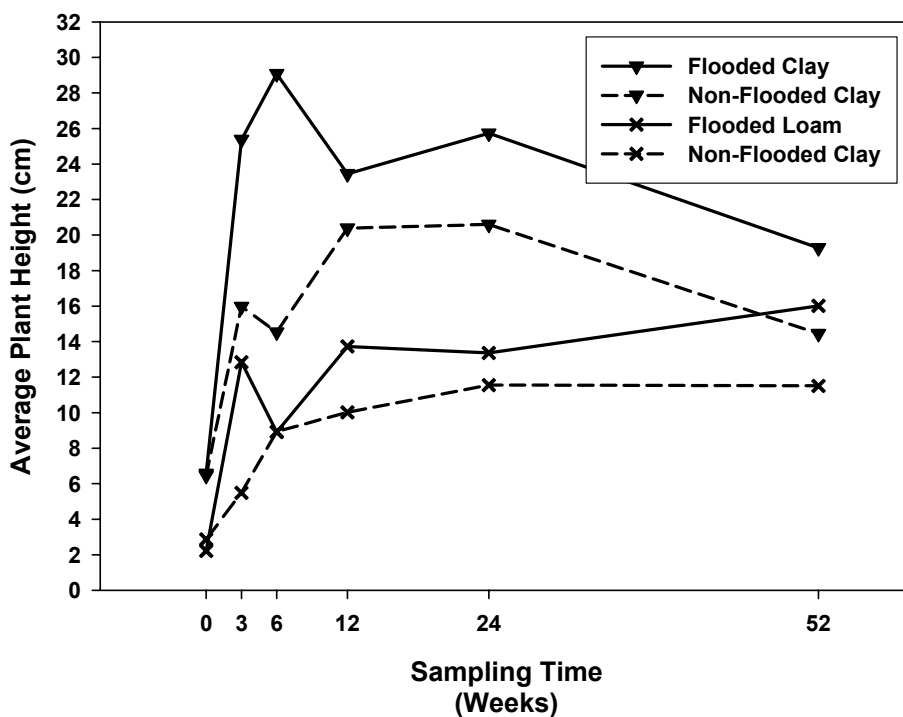


Figure A.29. Average height per rice plant from pots receiving 0.07 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

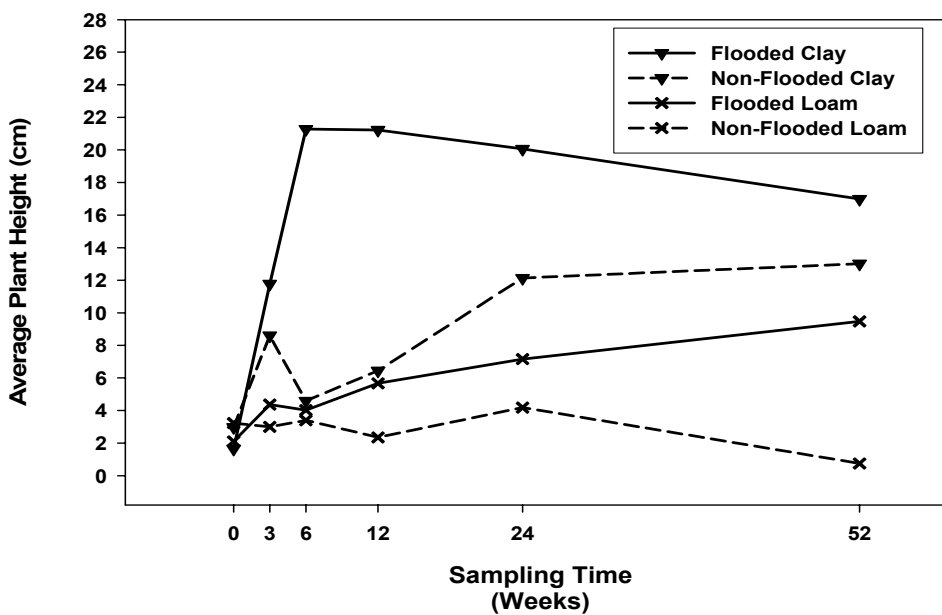


Figure A.30. Average height per rice plant from pots receiving 0.7 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

