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DOI: 10.1017/wsc.2024.96

Pendimethalin Partition Rice

Water-Seeded Rice Seedling Response to Soil-Water Partitioning of Pendimethalin

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Abstract

Weed management in California water-seeded rice (*Oryza sativa* L.) is challenging due to herbicide-resistant weeds. Research on additional herbicide options is necessary to control herbicide-resistant weeds. Pendimethalin is a dinitroaniline herbicide commonly used in dry-seeded rice; however, it is not registered in water-seeded rice. This study was conducted to determine the pendimethalin fate in water-seeded rice after application at 1-, 3-, and 5-leaf stage rice. Ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was utilized to quantify pendimethalin and degradants in the water, soil and rice seedling tissue at 1, 5, and 14 days after treatment. Over 50% of recovered pendimethalin was observed in the rice tissue and over 25% in the soil and least observed in the water at all application timings and sampling dates. Three pendimethalin degradants were observed at low concentrations: p36 [1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole], p44 [4-[(1-ethylpropyl) amino]-2-methyl-3,5-dinitrobenzoic acid] and p48 [4,5-dimethyl-3-nitro-N²-(pentan-3-yl) benzene-1,2-diamine]. The degradant p36 was observed in all samples and most abundant in the soil. The degradants p36 and p44 increased in concentration in the water by 14 days after treatment. The degradants p44 and p48 were at low concentrations or below the lowest level of quantification in water, soil, and tissue samples. The pendimethalin parent molecule remained intact and was not readily metabolized in rice tissue. The crown region and shoots of the rice seedlings demonstrated greater pendimethalin concentrations compared to the roots at all rice stages; however, pendimethalin concentrations remained similar across the three sample timings. Rice root and shoot reduction was 16 and 13%, respectively, after the 1-leaf stage application averaged over sample timings, and 6 and 4% after the 5-leaf stage application. The results suggest the rice stage at the application timing is an important factor for pendimethalin tolerance; therefore, encouraging early root development can be beneficial for pendimethalin tolerance in water-seeded rice.

Keywords: absorption; crown tissue; degradants; liquid chromatography-tandem mass spectrometry; root tissue; shoot tissue.

Introduction

Rice (*Oryza sativa* L.) is a staple crop worldwide (Chauhan et al. 2017). In the US, rice is produced in the Mid-South and the California Sacramento Valley (USDA 2023). US rice is a major commodity of high quality with an export value of nearly 1.7 billion USD (USDA 2023). Therefore, US rice production must maintain the standard production levels to fulfill the global needs (Chauhan et al. 2017; USDA 2023). Weed management is a major challenge in rice production. Rice grain yields can be reduced up to 79% by interference of weedy grasses (Smith 1968). Weedy grass interference to rice growth is the major predictor for yield loss in mixed-weed species (Brim-DeForest et al. 2017).

In the California Sacramento Valley, water-seeded rice is the common production method where pregerminated rice is air-seeded onto fields with a 5- to 10-cm standing flood (Becerra-Alvarez et al. 2023). The continuous flood is maintained until harvest and utilized for control of weedy grasses, weedy rice, and non-aquatic weed species (Chauhan et al. 2017; Hill et al. 2006). However, years of continuous rice cultivation have shifted to flood-tolerant weed populations leading to increased difficultness in weed management (Becerra-Alvarez et al. 2023; Hill et al. 2006).

An overreliance on herbicides for weed control have selected for herbicide-resistant weedy grasses (*Echinochloa* spp. and bearded sprangletop [*Leptochloa fusca* (L.) Kunth spp. *fascicularis* (Lam.) N Snow], sedges (samflower umbrella sedge (*Cyperus difformis* L.) and bog bulrush (*Schoenoplectus mucronatus* L. Palla), and broadleaves (*Sagittaria* spp. and *Ammannia* spp.) in California water-seeded rice (Becerra-Alvarez et al. 2023; Hill et al. 2006). Resistance has reduced weed control efficacy making weed management challenging (Becerra-Alvarez et al. 2023). The herbicide-resistant weed management challenge has encouraged research on potential new herbicide introductions in water-seeded rice (Al-Khatib 2022). The introduction of new herbicides can be useful to manage herbicide-resistant populations through practices like mode of action rotations and mixtures (Beckie and Reboud 2009).

Pendimethalin is a mitotic inhibiting herbicide from the dinitroaniline chemistry that inhibits seedling growth shortly after germination (Appleby and Valverde 1989). Pendimethalin was successful in controlling local herbicide-resistant weedy grass populations in a greenhouse herbicide screening (Becerra-Alvarez and Al-Khatib 2024a); however, little data are available on

the use of pendimethalin in water-seeded rice fields. Our previous research showed that pendimethalin may cause injury to rice; however, rice injury was reduced when pendimethalin in a capsule suspension formulation was applied after 3- to 4-leaf stage rice compared to a 1-leaf stage rice (Becerra-Alvarez and Al-Khatib 2024a). Rice response was rate dependent with 2.3 kg ai ha⁻¹ rate of pendimethalin resulting in acceptable rice response and weed control compared to lower and higher application rates (Becerra-Alvarez and Al-Khatib 2024a). The results were encouraging; however, greater knowledge is needed to further understand the potential for pendimethalin injury in water-seeded rice.

There is limited research on the fate of pendimethalin in the water-seeded rice environment. Herbicide fate in the soil and water of a water-seeded rice field is integral to establish effective and environmentally practical practices (Becerra-Alvarez and Al-Khatib 2024b; Hill et al. 2006). Barrett and Lavy (1983) compared pendimethalin soil dissipation in soybean [*Glycine max* (L.) Merr.] (furrow-irrigated), upland rice (flush-irrigated and never continuously flooded) and lowland rice (flush-irrigated and subsequently flooded). Pendimethalin soil dissipation was rapid in lowland rice, followed by upland rice and slowest in soybeans. The observed soil dissipation pattern was most likely caused by alternate wetting intervals in rice cropping systems that accelerated pendimethalin dissipation in the soil through volatilization during flush irrigations (Barrett and Lavy 1983). The dry/wet soil cycles increased volatilization potential in the dry soil and rapidly reduced pendimethalin concentration in the soil (Weber 1990). Volatilization of pendimethalin from the soil decreases in anaerobic or flooded conditions because of lower movement of the vapor phase in the wet soil compared to when the soil is dry, then, the vapor phase moves more readily (Weber 1990). Gaining information on the fate of pendimethalin in the soil-water interface in the water-seeded system can provide knowledge to reduce rice injury and improve weed control from pendimethalin.

