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Water-seeded rice seedling response to soil–water partitioning of pendimethalin

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Research Article

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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 to 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 d after treatment (DAT). More than 50% of recovered pendimethalin was observed in the rice tissue and more than 25% in the soil, with the least amounts 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-benximidazole]; p44, [4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid]; and p48 [4,5-dimethyl-3-nitro-N^2-(pentan-3-yl) benzene-1,2-diamine]. Degradant p36 was observed in all samples and most abundant in the soil. Degradants p36 and p44 increased in concentration in the water by 14 DAT. 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 with 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%, respectively, 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.

Introduction

Rice (*Oryza sativa* L.) is a staple crop worldwide (Chauhan et al. 2017). In the United States, rice is produced in the Midsouth and the California Sacramento Valley (USDA-FAS 2023). U.S. rice is a major commodity of high quality with an export value of nearly US$1.7 billion (USDA-FAS 2023). Therefore, U.S. rice production must maintain the standard production levels to fulfill the global need (Chauhan et al. 2017; USDA-FAS 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 with 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 nonaquatic weed species (Chauhan et al. 2017; Hill et al. 2006). However, years of continuous rice cultivation have shifted weed populations to flood-tolerant weeds, leading to increased difficulty in weed management (Becerra-Alvarez et al. 2023; Hill et al. 2006).

An overreliance on herbicides for weed control has selected for herbicide-resistant weedy grasses (*Echinochloa* spp. and bearded sprangletop [*Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow]), sedges [sammflower umbrella sedge (*Cyperus difformis* L.) and bog bulrush [*Schoenoplectus mucronatus* L. Palla; syn.: *Schoenoplectiella mucronata* (L.) J. Jung & H.K. Choi], 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

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manage herbicide-resistant populations through practices like mode of action rotations and mixtures (Beckie and Reboud 2009).

Pendimethalin is a mitosis-inhibiting herbicide from the dinitroaniline chemistry that inhibits seedling growth shortly after germination (Appleby and Valverde 1989). It was successful in controlling local herbicide-resistant weedy grass populations in a greenhouse herbicide screening (Becerra-Alvarez and Al-Khatib 2024b); however, few 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 to rice after the 3- to 4-leaf stage compared with rice at the 1-leaf stage (Becerra-Alvarez and Al-Khatib 2024b). Rice response was rate dependent, with 2.3 kg ai ha^{-1} rate of pendimethalin resulting in acceptable rice response and weed control compared with lower and higher application rates (Becerra-Alvarez and Al-Khatib 2024b). 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 establishing effective and environmentally practical practices (Becerra-Alvarez and Al-Khatib 2024a; 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 anerobic or flooded conditions because of lower movement of the vapor phase in the wet soil compared with dry soil, in which 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 (Barrett and Lavy 1983; Chen et al. 2021; Weber 1990). Previous research suggests the pendimethalin parent molecule is the abundant remaining residue in soils (Appleby and Valverde 1989; Parka and Soper 1977; Weber 1990). However, advances in chromatographic technology allow quantification of 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 creates the degradant p44 [4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid], which is commonly found in soil and water (USEPA 2013a, 2013b). The substitution of a nitro group for an amino group creates degradant p48 [4,5-dimethyl-3-nitro-N^2-(pentan-3-yl) benzene-1,2-diamine], which is common in water but less common in soil; while the cyclization between the nitro groups creates p36 [1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benxi-midazole], which has been most commonly observed in soils (USEPA 2013a, 2013b).

Dinitroanilines possess physicochemical characteristics that create a molecule greatly attracted to organic matter and will typically remain on the topsoil surface after an application (Makkar et al. 2020). 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 creates an environment for injury potential. The postemergence 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 2024b). Dinitroaniline herbicides typically need to be absorbed at the plant growth 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 (Khaliq and Matloob 2012; Koger et al. 2006). Koger et al. (2006) observed cultivar tolerance differences in which shorter mesocotyl lengths led to less injury in drill-seeded rice seedlings by avoiding direct contact with the herbicide on the soil surface compared with 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 the fate of dinitroaniline herbicides inside rice seedlings only measured amino acid content after an application but did not observe 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 postemergence 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 University of California Davis Orchard Park Greenhouses (38.5425°N, 121.7631°W). Natural lighting was supplemented with 1,000-W metal-halide lamps providing 400 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density 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, Bourne, MA) (Supplementary Material 1).

