

Responses of rice genotypes to foliar-applied metribuzin

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Research Article

Cite this article: Marsh SL and Al-Khatib K (2023) Responses of rice genotypes to foliar-applied metribuzin. *Weed Technol.* **37**: 537–544. doi: [10.1017/wet.2023.76](https://doi.org/10.1017/wet.2023.76)

Received: 5 August 2023
Revised: 29 September 2023
Accepted: 2 October 2023
First published online: 13 October 2023

Associate Editor:

Jason Bond, Mississippi State University

Nomenclature:

Metribuzin; rice, *Oryza sativa* L.; soybean, *Glycine max* (L.) Merr.; wheat, *Triticum aestivum* L.; potato, *Solanum tuberosum* L.; peas, *Pisum sativum* L.

Keywords:

Photosystem II-inhibiting herbicides; visible injury

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Abstract

The increasing development of herbicide resistance in weeds found in rice cropping systems has encouraged researchers to evaluate alternate herbicides to prevent and manage herbicide-resistant weed biotypes. Metribuzin is a photosynthetic-inhibiting herbicide that controls various important grass and broadleaf weeds. Several crops, including soybean, wheat, peas, and potato, have shown differential varietal responses to metribuzin. To determine whether rice has differential varietal responses to metribuzin for potential utilization in a rice breeding program, greenhouse experiments were conducted to evaluate the responses of 142 long-, medium-, and short-grain rice genotypes to the herbicide. Metribuzin was applied at 0, 22, 44, 88, 176, and 352 g ai ha⁻¹ when rice plants were in the 3- to 4-leaf stage. Crop response regarding phytotoxicity, height reduction, and biomass reduction was evaluated. Metribuzin caused significant injury to all rice genotypes tested, but short-grain rice genotypes were, on average, more susceptible than medium- and long-grain rice genotypes. Short-grain rice genotypes generally had greater height reduction and produced less biomass than long-grain or medium-grain rice genotypes. Crop visual injury ratings were correlated with plant height reductions and biomass reductions. The results indicate that the level of metribuzin tolerance in rice is inadequate for commercial use; however, further research is needed to develop higher levels of herbicide resistance by mutagenized rice cultivars.

Introduction

Rice is one of the most commonly grown agricultural commodities in the world (Childs 2022) and contributes significantly to sources of human energy across the globe (Kondhia et al. 2015). Global rice production is estimated to reach 467.2 million metric tons for the 2022 to 2023 year (Childs and LeBeau 2022). In the United States, long-grain *indica* rice accounts for almost 75% of rice production, and *japonica* medium-grain and short-grain rice production make up the remainder (Childs 2022). All U.S. rice is grown under irrigated conditions, which may vary by geographic distribution. The majority of California's rice production, which accounts for 200,000 ha, is grown in a continuously flooded cropping system, where rice is pregerminated and seeded by airplane onto fields with a 10- to 15-cm standing flood (Ceseski and Al-Khatib 2021; Espino et al. 2019).

Continuous flooding to suppress grass, sedge, and broadleaf weeds in rice fields is a method of weed control that has been extremely effective (Hill et al. 1994). However, decades of using continuous flooding, in addition to a lack of robust crop rotation in rice production areas, have selected weed species that exhibit ecological requirements and growing patterns that are similar to rice and can compete with rice resources (Hill et al. 1994). These flooded conditions favor weedy grasses that are well adapted to flood, which include watergrass species (*Echinochloa* spp.), bearded sprangletop [*Leptochloa fusca* (L.) Kunth ssp. *fascicularis* (Lam.) N. Snow], and weedy rice (*Oryza sativa* f. *spontanea* Rosh.) (Brim-DeForest et al. 2017; Ceseski et al. 2022).

Crop yields and harvest quality face the highest biological constraints because of weed infestations, and farmer inputs to weed management are expected to increase as herbicide resistance spreads worldwide (Brim-DeForest et al. 2017). Certain weeds and weed groups cause more yield loss than others, even at lower infestation densities (Smith 1988). In rice systems, grasses are considered the most difficult weeds to control owing to the narrow selectivity between the crop and the grass weeds (Carey et al. 1995). Rice yield losses can amount to 79% after season-long interference from barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] and have been recorded as high as 59% due to season-long competition with late watergrass [*Echinochloa phyllopogon* (Stapf.) Koso-Pol.] (Gibson et al. 2001; Smith 1968). Weedy rice is an increasingly problematic weed in rice-growing regions around the world, causing yield loss and contamination due to the critical weedy traits of seed shattering and seed dormancy (de Leon et al. 2019), which build up a large soil seed reservoir for future years (Ziska et al. 2015). The weedy rice infestation threshold is 1 to 3 plants m⁻², with higher ratios causing significant

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yield loss; weedy rice densities of 30 to 40 plants m^{-2} can reduce rice yields by 60% to 90%, depending on the height of the cultivar (Smith 1988; Ziska et al. 2015). In California, six biotypes of weedy rice have thus far been identified (de Leon et al. 2019). Infestations of weedy rice cause harvest quality problems, increased production costs, and reduced yield, so an effective method of control is needed (de Leon et al. 2019).

There are few options for weed control in California rice production. Although crop rotation would allow for alternative herbicides that may be able to manage resistant weeds, it is not a commonly used tactic in many rice production regions owing in part to the heavy clay or hardpan soils that typify many rice fields and result in low water drainage (Hill et al. 2006). Weed removal on field levees and ditches and the California statewide mandate on using certified clean seed assist in integrated weed management practices, but most rice growers rely solely on in-season herbicide applications and deepwater flooding for weed management (California Crop Improvement Association 2019; Hill et al. 2006).