There is limited information on pendimethalin degradants in soil and water (Chen et al. 2021; Barrett and Lavy 1983; Weber 1990). Previous research suggest pendimethalin parent molecule is the abundant remaining residue in soils (Parka and Soper 1977; Appleby and Valverde 1989; Weber 1990). However, advances in chromatography technology allow to quantify lower residue levels and may demonstrate new knowledge in pendimethalin degradation (De Vos et al. 2016). The oxidation of one of the methyl groups to a carboxylic acid create the

degradant p44 [4-[(1-ethylpropyl) amino]-2-methyl-3,5-dinitrobenzoic acid] which is commonly found in soil and water (USEPA 2013a; USEPA 2013b). The substitution of a nitro group for an amino group creates degradant p48 [4,5-dimethyl-3-nitro-N²-(pentan-3-yl) benzene-1,2-diamine] which is common in water but less common in soil; while the cyclization between the nitro groups create p36 [1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole], which has been most commonly observed in soils (USEPA 2013a; USEPA 2013b).

Dinitroanilines possess physico-chemical characteristics that create a molecule greatly attracted to organic matter and will typically remain on the topsoil surface after an application (Makkar et al. 2019). Therefore, crop placement is a means of ensuring crop tolerance to pendimethalin in drill-seeded rice. In drill-seeded rice, pendimethalin is commonly utilized after placing the rice seed 3.2 cm deep into the soil followed by the herbicide application on the soil surface (Bond et al. 2009). The depth of the seed placement allows for germination and early growth before contact with the herbicide and therefore, avoids herbicide absorption by the rice coleoptile and roots (Bond et al. 2009). However, in water-seeded rice the rice seeds are placed on the soil surface in a high moisture environment which create an environment for injury potential. The post-emergence application of pendimethalin in water-seeded rice is suspected to induce tolerance by avoiding concentrated herbicide absorption by larger established rice seedlings (Becerra-Alvarez and Al-Khatib 2024). Dinitroaniline herbicides typically need to be absorbed at the plant growing points to cause significant injury in plants (Knake et al. 1967; Parka and Soper 1977). In dry-seeded rice systems, it has been suggested that the rice seedling coleoptile and mesocotyl are most sensitive to absorption of pendimethalin (Koger et al. 2006; Khaliq and Matloob 2012). Koger et al. (2006) observed cultivar tolerance differences where shorter mesocotyl lengths led to less injury in drill-seeded rice seedlings by avoiding direct contact with the herbicide on the soil surface compared to cultivars with a longer mesocotyl length which encountered the herbicide on the soil surface.

In water-seeded rice, the roots may be a significant absorption area apart from the coleoptile region because the rice seed is placed on the soil surface and shallow root systems are developed in the flooded environment (UCANR 2023). There has been no previous work observing water-seeded rice absorption of pendimethalin. Research on herbicide absorption and fate in plants can support understanding of tolerance mechanisms toward herbicide injury in rice

by observing what degradants are formed or where the herbicide is translocated inside the plant (Tenbrook and Tjeerdema 2006). Previous research on dinitroaniline herbicides fate inside rice seedlings only measured amino acid contents after an application but have not observed the formation of degradants (Parka and Soper 1977). Therefore, understanding pendimethalin absorption and degradation in rice seedlings can be useful to understand the tolerance mechanisms and develop management that may help reduce rice injury and improve weed control when pendimethalin is applied post-emergence to water-seeded rice. The objectives of this study were to characterize pendimethalin fate as it affects water-seeded rice seedling response after application at different seedling growth stages.

Materials and Methods

Experimental design

A greenhouse study was carried out in late 2023 to early 2024 at the UC Davis Orchard Park Greenhouses (38°32'33"N, 121°45'47"W). Natural lighting was supplemented with 1000 W metal halide lamps providing $400 \mu\text{mol m}^{-2}\text{s}^{-1}$ photosynthetic photon flux density (PPFD) to fulfill a 16 h photoperiod when necessary. Air and water temperature data were collected and averaged over three data loggers at each experimental run (HOBO Pendant Temp/Light 8K) (Supp. Material 1).

Plastic pots with no drain openings of 11.4 cm width by 11.4 cm length by 15 cm height were filled with 1.4 kg of field soil. Soil type was characterized as Esquon-Neerdobe (fine, smectitic, thermic Xeric Epiaquerts and Duraquerts), silty clay, made up of 27% sand, 39% silt, and 34% clay, with a pH of 5.1, and 2.8% organic matter. The soil was saturated with water in all pots prior to sowing seeds. 'M-206' rice was pregerminated by placing the seed in water for 24 h and then drained and dried for 12 h at room temperature. About 40 seeds were evenly broadcasted by hand to each pot. The soil was maintained saturated and after three days a 10-cm flood above the soil surface was added and maintained for the remaining of the study by continuously adding water every 24 h. The 1-leaf stage application remained with 40 seeds in each pot. Prior to herbicide treatment at the 3- and 5-leaf stage, pots were thinned to 20 seedlings per pot with even growth. The study was organized as a completely randomized design with three replications and rerandomized every seven days. Experiments were repeated three times, initiated on September 13th, November 5th, and December 6th, 2023, respectively.

Herbicide application

Pendimethalin (Prowl H₂O, BASF, Florham Park, NJ) in a capsule suspension formulation was applied to the respective pots at the 1-, 3- and 5-leaf stage rice. A mixture of pendimethalin in water was continuously being stirred with a magnetic stirrer in a 200 ml-Erlenmeyer glass flask placed on a stir plate prior to applications. The flood water was drained by pouring the water out of the pots and pendimethalin was applied on the soil surface. Pendimethalin was evenly applied at a rate of 2.3 kg ai ha⁻¹ with a 5 ml pipette (Eppendorf Research Plus, Hamburg, Germany) on the soil surface for a total of 20 ml in each pot. At the 1-leaf stage rice, pendimethalin was applied over the top of the 40 rice seedlings in each pot. At the 3- and 5-leaf stage rice, pendimethalin was directly applied on the soil surrounding the 20 rice seedlings in each pot. The flood water was raised back to 10 cm above the soil immediately after the herbicide applications.