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Plastic pots (11.4-cm wide by 11.4-cm long by 15-cm high) with no drain openings 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 before sowing of 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 broadcast by hand to each pot. The soil was maintained saturated, and after 3 d, a 10-cm flood above the soil surface was added and maintained for the remainder of the study by continuously adding water every 24 h. The 1-leaf stage application was maintained at 40 seeds per pot. Before 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 7 d. Experiments were repeated three times, initiated on September 13, November 5, and December 6, 2023, respectively.

Herbicide Application

Pendimethalin (Prowl® H_2O, BASF, Florham Park, NJ) in a capsule suspension formulation was applied to the respective pots at the 1-, 3-, and 5-leaf stages of 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 before 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 of rice, pendimethalin was applied over the top of the 40 rice seedlings in each pot. At the 3- and 5-leaf stage of rice, pendimethalin was directly applied on the soil surrounding the 20 rice seedlings in each pot. The floodwater 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 d after treatment (DAT). The pots were sampled by first pouring the floodwater into 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 the soil was gently washed from the roots. The soil in the pot was poured out, homogenized, and laid flat in the greenhouse to air-dry for up to 3 d. 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 until 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 lengths were recorded from three randomly selected seedlings in each pot. Each seedling was then separated into shoot and root after the 1-leaf stage application, and into 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 being flash frozen 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.

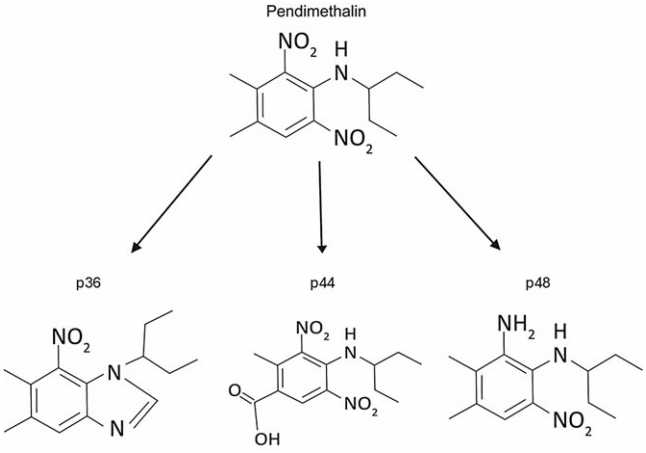

Figure 1. The chemical structure of pendimethalin and pendimethalin degradants observed in the water-seeded rice environment. Pendimethalin: *N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine; p36: 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benximidazole; p44: 4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic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

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-benximidazole], p44 [4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid], and p48 [4,5-dimethyl-3-nitro-N^2-(pentan-3-yl) benzene-1,2-diamine] (Figure 1; Supplementary Material 2–4).

The extraction and quantification of pendimethalin in water and soil samples was conducted referencing the U.S. Environmental Protection Agency methods (USEPA 2013a, 2013b) and previous methods for water samples (Becerra-Alvarez and 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 (Supplementary 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 with 20 rice seedlings in the 3- and 5-leaf stage applications. Pendimethalin in rice tissue was quantified with an internal standard compared with 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 (Supplementary Material 4).