Flooded rice agroecosystems are common worldwide in most rice production areas; however, certain regions have restrictions on the available herbicides to control weeds. For example, in California, largely owing to ecotoxicity concerns and strict regulatory structures (Cesek and Al-Khatib 2021; Hill et al. 1994), only 13 registered active ingredients across nine modes of action (MOAs) are available for use in flooded rice fields, which creates few opportunities for herbicide rotation to inhibit herbicide resistance development (Espino et al. 2019). By contrast, 60 active ingredients are registered for use in corn in the U.S. Midwest (Gerber 2021). Current herbicides in use in California rice systems include acetolactate synthase (ALS) inhibitors, protoporphyrinogen oxidase inhibitors, acetyl CoA carboxylase (ACCase) inhibitors, tubulin inhibitors, photosystem II (PSII) inhibitors, very-long-chain fatty-acid (VLCFA) inhibitors, auxin-mimics, 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors, and 1-deoxy-D-xylulose 5-phosphate inhibitors (Espino et al. 2019). Rice herbicides require proper selection in combination and sequence to provide adequate weed control. Early-season grass control applications commonly consist of field rates of carotenoid biosynthesis, HPPD, ALS, or VLCFA inhibitors (Brim-DeForest 2021). Late-season cleanup applications often use PSII, ALS, or ACCase inhibitors to control later-emerging grasses (Brim-DeForest 2021).

The continuous use of herbicides with similar MOAs has contributed to herbicide resistance evolution in several weeds found in rice systems. California arrowhead (*Sagittaria montevidensis* Cham. & Schltdl.) and smallflower umbrellasedge (*Cyperus difformis* L.) were the first confirmed cases of rice weeds with resistance to bensulfuron-methyl, an ALS inhibitor, in 1993 (Busi et al. 2006). Since then, eight other rice weed species have been identified, some with resistance to more than one MOA (Becerra-Alvarez and Al-Khatib 2022). The rise in herbicide resistance has increased the cost and difficulty of weed management, necessitating demand for novel herbicide development to postpone resistance expansion and assist in managing current herbicide-resistant weed biotypes (Qu et al. 2021).

Metribuzin is a selective and systemic herbicide that controls many broadleaf and some grass weeds (Armendáriz et al. 2014). Metribuzin is a PSII inhibitor that belongs to the triazinone family and functions by binding to the Q_B binding site on the D1 protein of the PSII complex in the chloroplast thylakoid membranes. Once the chemical binds to the site, electron transport from Q_A to Q_B is blocked, and CO_2 fixation and adenosine triphosphate

and nicotinamide adenine dinucleotide phosphate diaphorase production are stopped, halting necessary resources for plant growth (Lambrevia et al. 2014). Foliar-applied metribuzin is absorbed into the plant at moderate rates with apoplastic translocation.

To date, metribuzin is labeled for use in alfalfa (*Medicago sativa* L.), asparagus (*Asparagus* L.), cereals, field corn (*Zea mays* L.), garbanzo bean (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), peas, potatoes, sainfoin (*Onobrychis viciifolia* Scop.), soybean, sugarcane (*Saccharum officinarum* L.), and tomato (*Solanum lycopersicum* L.). Metribuzin has been successfully used to control broadleaf and grass weeds in wheat (Javaid et al. 2022) and barley (*Hordeum vulgare* L.) (Volova et al. 2020). There is no label for metribuzin for use in rice in California. Although information regarding the effect of metribuzin application rates and timing on weed control in rice is scant, recent studies from Mississippi have indicated that metribuzin applied post rice emergence at 42 g ai ha^{-1} caused 36% injury by 28 d after treatment (DAT) (Lawrence et al. 2021). The same study found no correlation between rice injury from metribuzin and yield reduction, dry weight reduction, maturity delays, or seed germination (Lawrence et al. 2021). Mahajan and Chauhan (2022) evaluated metribuzin at rates 72 and 144 g ai ha^{-1} and were able to reduce jungle rice [*Echinochloa colona* (L.) Link] biomass by 70% and 100%, respectively, compared to the untreated control.

Crop tolerance to herbicides may result from the ability of a crop to metabolize the chemical (Wright et al. 2021). Selectivity differences among genotypes depends on accumulation of a critical amount of the active ingredient at the target site of action and a sufficient differential in chemical uptake, in-plant movement, and arrival of the chemical at the correct location in the active form (Cole 1994). Although several factors may be involved in selectivity, the most imperative function is that of tolerant plants metabolizing and detoxifying herbicides rapidly and susceptible plants having reduced or no ability to do so (Cole 1994).

Differential tolerance responses of soybean, pea, and wheat genotypes to foliar-applied metribuzin have been noted (Al-Khatib et al. 1997; Barrentine et al. 1976; Hardcastle 1974; Javaid et al. 2022). In rice, cultivar-specific responses to herbicide treatments have been previously identified after parent material was mutagenized using ethyl methanesulfonate (Shoba et al. 2017). Herbicide-resistant rice lines, such as Clearfield® (BASF, Research Triangle Park, NC, USA) or FullPage® (ADAMA, Raleigh, NC, USA) and Provisia® (BASF) or Max-Ace® (ADAMA) rice, were developed using this genetic material, which conferred resistance to imidazolinones and quizalofop, respectively. Differing levels of sensitivity to triclopyr (Pantone and Baker 1992) and floryprauxifen-benzyl (Wright et al. 2021), synthetic auxin herbicides, have also been observed in various rice genotypes. The inherent genetic variability in rice genotypes may provide a resource for crop improvement through breeding (Okoshi et al. 2018).