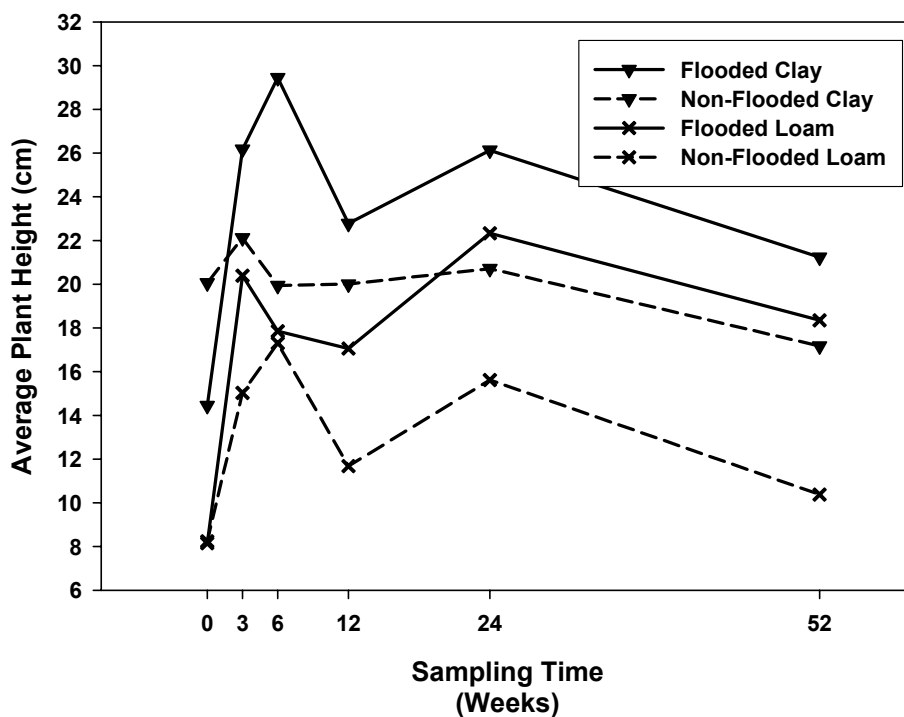


Figure A.31. Average height per rice plant from pots receiving 0.23 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

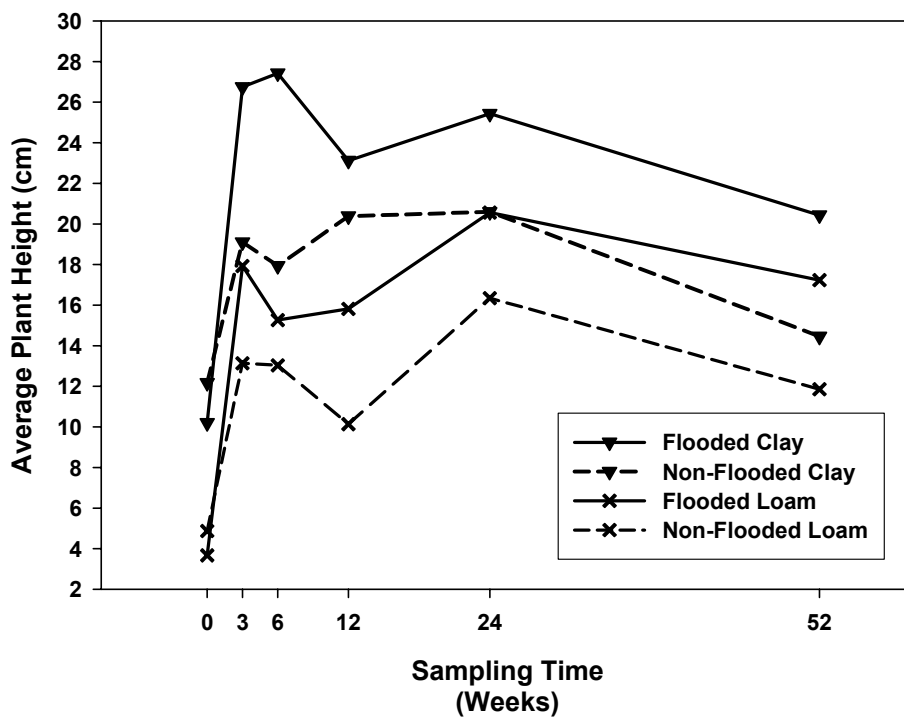


Figure A.32. Average height per rice plant from pots receiving 0.035 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