Data Analysis

The concentrations of pendimethalin and relative degradants in extracts were converted (to μg L^{-1} in water) with the following equation:

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$$R = C_{end} \times \frac{V_{Fex}}{V_{Iex}} \times 1,000 \times 0.001 \quad [1]$$

where R is the analyte residue (μg L^{-1}), C_{end} is the concentration of analyte in the final sample (ηg L^{-1}), V_{Fex} is the volume of final extract (ml), and V_{Iex} is the volume of initial extract (ml). The volume of the final extract was 0.8 ml extracted from the initial 10-ml water subsample. The concentration in the soil samples was converted (to μg g^{-1} of soil) with the following equation:

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

where R is the analyte residue (μg g^{-1}) C_{end} is the concentration of analyte in the final sample (ηg ml^{-1}), DF is the dilution factor, V_{Fex} is the volume of final extract (ml), and W_s is the weight of initial soil sample (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 was converted (to μg g^{-1} of fresh weight) with the following equation:

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

where R is the analyte residue (μg g^{-1}), C_{end} is the concentration of analyte in the final sample (ηg ml^{-1}), V_{Fex} is the volume of final extract (ml), and W_{fw} is the fresh weight of tissue sample (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 were analyzed with regression and Tukey's honestly significant difference at $\alpha = 0.05$ where applicable using R v. 4.4.0 (Kuznetsova et al. 2017; R Core Team 2024).

Results and Discussion

Total Soil–Water–Rice Partitioning

Pendimethalin was quantified with an internal standard of pendimethalin-d_5 and external calibration curves were determined 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 concentrations 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 (Supplementary Material 2 and 3). The average recoveries for the shoot, crown region, and root were 89%, 87%, and 91%, respectively (Supplementary Material 4).

The data from water, soil, and rice tissue on the recovered pendimethalin were combined to compare pendimethalin concentrations. More than 50% of recovered pendimethalin was observed in the rice tissue across the three application timings (Figure 2). Pendimethalin was observed second-most in the soil, and less than 20% of pendimethalin was observed in the floodwater (Figure 2). The results are not surprising, because pendimethalin's physiochemical 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. 2020; 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 is 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 (Appleby and Valverde 1989; Chen et al. 2021; Parka and Soper 1977).

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 observed for the 3- and 5-leaf stage applications (Figure 2). The absence of available lipids for pendimethalin to partition to the rice seedling at the 1-leaf stage would have resulted in pendimethalin partitioning to the soil (Vighi et al. 2017).

The p36 degradant was the only degradant observed in quantifiable levels in all three sample types. Degradant p36 was found in greater concentrations in the soil and water samples compared with the rice tissue (Figure 3). More than 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 to similar levels in water samples (Figure 3). The low or lack of observed degradants supports the strong bonding behavior of pendimethalin to lipids or organic matter (Weber 1990). 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 (Appleby and Valverde 1989; Pal et al. 1991). 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 value observed was 1.058 μg L^{-1} at 1 DAT (Table 1). At 14 DAT, pendimethalin residue in water was 0.124 to 0.270 μg L^{-1} (Table 1). The values observed in this study at late sampling dates agree with field studies by Becerra-Alvarez and Al-Khatib (2024a); however, samples at 1 DAT did not result in similar concentration levels. In the field study, a pendimethalin capsule suspension was applied onto the water of a water-seeded rice field environment, which recorded 10.9 μg L^{-1} of residue in the water at 1 DAT and 0.3 μg L^{-1} residue in water by 15 DAT (Becerra-Alvarez and Al-Khatib 2024a). In this greenhouse study, pendimethalin was applied on the soil, which was then reflooded; this 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 degradant p48 fell below the lowest level of quantification. There were only 11 samples that were quantified for degradant p48 from 0.009 to 0.05 μg L^{-1}. The p36 and p44 degradants increased after the first sample date and were up to 0.261μg L^{-1} for p36 and up to 0.068 μg L^{-1} for p44 at 14 DAT