There is a need for additional and alternative herbicide programs to complement sustainable chemical weed control in rice systems. Investigation of differential responses to a chemical can reveal susceptible and tolerant crop genotypes that may prove useful in breeding programs. With limited knowledge of the response of rice genotypes to metribuzin, the objectives of this research were to evaluate the response of various rice genotypes to post rice emergence-applied metribuzin and to determine if early-season injury symptoms from foliar metribuzin application are correlated with reduced shoot biomass.

| | Long grain | | Medium grain | | | Short grain | |
|--------------|------------|--------|--------------|---------|--------|-------------|--------|
| A-201 | RES7 | RES32 | CL271 | RES157 | RES329 | RES209 | RES245 |
| A-202 | RES8 | RES34 | M-105 | RES159 | RES333 | RES212 | RES247 |
| Calaroma-201 | RES9 | RES35 | M-205 | RES160 | RES340 | RES213 | RES251 |
| Calmati-201 | RES10 | RES36 | M-206 | RES161 | RES341 | RES214 | RES256 |
| Calmati-202 | RES11 | RES37 | RES107 | RES167 | RES342 | RES216 | RES257 |
| Cheniére | RES12 | RES38 | RES110 | RES171 | RES344 | RES218 | RES259 |
| Cocodrie | RES13 | RES39 | RES111 | RES172 | RES358 | RES220 | RES261 |
| Della-2 | RES14 | RES40 | RES114 | RES173 | RES359 | RES221 | S-102 |
| Jazzmin-2 | RES15 | RES41 | RES117 | RES174 | RES360 | RES223 | S-201 |
| L-201 | RES16 | RES42 | RES119 | RES175 | Titan | RES226 | S-301 |
| L-202 | RES17 | RES43 | RES120 | RES176 | | RES227 | |
| L-203 | RES19 | RES44 | RES121 | RES179 | | RES228 | |
| L-204 | RES20 | RES45 | RES125 | RES183 | | RES230 | |
| L-205 | RES21 | RES46 | RES130 | RES185 | | RES233 | |
| L-206 | RES22 | RES324 | RES131 | RES187 | | RES236 | |
| RES1 | RES24 | Rex | RES135 | RES190 | | RES237 | |
| RES2 | RES25 | Rondo | RES140 | RES199 | | RES238 | |
| RES3 | RES28 | | RES142 | RES2000 | | RES241 | |
| RES4 | RES29 | | RES146 | RES201 | | RES242 | |
| RES5 | RES30 | | RES153 | RES204 | | RES243 | |
| RES6 | RES31 | | RES154 | RES205 | | RES244 | |

Figure 1. Rice line and grain type for 142 genotypes used in the greenhouse study to evaluate the differential rice response to postemergence foliar-applied metribuzin. Plant material was sourced from the Rice Experiment Station, Biggs, CA.

Materials and Methods

Growing Conditions

Experiments were conducted during 2021 to 2022 in greenhouses at the Rice Experiment Station (RES) in Biggs, CA (39.45°N, 121.72°W). Plastic perforated flats measuring 28 × 54 × 6 cm were prefilled with a Esquon-Neerdobe (fine, smectitic, thermic Xeric Epiaquerts and Duraquerts) silty clay with a pH of 5.11 and 2.6% organic matter that was sieved through a 2-cm mesh. One hundred forty-two rice genotypes sourced from the RES representing long-grain, medium-grain, and short-grain rice were selected, and 15 seeds of each genotype were sown in rows in the flats, with eight rice genotypes per flat and each row serving as a single experimental unit (Figure 1). Flats were placed in large basins filled with 5 cm of standing water for irrigation. Plants were grown in greenhouse conditions with average day/night temperatures of 32/18 C and 16-h photoperiod with supplemental light intensity of 250 mmol m² s⁻¹ photosynthetic photon flux density.

Metribuzin Treatments

Rice seedlings at the 3- to 4-leaf stage were treated with 0, 22, 44, 88, 176, and 352 g ai ha⁻¹ metribuzin (Glory® 4L, ADAMA). Rates were 0X, 1/8X, 1/4X, 1/2X, 1X, and 2X the label rate for use in peas (Anonymous 2014). Treatments were applied with a research track bench sprayer (DeVries Manufacturing, Hollandale, MN, USA) equipped with a flat-fan TP8001EVS TeeJet® nozzle (TeeJet® Technologies, Wheaton, IL, USA) and calibrated to deliver

187 L ha⁻¹ at 180 kPa. Control plants were treated with water. Each flat was sprayed at a height of 45 cm above plant canopy.

Data Collection

Visible rice injury was rated at 7, 14, 21, and 28 DAT. Visible injury ratings were based on a scale where 0% was no injury and 100% was plant death. At 28 DAT, rice height was recorded by measuring the plant from top leaf to soil, and plant biomass was harvested by removing all aboveground tissue. Hand-harvested samples were dried at 65 C for 10 d and weighed. Biomass and height data were reported as percent biomass and height reduction and were calculated as

$$\% \text{ reduction} = \left[\frac{\text{UTC} - B}{\text{UTC}} \right] \times 100 \quad [1]$$

where UTC is the mean biomass (g) or height (cm) of the untreated control for each respective rice cultivar and *B* is the biomass (g) or height (cm) of the experimental unit of interest (Ortmeier-Clarke et al. 2022).

Statistical Analysis

The experiment was a randomized complete-block design with a split-plot arrangement of treatments in which each treatment was replicated three times and the experiment was conducted twice; the experiment was planned so as to avoid climatic interference in the greenhouse during June to August 2021 (Abit et al. 2009).