Soil-water and rice tissue sampling

The water, soil and rice seedlings were sampled at 1, 5, and 14 days after treatment (DAT). The pots were sampled by first pouring the flood water in separate plastic containers and quickly subsampled 40 ml into 50 ml conical centrifuge plastic containers and stored at -20 C immediately after sampling before further analysis. Then, the seedlings were carefully removed and gently washed the soil from the roots. The soil in the pot was poured out, homogenized, and laid flat in the greenhouse to air dry for up to three days. The dry soil was then further homogenized. Then, 50 ml of the soil was subsampled and placed in 50 ml conical centrifuge containers stored at -20 C before further analysis.

The seedlings were immediately taken to the lab for further preparation of samples. The entire seedlings were washed by dipping the roots and shoots in a container filled with a 50:50 methanol: distilled water mixture, then, further rinsed with only distilled water. Rice seedlings shoot and root length were recorded from three randomly selected seedlings in each pot. Each seedling was then separated by shoot and root after the 1-leaf stage application, and by shoot, crown region, and root after the 3- and 5-leaf stage applications. The fresh weight of each region was recorded, and the tissue was further homogenized by crushing with a mortar and pestle to pieces smaller than 2 mm after flash freezing with liquid nitrogen. An aliquot of 100 mg ± 2 mg of fresh weight was placed in 2 ml tubes and stored at -80 C until further analysis.

Pendimethalin and degradant quantification

Ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was utilized to quantify the absolute concentration of pendimethalin using reference standards and to quantify relative quantities of pendimethalin degradants p36 [1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole], p44 [4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid] and p48 [4,5-dimethyl-3-nitro-N²-(pentan-3-yl) benzene-1,2-diamine] (Figure 1; Supp. Material 2; Supp. Material 3; Supp. Material 4).

The extraction and quantification of pendimethalin in water and soil samples was conducted referencing the US Environmental Protection Agency methods (USEPA 2013a; USEPA 2013b) and previous methods with water samples (Becerra-Alvarez, Al-Khatib 2024b). There is no regulatory method for analysis of pendimethalin in rice plant tissue; therefore, a modified extraction method was established for pendimethalin and its degradants by conducting a preliminary analysis (Supp. Material 4). The finalized protocol was two aliquot 100 mg of tissue from a total mass of at least 500 mg or higher from each treated pot. Therefore, at the 1-leaf stage rice treatment, where tissue biomass was much smaller per individual plant, 40 rice seedlings per pot were allowed to increase the tissue biomass compared to 20 rice seedlings in the 3- and 5-leaf stage applications. Pendimethalin in rice tissue was quantified with an internal standard compared to an external calibration curve in the shoot, crown region, and root samples from each pot and the degradant's quantities were quantified relative to the parent molecule (Supp. Material 4).

Data Analysis

The concentrations of pendimethalin and relative degradants in extracts were converted to $\mu\text{g L}^{-1}$ in water with the following equation:

$$R = C_{\text{end}} \times \frac{V_{\text{Fex}}}{V_{\text{Iex}}} \times 1000 \times 0.001 \quad [1]$$

Where R = analyte residue in $\mu\text{g L}^{-1}$, C_{end} = concentration of analyte in final sample in ng L^{-1} , V_{Fex} = volume of final extract in ml, V_{Iex} = volume of initial extract in ml. The volume of the final extract was 0.8 ml and the volume of the initial water sample were all a 10 ml subsample.

The concentration in the soil samples were converted to $\mu\text{g g}^{-1}$ of soil with the following equation:

$$R = C_{\text{end}} \times DF \times \frac{V_{\text{Fex}}}{W_s} \times 0.001 \quad [2]$$

Where R = analyte residue in $\mu\text{g g}^{-1}$, C_{end} = concentration of analyte in final sample in ng ml^{-1} , DF = dilution factor, V_{Fex} = volume of final extract in ml, W_s = weight of initial soil sample in g. The column of the total extract was 30 ml, and the sample weight was 5 g subsampled from each pot. The concentration in the tissue were converted to $\mu\text{g g}^{-1}$ of fresh weight with the following equation:

$$R = C_{\text{end}} \times \frac{V_{\text{Fex}}}{W_{\text{fw}}} \times 0.001 \quad [3]$$

Where R = analyte residue in $\mu\text{g g}^{-1}$, C_{end} = concentration of analyte in final sample in ng ml^{-1} , V_{Fex} = volume of final extract in ml, W_{fw} = fresh weight of tissue sample in g. Most subsamples were 100 mg of fresh weight and 1 ml of extract was added; however, there were 10 samples that resulted in lower than 100 mg of fresh weight and the extract volume was reduced accordingly to the total fresh weight. All data was analyzed with regression and Tukey's post-hoc analysis at $\alpha=0.05$ where applicable using R v.4.4.0 (R Core Team 2024; Kuznetsova et al. 2017).

Results and Discussion

Total soil-water-rice partitioning

Pendimethalin was quantified with an internal standard of pendimethalin- d_5 and external calibration curves separately for water, soil, and rice tissue. The external calibration curves were prepared in 50% acetonitrile, 50% water and 0.1% formic acid with pendimethalin concentration from 0.03 ng ml^{-1} to 250 ng ml^{-1} and each standard concentration at 2 ng ml^{-1} of pendimethalin- d_5 . The average recovery was 89% in water samples and 113% in soil samples. The pendimethalin degradants were quantified as relative to the parent molecule with external calibration curves of low, medium, and high concentration ranges (Supp. Material 2; Supp. Material 3). The average recoveries for the shoot, crown region, and root were 89, 87, and 91%, respectively (Supp. Material 4).