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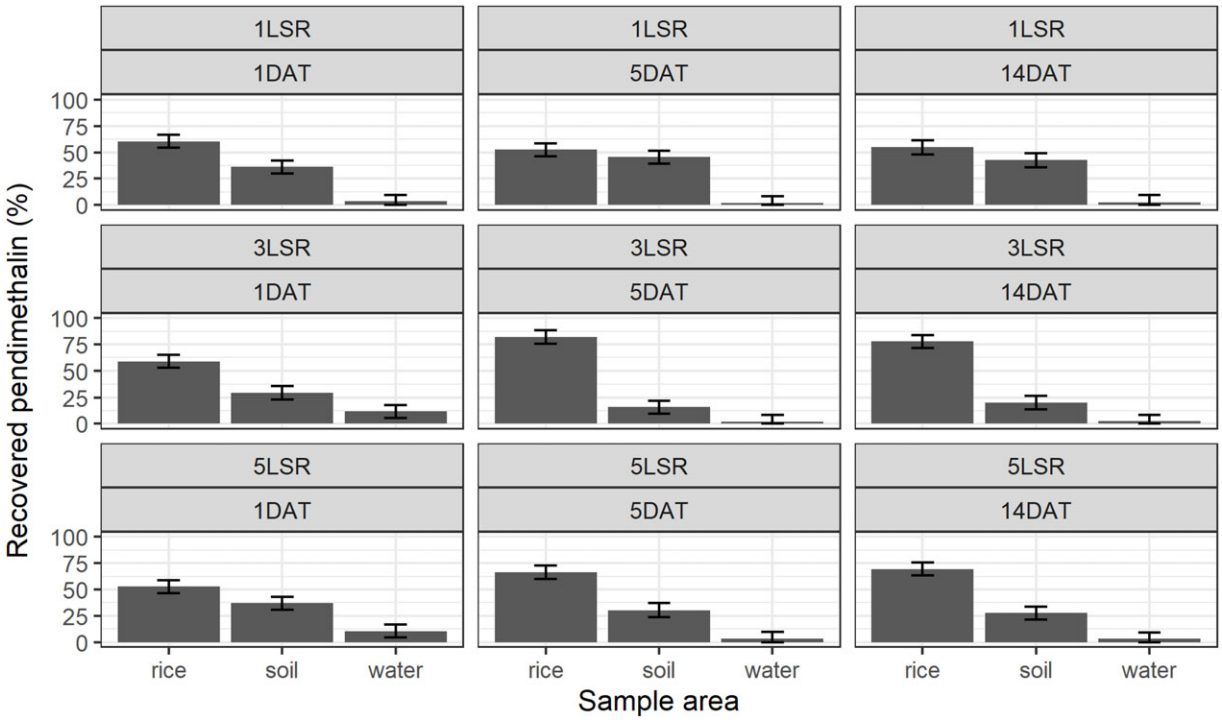

Figure 2. Total percentage of pendimethalin recovered in rice, soil, and water after application at 1-, 3, and 5-leaf stage rice (LSR), sampled at 1, 5, and 14 d after treatment (DAT) in water-seeded rice in plastic pots in a controlled environment. Pendimethalin: *N*-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine.