The main plots were the rice genotypes, and the subplots were the herbicide rates. The experimental unit of interest was the row of plants representing each rice line in each flat. Secondary analysis based on averaged values from the rice genotypes combined within their respective grain types was also performed. Data from the two experimental runs and the three replications were combined, as the experimental runs and replications were considered random effects. The data were fitted to the four-parameter logistic model

$$y = a + \frac{(a - c)}{[1 + (x/x_0)^b]} \quad [2]$$

where a is the lower limit representing plant survival at increasingly large herbicide rates, c is the upper limit representing plant survival at low herbicide rates close to untreated controls, x_0 is the rate giving 50% plant response, and b is the slope around x_0 . Metribuzin application rates that caused 50% visible injury (ID_{50}), biomass reduction (GR_{50}), and height reduction (HR_{50}) were estimated for each rice line and each grain type using the *ed* function in the DRC package in R (Ritz et al. 2015) to create nonlinear regression models (R Development Core Team 2022). ID_{50} , GR_{50} , and HR_{50} values were analyzed using analysis of variance, and means were separated using Tukey–Kramer’s honestly significant difference at a 95% significance level. Correlation coefficient analysis on phytotoxicity versus height reduction and biomass reduction was estimated using JMP® Pro16 (SAS Institute, Cary, NC, USA).

Results and Discussion

There was no interaction across experimental runs for rice injury, height reduction, and biomass reduction, so the data were averaged over two experimental runs. Foliar application of metribuzin injured all rice genotypes at all rates. Metribuzin injury symptoms were characterized by stunting and leaf chlorosis originating at leaf margins, followed by necrosis. Estimations of injury were similar to the symptoms observed from other PSII-inhibiting herbicides (Smith 1965). As the study progressed, the damage symptoms became more apparent; symptoms on treated plants became more severe at 14 DAT than at 7 DAT (data not shown). Crop damage peaked at 21 DAT, with treated plants that remained alive at 21 DAT showing some recovery from injury by producing new, normal growth by 28 DAT. Crop phytotoxicity from metribuzin at the 352 g ha⁻¹ use rate was more pronounced than it was at the use rate of 176 g ha⁻¹ at all rating dates.

Phytotoxicity

There was no significant difference among rice genotypes in metribuzin injury response at any rate tested because there was significant variability among the phytotoxicity responses of the rice genotypes to the rates of metribuzin (Supplementary Table S1). Across all 142 rice genotypes tested, crop injury at 21 DAT ranged from 30% to 88% at the use rate of 176 g ha⁻¹ and from 53% to 100% at the 352 g ha⁻¹ use rate (data not shown). The average metribuzin application rate required to cause ID_{50} across all rice accessions was 163 g ha⁻¹ metribuzin ($P < 0.0001$).

Differing grain type (long, short, and medium) was represented among the 142 rice genotypes tested. There were differences between crop injury response to metribuzin and the grain type of the rice genotypes (Figure 2). The average ID_{50} value for the short-grain rice genotypes was 136 g ha⁻¹, which was significantly lower

than the average ID_{50} for either long-grain or medium-grain rice genotypes, which were 172 g ha⁻¹ and 182 g ha⁻¹, respectively ($P = 0.009$) (Table 1). These results indicate that short-grain genotypes are more susceptible to phytotoxicity injury from foliar-applied metribuzin than are long-grain or medium-grain rice genotypes. Differences in grain type response to metribuzin may result from inherent differences in genetic background among the different grain types. Maeda et al. (2019) found a rice gene, *HIS1*, that was found to confer resistance to benzobicyclon and other β -triketone herbicides through chemical metabolism and detoxification; susceptible rice genotypes carried a defunct allele from a long-grain *indica* rice line that disabled functionality of the gene. The difference in grain types resulted in a genetic difference that altered the metabolic conversion of the toxic chemical and resulted in tolerant and susceptible rice genotypes (Lv et al. 2021).

Height Reduction

Correlation coefficient analysis showed that rice phytotoxicity is highly correlated with rice cultivar height response ($r = 0.727$, $P < 0.0001$) (data not shown). There was no difference among any individual rice cultivar height response and rate of applied metribuzin, except at the 88 g ha⁻¹ rate ($P = 0.0407$). When all the rice genotypes were tested, long-grain cultivar ‘RES14’ displayed an average 36% height increase at 88 g ha⁻¹ metribuzin, contrary to expected results (data not shown). This height increase was different from short-grain rice line ‘RES223’ and long-grain rice line ‘Calmati-202,’ which displayed the highest amount of height reduction, 45% and 33%, respectively, at the 88 g ha⁻¹ metribuzin application rate (data not shown). The average metribuzin application rate required to cause HR_{50} across all rice accessions was 187 g ha⁻¹ ($P < 0.0001$) (Table 1).

The average height HR_{50} results for the rice genotypes showed no differences among the grain types evaluated ($P = 0.002$) (Table 1). Long-grain, medium-grain, and short-grain rice all required 173 to 198 g ha⁻¹ for a 50% height reduction response (Table 1). These results indicate that metribuzin has an equivalent effect on height reduction across all grain types. The symptoms displayed after treatment with metribuzin correlate with Abou-Khater et al. (2021) and Bhoite et al. (2019), who noted similar symptoms in fava bean (*Vicia faba* L.) and wheat, respectively.

There were differences among the rice grain types and height reduction responses as a result of differing application rates of metribuzin. At 88, 176, and 352 g ha⁻¹ metribuzin, all three grain types had significantly different types of height reduction responses ($P < 0.0001$) (Table 2). Long-grain and medium-grain rice accessions exhibited 33% and 14% less height reduction, respectively, than did short-grain accessions at 352 g ha⁻¹ metribuzin. Short-grain rice genotypes consistently displayed the greatest crop height reduction in response to increasing rates of metribuzin, ranging from 17% to 87% height reduction at 88, 176, and 352 g ha⁻¹ metribuzin.

Biomass Reduction

Correlation coefficient analysis showed that rice phytotoxicity is moderately correlated with rice cultivar biomass response ($r = 0.657$, $P < 0.0001$) (data not shown). Reduction in plant biomass was observed for all genotypes at metribuzin application rates 176 and 352 g ha⁻¹ (data not shown). The average metribuzin application rate required to cause GR_{50} across all rice accessions was 118 g ha⁻¹ ($P < 0.0001$) (Table 1).