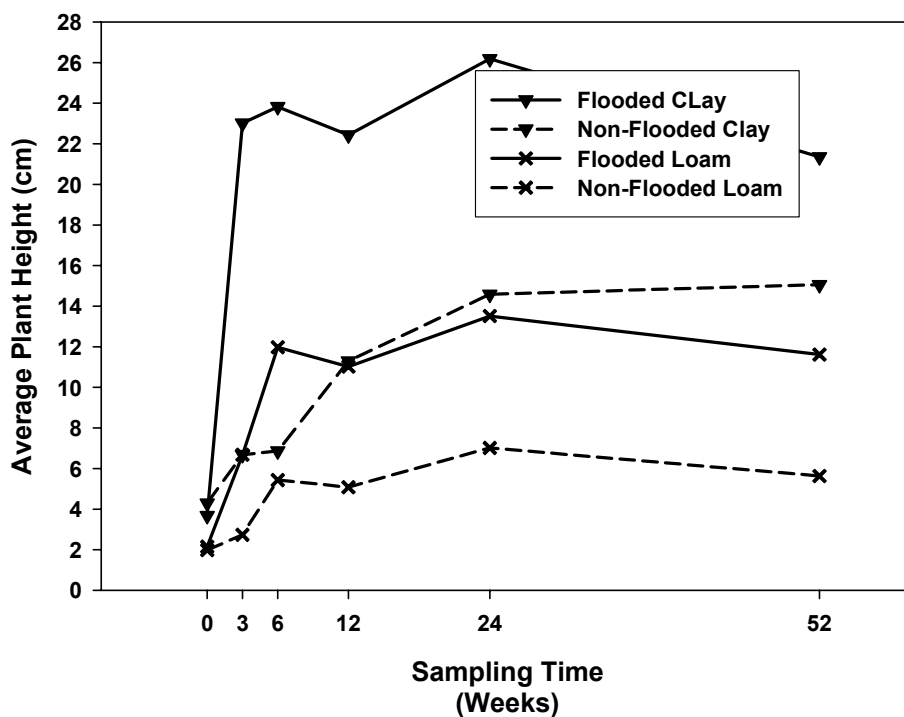


Figure A.33. Average height per rice plant from pots receiving 0.35 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

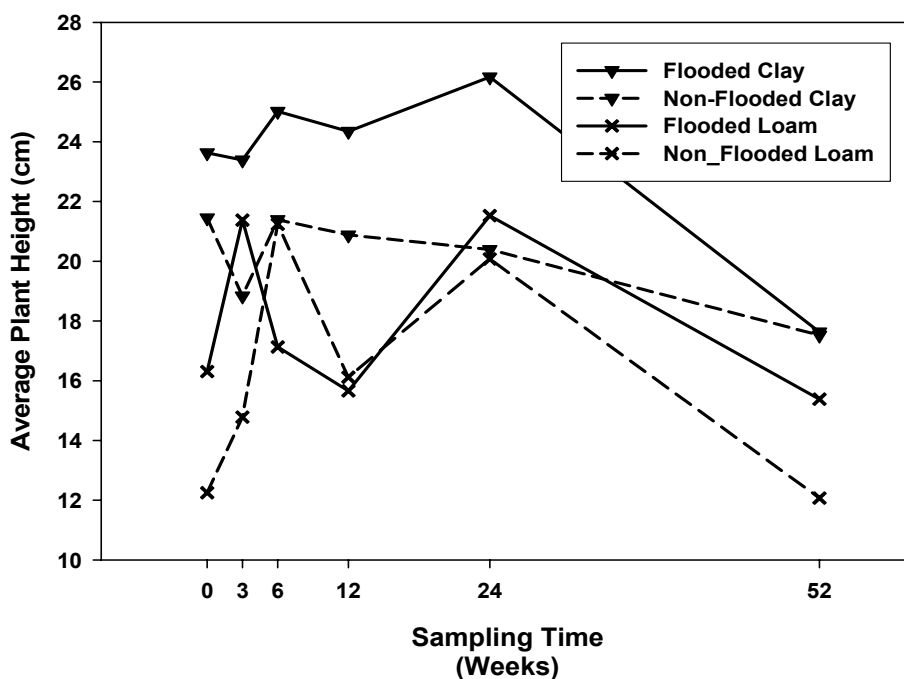


Figure A.34. Average height per rice plant from pots receiving 0.011 kg ai ha⁻¹ imazamox taken from 0 to 52 wks after application on two flooded and non-flooded soils.