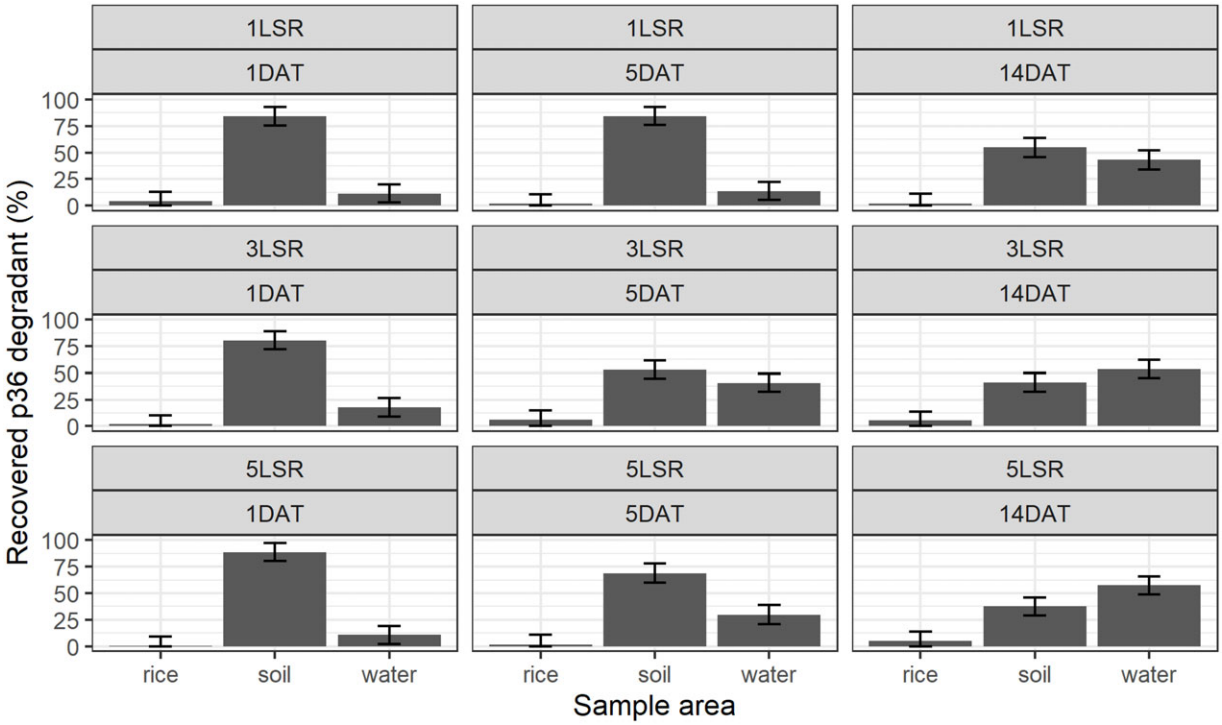

Figure 3. Total pendimethalin degradant p36 recovered in rice, soil, and water after application at 1-, 3-, and 5-leaf stage rice (LSR), sampled at 1, 5, and 14 d after treatment (DAT) in water-seeded rice in plastic pots in a controlled environment. Pendimethalin degradant p36: 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benximidazole.

(Table 1). The degradant synthesis pathway is most likely through photodegradation in the water (Pal et al. 1991; Vighi et al. 2017).

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 (Lehotay et al. 1998; Zimmerman et al. 2000). However, a

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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.[a,b]

Application stage	Sample timing	Observed concentration	Relative concentration	
		Pendimethalin	p36	p44
		μg 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

[a]DAT, days after treatment; <LLOQ, less than the lowest level of quantification; LSR, leaf stage rice; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benximidazole; pendimethalin degradant p44, 4-[(1-ethylpropyl) amino]-2-methyl-3,5-ditrobenzoic acid; pendimethalin degradant p48 [4,5-dimethyl-3-nitro-N^2-(pentan-3-yl) benzene-1,2-diamine].
[b]Values with the same letter within the same column are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$. Pendimethalin 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.

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

Factors[b]	Pendimethalin concentration
Application stage	μg 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

[a]Values with the same letter within the same column and within each factor are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$. Application stage is averaged over sample timing, and sample timing is averaged over application timing.
[b]ANOVA of factors resulted in application stage, $P < 0.001$, sample timing, $P < 0.001$, and interaction of application stage with sample timing $P = 0.728$. LSR, leaf stage rice; DAT, days after treatment.

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 2024a).

Soil Partitioning

Pendimethalin soil persistence was similar across the interaction of the 1-, 3-, and 5-leaf stage timings and sample timings 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 bind to soils (Helling and Krivonak 1978; Weber 1990). Previous research investigated partitioning behavior of pendimethalin on sediment in water/sediment dark environments, which demonstrated 50% allocation of pendimethalin onto sediments occurring within 0.4 to 1.6 d (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 in pendimethalin concentrations in the soil at 14 DAT averaged over application stages (Table 2). Barrett and Lavy (1983) demonstrated that pendimethalin dissipation is affected by soil–water content and that pendimethalin dissipated more rapidly in alternate flooding conditions than in continuously flooded conditions. Makkar et al. (2020) recorded pendimethalin in the soil of dry-seeded and transplanted rice fields dissipating rapidly early on and then 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 whether the pendimethalin in the soil continues to have herbicidal activity or is bound to the soil matter (Helling and Krivonak 1978). Helling and Krivonak (1978) 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). Degradant p48 was observed only in four soil samples at 0.002 μg g^{-1}. The p36 concentrations were relatively similar across application and sample timings; however, concentration did decrease by 14 DAT compared with the 1 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 al. (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 1 DAT was similar across application stages and seedling regions from 0.012 to 0.083 μg per seedling (Table 4). Pendimethalin concentration in the seedlings increased by 14 DAT, 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 and Wax (1968) demonstrated the early shoot region was the greatest area of trifluralin (a dinitroaniline herbicide) absorption in giant foxtail (*Setaria faberii* Herrm.), 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 μg g^{-1} similarly across the tissue regions; however, values were different across sample timings, $P < 0.001$. There was an increase in concentration by 14 DAT of p36 (Table 5). Despite the increase, the concentration was relatively low and demonstrates the majority of the pendimethalin parent molecule remains intact in the rice tissue. Degradant p48 was similarly observed in samples at very low concentrations from 0.0002 to 0.003 μg 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 μg g^{-1} or below the lowest level