The data from water, soil, and rice tissue on the recovered pendimethalin were combined to compare pendimethalin concentrations. Over 50% of recovered pendimethalin was observed in the rice tissue across the three application timings (Figure 2). Pendimethalin was secondly most observed in the soil and less than 20% of pendimethalin was observed in the flood water (Figure 2). The results are not surprising since pendimethalin physio-chemical characteristics demonstrate low water solubility (0.275 mg L^{-1}) and an affinity for lipids and organic matter ($\log K_{ow}=5.20$) (Makkar et al. 2019; Vighi et al. 2017). The pendimethalin molecule is highly lipophilic and will rapidly partition to the lipid contents found in plants or organic matter (Vighi et al. 2017). The strong bonds between the nitro groups of pendimethalin and lipids or organic matter created by hydrogen bonding characterize pendimethalin's strong bond to soil organic matter (Weber 1990). The binding characteristic of the pendimethalin molecule are probably the reason similar levels were observed throughout the three sampling times in this study. All previous research has agreed that the pendimethalin parent molecule is the dominant concentration recovered after application in soils and plant tissues (Parka and Soper 1977; Appleby and Valverde 1989; Chen et al. 2021).

At the 1-leaf stage application, 30 to 50% of pendimethalin was recovered in the soil and similar levels in the rice seedlings were observed (Figure 2). The observed partition behavior at the early rice growth stage is probably due to the reduced size of rice seedlings which did not absorb pendimethalin at quantities like the 3- and 5-leaf stage applications (Figure 2). The absence of available lipids for pendimethalin to partition to rice seedling at the 1-leaf stage would have resulted in pendimethalin to partition to the soil (Vighi et al. 2017).

The p36 degradant was the only degradant observed in quantifiable levels on all the three sample types. Degradant p36 was in greater concentrations in the soil and water samples compared to the rice tissue (Figure 3). Over 50% of p36 was observed in the soil at 1 and 5 DAT at all application timings; however, by 14 DAT the degradant observed concentration increased in the water samples to similar levels (Figure 3). The low or lack of observed degradants support the strong bonding behavior of pendimethalin to lipids or organic matter (Weber 1990). The degradant p36 is created through cyclization initiated by soil microbes (Kulshrestha et al. 2000) or photodegradation in soils (Pal et al. 1991). The cyclization process is its own pathway with no intermediary metabolites in between (Pal et al. 1991; Appleby and Valverde 1989). The p36

molecule is a known stable pendimethalin degradant in the soil (USEPA 2013b); however, it is not a common degradant of water (USEPA 2013a). It is possible photodegradation in the water led to the synthesis of p36 (Pal et al. 1991).

Water partitioning

Overall, observed pendimethalin concentration levels in the water were relatively low and similar across application stage and sample timing (Table 1). The highest values observed was $1.058 \mu\text{g L}^{-1}$ at one DAT (Table 1). At 14 DAT, pendimethalin residue in water were 0.124 to $0.270 \mu\text{g L}^{-1}$ (Table 1). The values observed in this study at late sampling dates agree with field studies by Becerra-Alvarez and Al-Khatib (2024b); however, samples at one DAT did not result in similar levels of concentration. In the field study, pendimethalin capsule suspension was applied onto the water of a water-seeded rice field environment which recorded $10.9 \mu\text{g L}^{-1}$ of residue in the water at one day after treatment and $0.3 \mu\text{g L}^{-1}$ residue in water by 15 DAT (Becerra-Alvarez and Al-Khatib 2024b). In this greenhouse study, pendimethalin was applied on the soil then reflooded which would have resulted in greater pendimethalin adsorption onto the soil and less concentration in the water.

The p36 and p44 degradants were observed at quantifiable levels in the water, while the degradant p48 was below the lowest level of quantification. There were only 11 samples that were quantified for degradant p48 from 0.009 to $0.05 \mu\text{g L}^{-1}$. The p36 and p44 degradants increased after the first sample date and were up to $0.261 \mu\text{g L}^{-1}$ for p36 and up to $0.068 \mu\text{g L}^{-1}$ for p44 at 14 DAT (Table 1). The degradant synthesis pathway is most likely through photodegradation in the water (Vighi et al. 2017; Pal et al. 1991).

Pendimethalin degradants did increase in the water after an application relative to the initial sample date; however, concentrations were very low overall (Table 1). Pendimethalin is not highly water soluble and the low concentrations in the water were expected despite this study not being an environmental residue study (Lehotoy et al. 1998; Zimmerman et al. 2000). However, a water-holding period after a pendimethalin application in water-seeded rice may still be important and useful to allow the herbicide molecule to remain in the field where the herbicidal activity is needed and degrade in the field (Becerra-Alvarez and Al-Khatib 2024b).

Soil partitioning

Pendimethalin soil persistence was similar across the interaction of the 1-, 3-, and 5-leaf stage timing and sample timing of 1, 5, and 14 DAT. Only application timing, $P < 0.001$, and sample timing, $P < 0.001$, as factors alone showed a difference (Table 2). The results are not surprising because of the characteristics of dinitroaniline molecules which create an affinity for organic matter and bound to soils (Weber 1990; Helling and Krivonak 1978). Previous research investigated partitioning behavior of pendimethalin onto sediment in water/sediment dark environments which demonstrated 50% allocation of pendimethalin onto sediments to occur within 0.4 to 1.6 days (Vighi et al. 2017). The observations from Vighi et al. (2017) demonstrate a rapid and strong affinity to the soil matter which could explain the lack of interaction effects observed in this study.

The results demonstrated a 22% decrease of pendimethalin concentrations in the soil at 14 DAT, averaged over application stages (Table 2). Barnett and Lavy (1983) demonstrated pendimethalin dissipation are affected by soil-water content and pendimethalin dissipated rapidly in alternate flooding conditions than in continuously flooded conditions. Makkar et al. (2020) recorded pendimethalin dissipation in the soil of dry-seeded and transplanted rice fields to dissipate rapidly early on and then dissipate at a slower rate. In the water-seeded conditions of this study, pendimethalin concentration was low and decreased slowly over time by 14 DAT. The results are consistent with other studies; however, it is difficult to know if the pendimethalin in the soil is continuing herbicidal activity or is bound to the soil matter (Helling 1976). Helling (1976) suggested faster degradation of dinitroanilines in anaerobic soils than aerobic soils; however, any visual injury on the rice seedlings was most visible until 14 DAT.