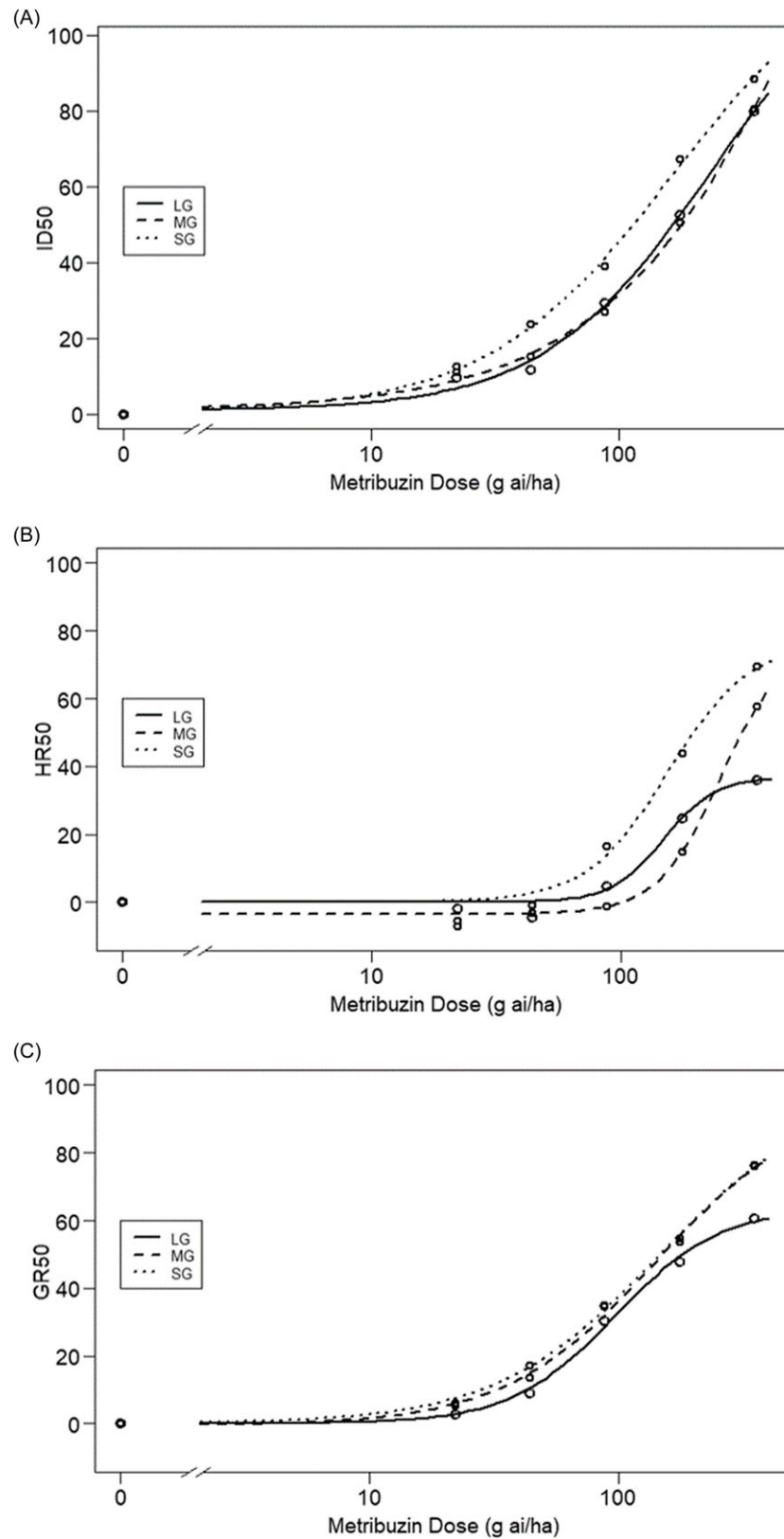


Figure 2. Rice phytotoxicity, height reduction, and biomass reduction as a result of increasing rates of metribuzin on long-grain, medium-grain, and short-grain rice, shown as ID₅₀ (A), HR₅₀ (B), and GR₅₀ (C). The data are averaged from two experimental runs with three replicates. Curves represent four-parameter logistic regression. Equation is $Y = a + (a - c) / [1 + (x/x_0)^b]$, where a and d are the maximum and minimum estimated values, respectively; b is the relative slope of regression about x_0 ; and x_0 is the rate giving 50% plant response.

Table 1. Average metribuzin application rate required to cause 50% visible injury, height reduction, and biomass reduction in the three rice grain types studied.^{a,b,c}

| Grain type | ID ₅₀ | HR ₅₀ | GR ₅₀ |
|------------|------------------|------------------|------------------|
| | | | |
| SG | 136 (±11) b | 198 (±15) a | 95 (±8) ab |
| LG | 172 (±8) a | 186 (±10) a | 114 (±5) a |
| MG | 182 (±9) a | 173 (±11) a | 94 (±6) b |

^aPlants were treated at the 3- to 4-leaf stage. Visible injury information was recorded at 21 d after treatment (DAT), and dry weights and heights were collected at 28 DAT. Standard errors are in parentheses.

^bAbbreviations: GR₅₀, rate required to cause 50% biomass reduction; HR₅₀, rate required to cause 50% height reduction; ID₅₀, rate required to cause 50% visible injury; LG, long grain, 59 genotypes; MG, medium grain, 52 genotypes; SG, short grain, 31 genotypes.

^cMeans accompanied by the same letter do not significantly differ with Tukey's honestly significant difference at $\alpha = 0.05$.