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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.[a,b]

Application stage	Sample timing	p36[c]
		μg 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

[a]LSR, 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-benximidazole.
[b]Degradants are presented as relative concentration of recovered pendimethalin. Only four samples were within the detection range for p48, and the remaining were <LLOQ. All samples were <LLOQ for p44.
[c]Values with the same letter within the same column are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$.

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.[a]

Application stage	Rice seedling region	Observed concentration at different sample dates[b]		
		1 DAT	5 DAT	14 DAT
		μg 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

[a]LSR, leaf stage rice; DAT, days after treatment; NA, not applicable (crown region was not sampled at 1 LSR); <LLOQ, less than the lowest level of quantification.
[b]Values with the same letter within the same column are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$. Interaction of application stage by seedling region was significant, $P = 0.021$.

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.[a]

Application stage	Sample timing	Observed concentration at different rice seedling region[b]		
		Shoot	Crown region	Root
		μg 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

[a]LSR, leaf stage rice; DAT, days after treatment; NA, not applicable; pendimethalin degradant p36, 1-(1-ethylpropyl)-5,6-dimethyl-7-nitro-1H-benximidazole.
[b]Values with the same letter within the same column are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$. Interaction of application stage by sample timing was significant, $P < 0.001$, and not significant across application stage by seedling region, $P = 0.137$. Mean values were log transformed to fulfill model parameters and mean comparison. Values were back-transformed for presentation.

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.[a,b,c]

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

[a]LSR, leaf stage rice; DAT, days after treatment.
[b]Values with the same letter within the same column and within each factor are not significantly different with Tukey's honestly significant difference $\alpha = 0.05$.
[c]Application stage is averaged over sample timing, and sample timing is averaged over application timing. Original values were measured in millimeters.

of quantification (data not shown). An interesting observation was that degradant p44 was not observed in shoot tissue, and observed concentrations occurred 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 demonstrate greater concentration in the shoots at all timings (Table 4). Knake and Wax (1968) demonstrated the shoot absorption of trifluralin was most limiting to growth compared with root absorption of trifluralin by *S. faberii* seedlings. Pendimethalin concentrations were considerably similar in the crown regions and shoots, indicating pendimethalin can translocate acropetally to some degree. However, application stage appeared to be 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 similar to the nontreated (Table 6). These observations were similar to 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 2024b). 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) (Malefyt and Duke 1984). Therefore, application timing is most important for water-seeded rice seedling tolerance of 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

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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 increased lipid contents, unlike most broadleaves (Ndon and Harvey 1981), there are lipids like suberin layers in rice seedling roots that 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 2024b).

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 suggests 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 remains for the most part intact; however, p36 was the 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 for tolerance of pendimethalin applications. Pendimethalin applied to 1-leaf stage rice resulted in great injury. At the 3-leaf stage, pendimethalin reduced shoot length in the rice seedlings. Tolerance to pendimethalin was observed at the 5-leaf stage rice application. These results may suggest establishing a strong root system early on could allow for greater tolerance to pendimethalin applied postemergence in water-seeded rice.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/wsc.2024.96

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