The only pendimethalin degradant observed at quantifiable levels in the soil was p36 (Table 3). The degradant p48 was observed only in four samples at $0.002 \mu\text{g soil}^{-1}$. The p36 concentrations were relatively similar across application timings and sample timing; however, concentration did decrease by 14 DAT compared to one DAT sampling time in the 3- and 5-leaf stage application (Table 3). Makkar et al. (2020) attempted to quantify pendimethalin degradants in the soil of dry-seeded and transplanted rice fields at time of harvest but did not find quantifiable levels of the pendimethalin metabolites. Vighi et (2017) state p48 is a major

degradant of anaerobic degradation; however, it can be further degraded to other degradants and as previously mentioned p36 may be more stable in the soil under the water-seeded environment.

Rice tissue partitioning

The pendimethalin concentration at one day after treatment was similar across application stages and seedling regions from 0.012 to 0.083 $\mu\text{g seedling}^{-1}$ (Table 4). Pendimethalin concentration in the seedlings increased by 14 days after treatment with the majority being observed in the crown region and shoot of the seedlings (Table 4). It is not surprising the crown region observed the greatest levels of pendimethalin because pendimethalin tends to not readily leach and remains on the soil surface where the crown region interfaces with the herbicide. Knake et al. (1968) demonstrated the early shoot region was the greatest area of trifluralin, a dinitroaniline herbicide, absorption in giant foxtail, a weedy grass species. This study demonstrates the crown region is the area of greatest concentration of pendimethalin after being absorbed by water-seeded rice.

The pendimethalin degradants were limited in the rice tissues and p36 was most observed among the degradants up to 0.007 $\mu\text{g g}^{-1}$ similarly across the tissue regions; however, values were different across sample timing, $P < 0.001$. There was an increase in concentration by 14 days after treatments of p36 (Table 5). Despite the increase, the concentration was relatively low and demonstrate the majority of the pendimethalin parent molecule remain intact in the rice tissue. The degradant p48 was similarly observed in samples at very low concentrations from 0.0002 to 0.003 $\mu\text{g g}^{-1}$ across all tissue regions (data not shown). No differences were observed across sample timings. Most samples demonstrated concentrations of degradant p44 to be less than 0.0009 $\mu\text{g g}^{-1}$ or below the lowest level of quantification (data not shown). An interesting observation was that degradant p44 was not observed in shoot tissue and observed concentrations were only in the crown region and roots. Parka and Soper (1977) state an increase of amino acids in the shoots and roots occurred after a trifluralin application onto rice seedlings. An increase in amino acids could potentially interact with dinitroaniline herbicides to create the associated metabolites (Parka and Soper 1977). However, metabolism of the pendimethalin parent molecule in rice tissue appears not to be a major mechanism of tolerance because of the low degradant concentrations observed. Appleby and Valverde (1989) similarly concluded pendimethalin was the greatest residue observed in various plant species.

The rice seedlings in this study did observe greater concentration in the shoots at all timings (Table 4). Knake et al. (1968) demonstrated the shoot absorption of trifluralin was most limiting to growth compared to root absorption of trifluralin by giant foxtail seedlings. Pendimethalin concentrations were considerably similar in the crown regions and shoots indicating pendimethalin can translocate acropetally to some degree. However, application stage appeared as the major factor affecting rice growth reductions. At the 1-leaf stage application, root and shoot reduction was up to 16% and the rice seedlings visually appeared greatly injured (Table 6). However, at the 3- and 5-leaf stage rice applications, rice growth was only reduced 7% and visually appeared like the nontreated (Table 6). The observations were like the field observations after a pendimethalin application in water-seeded rice at 3-leaf stage led to greater growth reductions than at the 4- to 5-leaf stage applications (Becerra-Alvarez and Al-Khatib 2024a). Pendimethalin application timing and plant size are limiting factors in observing injury from pendimethalin as observed with other plant species like velvetleaf (*Abutilon theophrasti* L.) and Powell amaranth (*Amaranthus powellii* S. Watson) (Malefy and Duke 1984). Therefore, application timing is most important for water-seeded rice seedlings to tolerate pendimethalin.

The crown region of rice seedlings is the area with the greatest pendimethalin concentration and tolerance is conferred by the seedling stage at time of application. When more biomass is present in the root and shoot, the seedlings have greater ability to tolerate pendimethalin applications at 2.3 kg ha⁻¹ in water-seeded environments. In various plants, greater lipid content is associated with tolerance to dinitroaniline herbicides (Ndon and Harvey 1981). While grasses do not have the increased lipid contents as most broadleaves do (Ndon and Harvey 1981), there are lipids like suberin layers in rice seedling roots which could play a role in tolerance (Kreszies et al. 2018). However, the lower concentration of pendimethalin observed in the roots would allow the roots to supplement the rice seedling growth and overcome the aboveground injury. Therefore, practices that encourage rice seedling root establishment in water-seeded rice before a pendimethalin application could allow for increased tolerance to pendimethalin. The practices to evaluate can include draining fields early on to allow root establishment in water-seeded rice or use of vigorous rice cultivars for rapid establishment (Becerra-Alvarez and Al-Khatib 2024a).

Pendimethalin concentrations were most abundant in the rice seedlings followed by the soil and least observed in the water in water-seeded rice. Pendimethalin behavior in the soil-water interface suggest concentrations in the soil are greatest and residue in water is not of concern for rice injury. However, pendimethalin degradant concentrations did increase in the water by 14 DAT. Pendimethalin degradants were not abundant in all soil-water-tissue interfaces and suggest the parent molecule to remain intact for the most part; however, p36 was most observed of all degradants evaluated in this study and is probably the most stable degradant in the water-seeded environment. Metabolism of pendimethalin in tissue was not a method of tolerance by the rice seedlings. Absorption of pendimethalin was most concentrated in the crown region and shoots of the seedlings at all stages and application timings. However, rice seedling stage was the important factor to tolerate the pendimethalin applications. Pendimethalin applied at 1-leaf stage rice resulted greatly injured. At 3-leaf stage, pendimethalin reduced shoot length in the rice seedlings. Tolerance to pendimethalin is observed at the 5-leaf stage rice application. The results may suggest establishing a strong root system early on could allow for greater tolerance to pendimethalin applied post-emergence in water-seeded rice.

Supplementary Materials

Supp. Material 1: Air and water temperature in the greenhouse after an application of pendimethalin in water-seeded rice at different rice growth stages.

Supp. Material 2: Analytical methods for quantification of pendimethalin and degradants p36, p44 and p48 in water samples after an application in water-seeded rice.