Table 2. Average rice height and biomass reduction for each grain type at 28 d after foliar-applied metribuzin at varying rates.^{a,b,c}

| Grain type | Height reduction | | | Biomass reduction | | |
|------------|----------------------------|-----------|-----------|-------------------|-----------|-----------|
| | 88 | 176 | 352 | 88 | 176 | 352 |
| | % g ai ha ⁻¹ | | | | | |
| SG | 17 (±3) a | 42 (±3) a | 69 (±4) a | 73 (±3) a | 78 (±3) a | 87 (±2) a |
| LG | 4 (±1) b | 25 (±2) b | 36 (±3) c | 26 (±2) b | 46 (±2) b | 54 (±2) c |
| MG | -2.2 (±2) c | 13 (±2) b | 55 (±2) b | 32 (±4) b | 53 (±3) b | 76 (±2) b |

^aPositive numbers indicate percent reduction as compared to the nontreated control plants, and negative numbers indicate percent increase as compared to the nontreated control plants. Standard errors are in parentheses.

^bAbbreviations: LG, long grain, 59 genotypes; MG, medium grain, 52 genotypes; SG, short grain, 31 genotypes.

^cMeans accompanied by the same letter do not significantly differ with Tukey's honestly significant difference at $\alpha = 0.05$.

At 176 g ha⁻¹ metribuzin, the biomass of short-grain genotype 'RES223' was significantly reduced, by 88% of the untreated control. Researchers in Australia found that rates of metribuzin as low as 36 g ha⁻¹ were required to reduce the negative effect on rice biomass (Mahajan and Chauhan 2022), so the results of the present study concur with this conclusion. Long-grain genotypes 'RES8', 'CL271', and 'RES19' produced the least biomass reduction, at 10%, 8%, and 4% of the untreated control, respectively. 'RES8' and 'RES19' are rice genotypes that were developed specifically for California water-seeded rice production. At 352 g ha⁻¹ metribuzin, six rice genotypes responded with biomass reductions ranging from 90% to 94%: 'RES223', 'RES213', 'RES226', 'RES216', 'RES230', and 'RES212', all of which are short grain. At the 352 g ha⁻¹ rate, seven genotypes had biomass reductions that were less than those previously mentioned; long-grain genotypes 'L-205', 'RES36', 'L-201', 'RES35', 'Rex', and 'Della-2' and medium-grain genotype 'CL271' had biomass reductions ranging from 6% to 20%. Of the long-grain rice genotypes that had fewer biomass reductions, four were developed for California rice conditions: 'L-205', 'RES36', 'L-201', and 'RES35'. 'CL271' is a medium-grain Clearfield® genotype that was developed to harbor resistance to imidazolinone herbicides through a forcibly mutated gene that prevents inhibition of the acetolactate synthase enzyme. Yean et al. (2021) suggests that although imidazolinone resistance is conferred primarily through target site resistance, non-target site resistance can be an alternate mechanism that leads to herbicide resistance, which may contribute to 'CL271' rice having biomass reductions that were less than other assessed medium-grain

rice genotypes. Abou-Khater et al. (2021) screened accessions of fava beans for innate tolerance to both metribuzin and imazethapyr and found three accessions that showed low visual damage and no reduction in yield after treatment with metribuzin and imazethapyr.

Biomass GR₅₀ values varied among the grain types of rice genotypes evaluated in this study (Figure 2). The average GR₅₀ for the medium-grain rice genotypes was 94 g ha⁻¹, which was significantly lower than the GR₅₀ for long-grain rice genotypes, which averaged 114 g ha⁻¹ ($P < 0.0001$) (Table 1). These results would indicate that medium-grain genotypes are more susceptible to rice biomass reduction at a lower rate of foliar-applied metribuzin than long-grain rice genotypes. These findings are similar to results from research that showed differential responses of annual ryegrass genotypes to foliar-applied metribuzin and atrazine (Ma et al. 2020). The differential response of annual ryegrass genotypes to metribuzin may have been due to differences in metabolism of the foliar-absorbed herbicide. Annual ryegrass genotypes that were more tolerant to foliar-applied metribuzin metabolized metribuzin twice as quickly as the more sensitive genotypes (Ma et al. 2020).

There were differences between the rice grain types and the rate of biomass reduction as a result of differing rates of metribuzin. At 88, 176, and 352 g ha⁻¹ metribuzin, all three grain types produced different biomass responses ($P < 0.0001$) (Table 2). Short-grain rice genotypes continually exhibited higher biomass reduction in response to increasing rates of metribuzin as compared to the other two grain types. Biomass reduction values for the short-grain genotypes ranged from 73% to 87% reduction at 88, 176, and 352 g ha⁻¹ metribuzin.

At all tested rates, short-grain rice genotypes were more susceptible to metribuzin than long-grain or medium-grain rice genotypes. In general, short-grain rice genotypes had greater height reduction and produced less biomass than long-grain or medium-grain rice genotypes with the same rates of foliar-applied metribuzin. Crop injury from metribuzin was moderately correlated with biomass reductions and highly correlated with plant height reductions. However, further research is required to verify the extent of crop injury and resiliency from foliar-applied metribuzin under field conditions. The results of this research show that the level of metribuzin tolerance in rice is not adequate for commercial use; however, further research is needed to develop higher levels of tolerance by mutagenized rice cultivars.