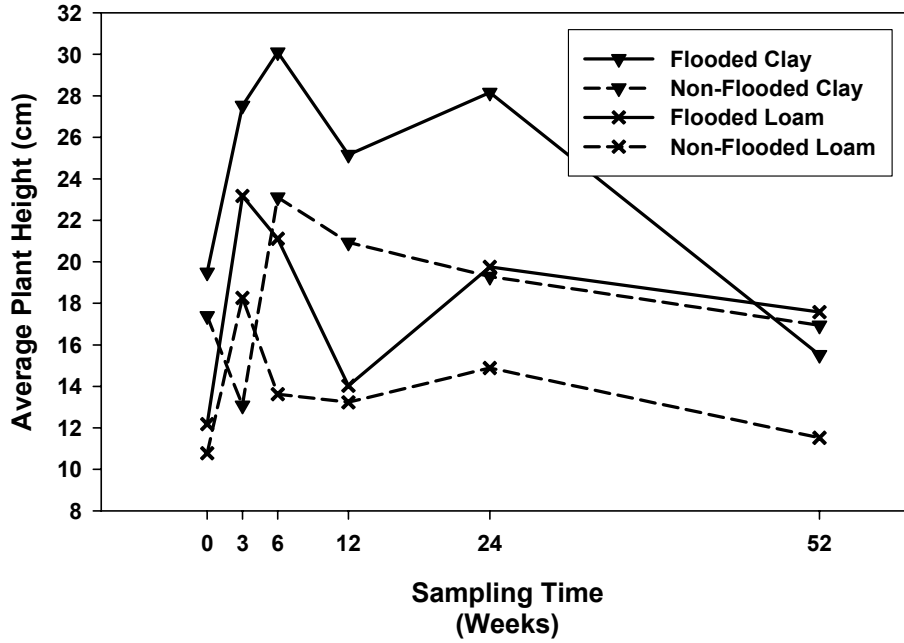


Figure A.35. Average height per rice plant from pots receiving 0.018 kg ai ha⁻¹ imazethapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.

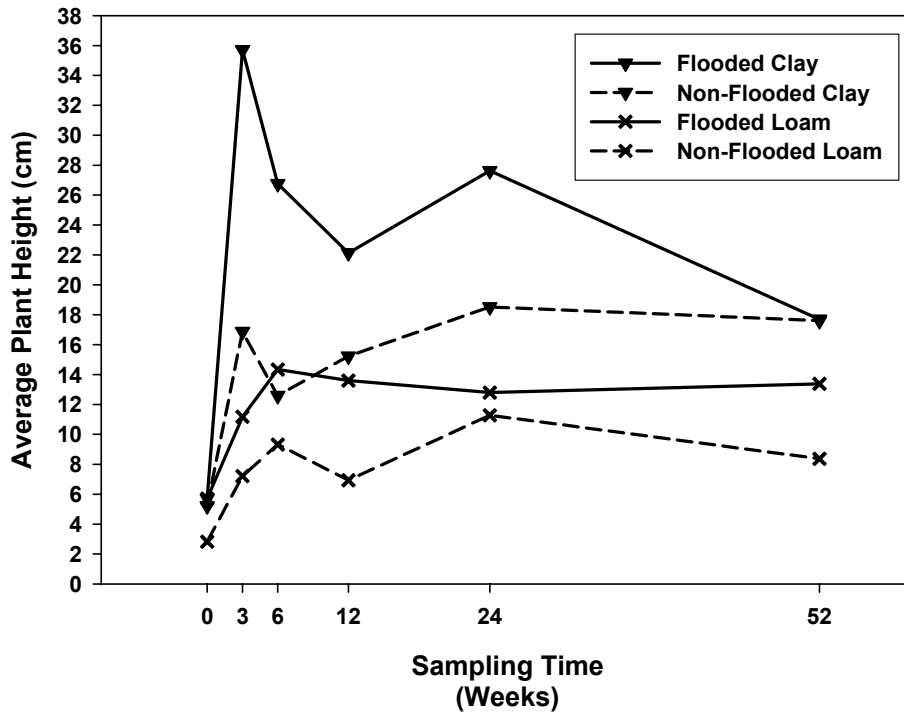


Figure A.36. Average height per rice plant from pots receiving 0.18 kg ai ha⁻¹ imazapyr taken from 0 to 52 wks after application on two flooded and non-flooded soils.