Supp. Material 3: Analytical methods for quantification of pendimethalin and degradants p36, p44 and p48 in soil samples after an application in water-seeded rice.

Supp. Material 4: Analytical methods for quantification of pendimethalin and degradants p36, p44 and p48 in rice shoot, crown region and root tissue samples after an application at three different growth stages in water-seeded rice.

Acknowledgments

The authors acknowledge the Multi-User Analytical lab in Clemson University which assisted with the analytical analysis of this study.

Funding

The study was funded by the California Rice Research Board. The authors also acknowledge the D. Marlin Brandon Rice Research Fellowship by the California Rice Research Trust, the Horticulture and Agronomy Graduate Group scholarships including the Bert and Nell Krantz Fellowship and the Jack Pickett Agricultural Scholarship, the William G. and Kathleen Golden International Agricultural Fellowship, and the Department of Plant Sciences, UC Davis for the award of a GSR scholarship funded by endowments, particularly the James Monroe McDonald Endowment, administered by UCANR which supported the first author as a student during the study.

Competing Interests

Competing interests: The authors declare none.

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Table 1. Pendimethalin and degradant concentrations observed in water samples after application at three different water-seeded rice stages in plastic pots in a controlled environment^{abc}

Application stage	Sample timing	Observed concentration		Relative concentration			
		Pendimethalin		P36		P44	
		$\mu\text{g L}^{-1}$					
1 LSR	1 DAT	0.404	bc	0.012	e	<LLOQ	
1 LSR	5 DAT	0.124	c	0.040	cde	<LLOQ	
1 LSR	14 DAT	0.157	c	0.223	ab	0.019	b
3 LSR	1 DAT	1.058	a	0.060	bcd	0.002	c
3 LSR	5 DAT	0.327	bc	0.244	a	0.037	ab
3 LSR	14 DAT	0.270	bc	0.261	a	0.068	a
5 LSR	1 DAT	0.655	ab	0.036	de	0.007	c
5 LSR	5 DAT	0.327	bc	0.095	abcd	0.058	a
5 LSR	14 DAT	0.208	bc	0.137	abc	0.055	a

^aValues with the same letter within the same column are not significantly different with Tukey's HSD $\alpha=0.05$.

^bPendimethalin degradant p36 and p44 values were log transformed to fulfill linear regression assumptions and back transformed for presentation. Degradant values are relative concentration of recovered pendimethalin. Pendimethalin degradant p48 was <LLOQ in many water samples, only 11 samples were within the detection range.

^cLSR, leaf stage rice; DAT, days after treatment; <LLOQ, less than the lowest level of quantification; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole; pendimethalin degradant p44, 4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid; p48 [4,5-dimethyl-3-nitro-N²-(pentan-3-yl) benzene-1,2-diamine].

Table 2. Pendimethalin concentrations observed in soil samples after application at three different water-seeded rice stages in plastic pots in a controlled environment^{abc}

Factors	Pendimethalin concentration	
Application stage	$\mu\text{g total soil weight}^{-1}$	
1 LSR	3,590	a
3 LSR	2,930	b
5 LSR	2,940	b
Sample timing		
1 DAT	3,520	a
5 DAT	3,220	a
14 DAT	2,730	b

^aValues with the same letter within the same column and within each factor are not significantly different with Tukey's HSD $\alpha=0.05$. Application stage is averaged over sample timing and sample timing is averaged over application timing.

^bAnalysis of variance of factors resulted in application stage, $P<0.001$, sample timing, $P<0.001$, and interaction of application stage with sample timing $P=0.728$.

^cLSR, leaf stage rice; DAT, days after treatment.

Table 3. Relative concentration of pendimethalin degradant p36 observed in soil samples after application at three different water-seeded rice stages in plastic pots in a controlled environment^{abc}

Application stage	Sample timing	P36	
		$\mu\text{g g}^{-1}$	
1 LSR	1 DAT	0.143	cd
1 LSR	5 DAT	0.356	ab
1 LSR	14 DAT	0.281	abc
3 LSR	1 DAT	0.337	ab
3 LSR	5 DAT	0.368	a
3 LSR	14 DAT	0.206	bcd
5 LSR	1 DAT	0.335	ab
5 LSR	5 DAT	0.249	abcd
5 LSR	14 DAT	0.092	d

^aValues with the same letter within the same column are not significantly different with Tukey's HSD $\alpha=0.05$.

^bDegradants are presented as relative concentration of recovered pendimethalin. Only 4 samples were within the detection range for p48 and the remaining were <LLOQ. All samples were for <LLOQ for p44.

^cLSR, leaf stage rice; DAT, days after treatment; <LLOQ, less than the lowest level of quantification; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole.

Table 4. Observed pendimethalin concentration in different rice tissue regions after application at three different growth stages in water-seeded rice in plastic pots in a controlled environment^{ab}

Application stage	Rice seedling region	Observed concentration at different sample dates					
		1 DAT		5 DAT		14 DAT	
		$\mu\text{g seedling}^{-1}$					
1 LSR	Shoot	0.047	abc	0.049	bc	0.088	c
1 LSR	Crown region	NA		NA		NA	
1 LSR	Root	0.012	bcd	0.014	c	0.053	c
3 LSR	Shoot	0.083	abc	0.185	a	0.208	a
3 LSR	Crown region	0.066	abc	0.168	a	0.191	ab
3 LSR	Root	<LLOQ		0.063	bc	0.086	c
5 LSR	Shoot	0.091	ab	0.132	ab	0.187	a
5 LSR	Crown region	0.107	a	0.147	a	0.203	a
5 LSR	Root	0.021	cd	0.061	c	0.117	bc

^aValues with the same letter within the same column are not significantly different with Tukey's HSD $\alpha=0.05$. Interaction of application stage by seedling region was significant, $P=0.021$.