Practical Implications

Weed infestations drive the greatest biological yield losses and quality reductions in rice production. However, owing to high costs of development and registration, few additional herbicides are currently available for rice growers, particularly herbicides that target grass weeds. The problem necessitates introducing novel active ingredients into existing weed control programs to allow for herbicide MOA rotation and reducing selection for herbicide resistance in weed populations. The work done in this research determines the effects of metribuzin applied at varying rates on several different genotypes of rice, indicating that innate metribuzin resistance in rice is not sufficient without substantial modification. If further research is conducted regarding metribuzin tolerance in California rice genotypes, focus should be placed on inducing herbicide resistance through mutagenesis in the long-grain or medium-grain lines. This work quantifies the correlation between metribuzin-induced leaf injury and biomass and height

reductions and establishes a dose–response curve for rice injury from metribuzin.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/wet.2023.76>

Acknowledgments. The authors acknowledge the California Rice Research Board for partial funding of this project and the Rice Experiment Station in Biggs, CA, for sourcing rice genotypes and providing greenhouse space. Also acknowledged are several past and present lab members, technicians, and student assistants who assisted with the labor and maintenance of this project. The authors declare no conflicts of interest.

References

- Abit MJM, Al-Khatib K, Regehr DL, Tuinstra MR, Claassen MM, Geier PW, Stahlman PW, Gordon BW, Currie RS (2009) Differential response of grain sorghum hybrids to foliar-applied mesotrione. *Weed Technol* 23:28–33
- Abou-Khater L, Maalouf F, Patil SB, Balech R, Nacouzi D, Rubiales D, Kumar S (2021) Identification of tolerance to metribuzin and imazethapyr herbicides in faba bean. *Crop Sci* 61:2593–2611
- Al-Khatib K, Libbey C, Boydston R (1997) Weed suppression with *Brassica* green manure crops in green pea. *Weed Technol* 45:439–445
- Anonymous (2014) Glory 4L® herbicide label. ADAMA-168. Raleigh, NC: Makhteshim Agan of North America. 24 p
- Armendáriz CR, de la Torre AH, Fernández IJG, González GL (2014) Metribuzin. Pages 327–329 in *Encyclopedia of Toxicology*. 3rd ed. Cambridge, MA: Academic Press
- Barrentine WL, Edwards CJ, Hartwig EE (1976) Screening soybeans for tolerance to metribuzin. *Agron J* 68:351–353
- Becerra-Alvarez A, Al-Khatib K (2022) Tracking herbicide-resistant weeds in California rice through a community-driven approach. Page 85 in *Proceedings of Weed Science Society of America/Canadian Weed Science Society Annual Meeting*. Vancouver, BC: Weed Science Society of America
- Bhoite R, Si P, Liu H, Xu L, Siddique KH, Yan G (2019) Inheritance of pre-emergent metribuzin tolerance and putative gene discovery through high-throughput SNP array in wheat (*Triticum aestivum* L.). *BMC Plant Biol* 19:1–12
- Brim-DeForest W (2021) Preliminary herbicide screening in rice on watergrass species. UC Rice Blog UCANR. <https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=45541>. Accessed: June 24, 2022
- Brim-DeForest WB, Al-Khatib K, Fischer AJ (2017) Predicting yield losses in rice mixed-weed species infestations in California. *Weed Sci* 65:61–72
- Busi R, Vidotto F, Fischer AJ, Osuna MD, de Prado R, Ferrero A (2006) Patterns of resistance to ALS herbicides in smallflower umbrella sedge (*Cyperus difformis*) and ricefield bulrush (*Schoenoplectus mucronatus*). *Weed Technol* 20:1004–1014
- California Crop Improvement Association (2019) Rice Seed Quality Assurance Program. <https://ccia.ucdavis.edu/quality-assurance-programs/rice-seed-quality-assurance-program>. Accessed: January 26, 2023
- Carey VF III, Hoagland RE, Talbert RE (1995) Verification and distribution of propanil-resistant barnyardgrass (*Echinochloa crus-galli*) in Arkansas. *Weed Technol* 9:366–372
- Ceseski AR, Al-Khatib K (2021) An evaluation of the stale-drill cropping system in California rice: implications for weed management, crop physiology, and yield potential. PhD dissertation, University of California, Davis. 115 p
- Ceseski AR, Godar AS, Al-Khatib K (2022) Combining stale seedbed with deep rice planting: a novel approach to herbicide resistance management? *Weed Technol* 36:261–269
- Childs N (2022) Rice sector at a glance. U.S. Department of Agriculture Economic Research Service. <https://www.ers.usda.gov/topics/crops/rice/rice-sector-at-a-glance/>. Accessed: November 11, 2022
- Childs N, LeBeau B (2022) Rice outlook: July 2022. RCS-22F. U.S. Department of Agriculture Economic Research Service. <https://www.ers.usda.gov/publications/pub-details/?pubid=104481>. Accessed: May 3, 2023
- Cole DJ (1994) Detoxification and activation of agrochemicals in plants. *Pestic Sci* 42:209–222
- de Leon TB, Karn E, Al-Khatib K, Espino L, Blank T, Andaya CB, Andaya VC, Brim-DeForest W (2019) Genetic variation and possible origins of weedy rice found in California. *Ecol Evol* 9:5835–5848
- Espino L, Greer C, Al-Khatib K, Godfrey L, Eckert J, Fischer AJ, Lawler S (2019) Rice: UC IPM Pest Management Guidelines. Publication 3465. Davis, CA: University of California Department of Agriculture and Natural Resources. 56 p
- Gerber CK (2021) 2023 Corn and Soybean Field Guide. Publication ID-179. West Lafayette, IN: Purdue University. 316 p
- Gibson KD, Hill JE, Foin TC, Caton BP, Fischer AJ (2001) Water-seeded rice cultivars differ in ability to interfere with watergrass. *Agron J* 93:326–332
- Hardcastle WS (1974) Differences in the tolerance of metribuzin by cultivars of soybeans. *Weed Res* 14:181–184
- Hill JE, Smith RJ Jr, Bayer DE (1994) Rice weed control: current technology and emerging issues in temperate rice. *Aust J Exp Agr* 34:1021–1029
- Hill J, Williams J, Mutters R, Greer C (2006) The California rice cropping system: agronomic and natural resource issues for long-term sustainability. *Paddy Water Environ* 4:13–19
- Javaid MM, Mahmood A, Bhatti MIN, Waheed H, Attia K, Aziz A, Nadeem MA, Khan N, Al-Doss AA, Fiaz S, Wang X (2022) Efficacy of metribuzin doses on physiological, growth, and yield characteristics of wheat and its associated weeds. *Front Plant Sci* 13:866793
- Kondhia A, Tabien RE, Ibrahim A, Kondhia A, Tabien RE, Ibrahim A (2015) Evaluation and selection of high biomass rice (*Oryza sativa* L.) for drought tolerance. *Am J Plant Sci* 6:1962–1972
- Lambrea MD, Russo D, Polticelli F, Scognamiglio V, Antonacci A, Zobnina V, Campi G, Rea G (2014) Structure/function/dynamics of photosystem II plastoquinone binding sites. *Curr Protein Pept Sci* 15:285–295
- Lawrence BH, Bond JA, Golden BR, Allen TW, Reynolds DB, Bararpour TM (2021) Rice response to sublethal rates of paraquat, metribuzin, fomesafen, and clorasulam-methyl at different application timings. *Weed Technol* 35:681–689
- Lv Q, Zhang X, Yuan D, Huang Z, Peng R, Peng J, Li Z, Tang L, Liu D, Zhou X, Wang L, Pan L, Shao Y, Mao B, Xin Y, Zhu L, Zhao B, Bai L (2021) Exploring natural allelic variations of the β -triketone herbicide resistance gene HIS1 for application in *indica* rice and particularly in two-line hybrid rice. *Rice* 14:7
- Ma H, Lu H, Han H, Yu Q, Powles S (2020) Metribuzin resistance via enhanced metabolism in a multiple herbicide resistant *Lolium rigidum* population. *Pest Manage Sci* 76:3785–3791
- Maeda H, Murata K, Sakuma N, Takei S, Yamazaki A, Karim Mdr, Kawata M, Hirose S, Kawagishi-Kobayashi M, Taniguchi Y, Suzuki S, Sekino K, Ohshima M, Kato H, Yoshida H, Tozawa Y (2019) A rice gene that confers broad-spectrum resistance to β -triketone herbicides. *Science* 365:393–396
- Mahajan G, Chauhan BS (2022) Screening of herbicides for rice seedling safety and *Echinochloa colona* management under Australian conditions. *Agron J* 12:1273
- Okoshi M, Nishikawa T, Akagi H, Fujimura T (2018) Genetic diversity of cultivated rice (*Oryza sativa* L.) and wild rice (*Oryza rufipogon* Griff.) in Asia, especially in Myanmar, as revealed by organelle markers. *Genet Resour Crop Evol* 65:713–726
- Ortmeier-Clarke HJ, Oliveira MC, Arneson NJ, Conley SP, Werle R (2022) Dose–response screening of industrial hemp to herbicides commonly used in corn and soybean. *Weed Technol* 36:245–252
- Pantone DJ, Baker JB (1992) Varietal tolerance of rice (*Oryza sativa*) to bromoxynil and triclopyr at different growth stages. *Weed Technol* 6:968–974
- Qu RY, He B, Yang JF, Lin HY, Yang WC, Wu QY, Li QX, Yang GF (2021) Where are the new herbicides? *Pest Manage Sci* 77:2620–2625
- R Development Core Team (2022) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. 16 p
- Ritz C, Baty F, Streibig JC, Gerhard D (2015) Dose–response analysis using R. *PLoS ONE* 10(12):e0146021
- Shoba D, Raveendran M, Manonmani S, Utharasu S, Dhivyapriya D, Subhasini G, Ramchandrar S, Valarmathi R, Grover N, Krishnan SG, Singh AK, Jayaswal P, Kale P, Ramkumar MK, Mithra SVA, Mohapatra T, Singh K, Singh NK, Sarla N, Sheshshayee MS, Kar MK, Robin S, Sharma RP

- (2017) Development and genetic characterization of a novel herbicide (imazethapyr) tolerant mutant in rice (*Oryza sativa* L.). *Rice* 10:10
- Smith RJ Jr (1965) Propanil and mixtures with propanil for weed control in rice. *Weeds* 13:236–238
- Smith RJ Jr (1968) Weed competition in rice. *Weed Sci* 16:252–255
- Smith RJ Jr (1988) Weed thresholds in southern U.S. rice, *Oryza sativa*. *Weed Technol* 2:232–241
- Volova T, Shumilova A, Zhila N, Sukovaty A, Shishatskaya E, Thomas S (2020) Efficacy of slow-release formulations of metribuzin and tribenuron methyl herbicides for controlling weeds of various species in wheat and barley stands. *ACS Omega* 5:25135–25147
- Wright HE, Norsworthy JK, Roberts TL, Scott R, Hardke J, Gbur EE (2021) Characterization of rice cultivar response to florypyrauxifen-benzyl. *Weed Technol* 35:82–92
- Yean RA, Dilipkumar M, Rahman S, Song BK (2021) A two-in-one strategy: target and nontarget site mechanisms both play important role in IMI-resistant weedy rice. *Int J Mol Sci* 22:982
- Ziska L, Gealy D, Burgos N, Caicedo A, Gressel J, Lawton-Rauh A, Avila L, Theisen G, Norsworthy J, Ferrero A, Vidotto F, Johnson D, Ferreira F, Marchesan E, Menezes V, Cohn M, Linscombe S, Carmona L, Tang R, Merotto A (2015) Weedy (red) rice: an emerging constraint to global rice production. Pages 181–228 *in* *Advances in Agronomy*. Cambridge, MA: Academic Press