^bLSR, leaf stage rice; DAT, days after treatment; NA, not applicable crown region was not samples at 1 LSR; <LLOQ, less than the lowest level of quantification

Table 5. Relative concentration to pendimethalin degradant p36 observed in different rice tissue regions after application at three different growth stages in water-seeded rice in plastic pots in a controlled environment^{abc}

Application stage	Sample timing	Observed concentration at different rice seedling region					
		Shoot		Crown region		Root	
		$\mu\text{g g}^{-1}$					
1 LSR	1 DAT	0.003	ab	NA		0.001	bcd
1 LSR	5 DAT	0.003	ab	NA		0.001	abcd
1 LSR	14 DAT	0.007	a	NA		0.003	ab
3 LSR	1 DAT	0.0009	cd	0.004	c	0.0008	de
3 LSR	5 DAT	0.005	a	0.025	a	0.004	a
3 LSR	14 DAT	0.003	ab	0.015	ab	0.002	ab
5 LSR	1 DAT	0.0004	d	0.001	d	0.0003	e
5 LSR	5 DAT	0.001	bc	0.003	c	0.0009	cd
5 LSR	14 DAT	0.002	ab	0.007	bc	0.002	abc

^aValues with the same letter within the same column are not significantly different with Tukey's HSD $\alpha=0.05$. Interaction of application stage by sample timing was significant, $P<0.001$, and not significant across application stage by seedling region, $P=0.137$.

^bMean values were log transformed to fulfill model parameters and mean comparison. Values were back transformed for presentation.

^cLSR, leaf stage rice; DAT, days after treatment; NA, not applicable; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole.

Table 6. Rice seedlings shoot and root reduction after application of pendimethalin at three different growth stages and three sample dates in water-seeded rice in plastic pots in a controlled environment^{abc}

Factor	Root length		Shoot length	
Application stage	% Reduction of the nontreated			
1 LSR	16	a	13	a
3 LSR	7	b	6	ab
5 LSR	6	b	4	b
Sample timing				
1 DAT	9	ab	3	b
5 DAT	6	b	9	ab
14 DAT	15	a	13	b

^aValues with the same letter within the same column and within each factor are not significantly different with Tukey's HSD $\alpha=0.05$.

^bApplication stage is averaged over sample timing and sample timing is averaged over application timing. Original values were measured in millimeter.

^cLSR, leaf stage rice; DAT, days after treatment.

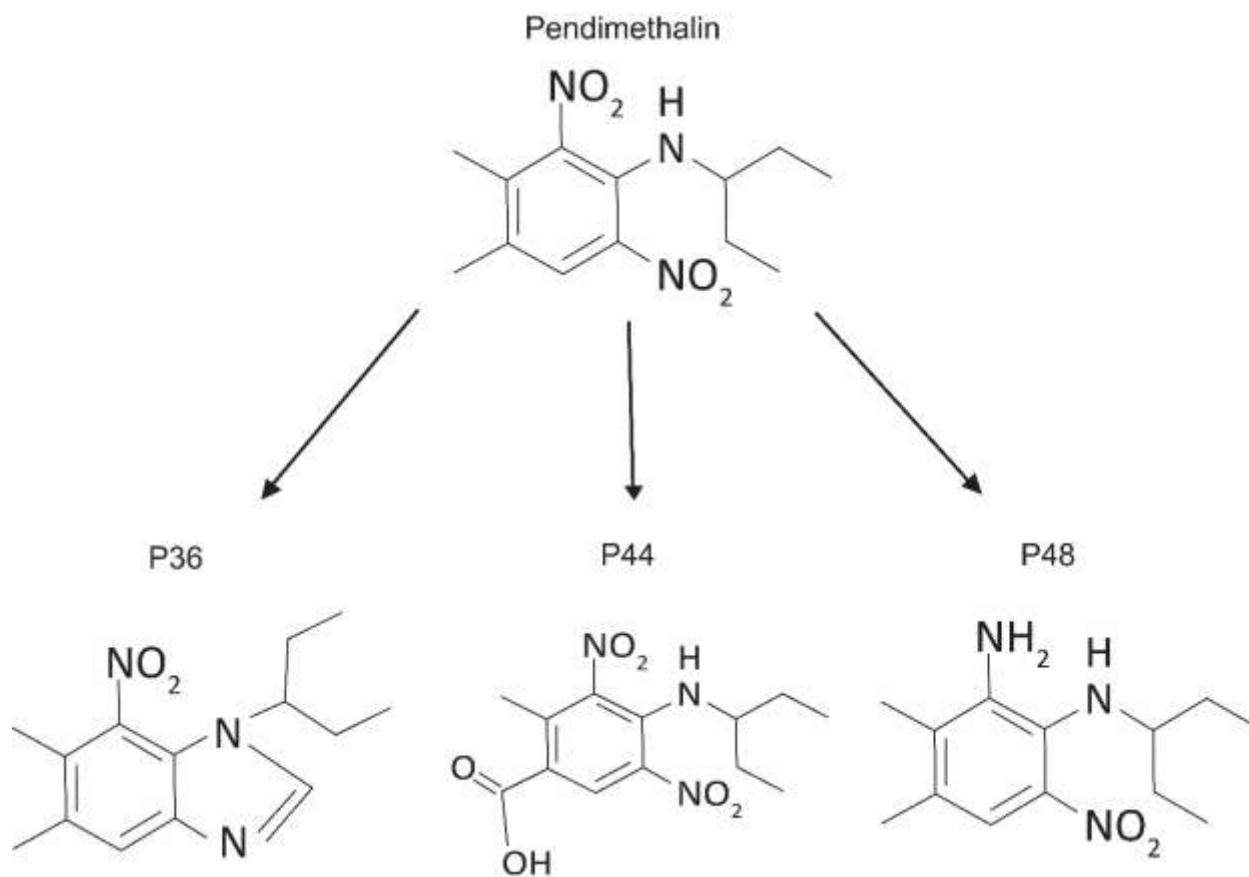


Figure 1. The chemical structure of pendimethalin and pendimethalin degradants observed in the water-seeded rice environment. Pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine; p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole; p44, 4-[(1-ethylpropyl) amino]-2-methyl-3,5-dinitrobenzoic acid; p48, 4,5-dimethyl-3-nitro-N²-(pentan-3-yl) benzene-1,2-diamine. Adapted from USEPA (2013a) and USEPA (2013b).

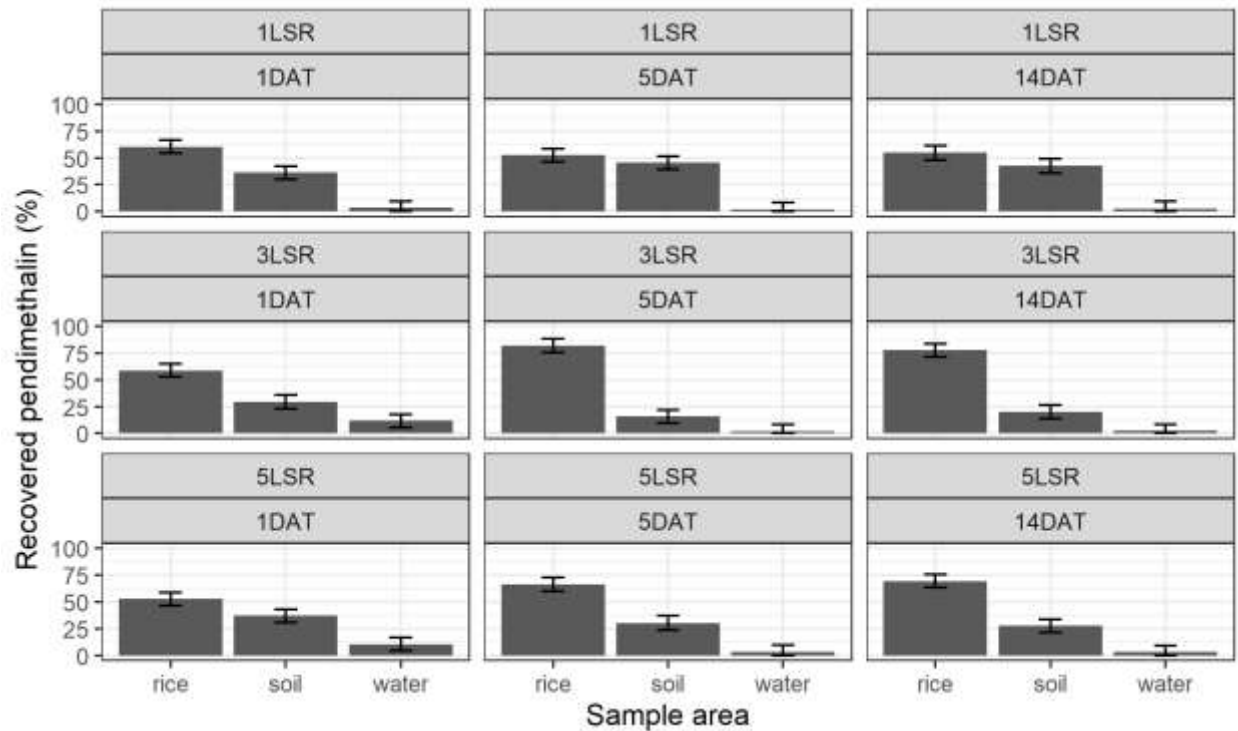


Figure 2. Total percent pendimethalin recovered in rice, soil and water after application at 1-, 3, and 5-leaf stage rice, sampled at 1, 5, and 14 days after application in water-seeded rice in plastic pots in a controlled environment. LSR, leaf stage rice; DAT, days after treatment. Pendimethalin, N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine.

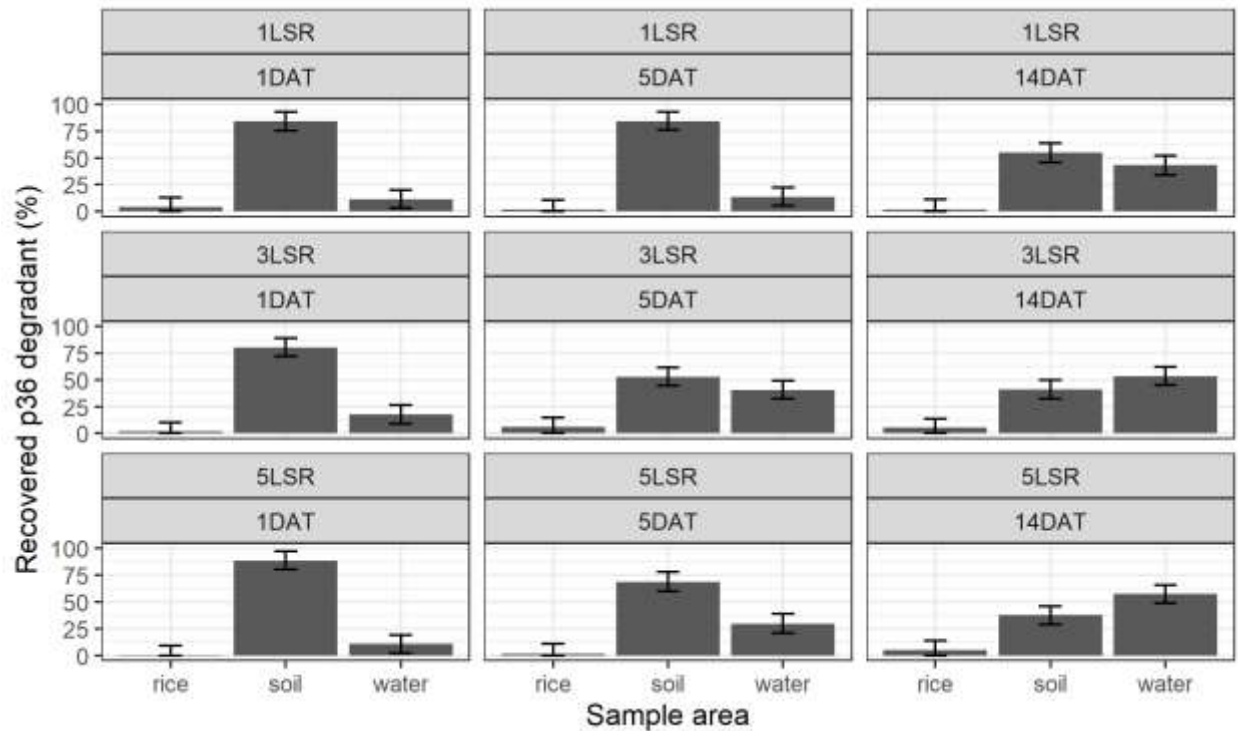


Figure 3. Total pendimethalin degradant, p36, recovered in rice, soil and water after application at 1-, 3-, and 5-leaf stage rice, sampled at 1, 5, and 14 days after treatment in water-seeded rice in plastic pots in a controlled environment. LSR, leaf stage rice; DAT, days after treatment; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole.