METABOLIC-BASED RESISTANCE IN HERBICIDE-RESISTANT GLOBE FRINGERUSH (*Fimbristylis miliacea*)

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INTRODUCTION

Fimbristylis miliacea (L.) Vahl is one of the most troublesome weeds in rice fields in southern Brazil and belongs to Cyperaceae family. Usually, this weed is controlled by herbicides in irrigated rice fields. Acetolactate synthase (ALS)-inhibiting herbicides are widely used to control weeds in rice fields because are highly effective, applied in low rates, presenting low toxicity to animals, wide control spectrum and some of them, long soil persistence (MAZUR; FALCO, 1989). However, the application of these herbicides for many years, in the same area, has resulted in selection of globe fringerush ALS-resistant biotypes NOLDIN; HEBERHARDT; RAMPELOTTI, 2002).

Overall, resistance to ALS-inhibiting herbicides can be attributed to target site alterations. Target site-based resistance is due to mutation(s) in the amino acid sequence resulting in conformational changes to the herbicide binding site of the target enzyme. Nontarget site-based resistance as enhanced metabolism of the herbicide is caused by mechanisms that reduce the amount of active herbicide that reaches the target enzyme or binding domain (POWLES; SHANER, 2001; MENENDEZ; BASTIDA; DE PRADO, 2006).

In a previous study to determine the level of resistance to ALS-inhibiting herbicides and compare the ALS gene sequences in two resistant and one susceptible globe fringerush biotypes, only one resistant biotype showed a single mutation thymine-adenine, resulting in an amino acid substitution $Asp_{376}Glu$, in the region F between the C, A, D and B, E domains in the ALS gene (SCHAEDLER et al., 2011a). Thus, the mechanism of resistance in globe fringerush was not completely elucidated.

Plants metabolize certain herbicides via the activity of enzymes belonging to the cytochrome P450 family. According to Preston et al. (1996), the organophosphate insecticide malathion is a cytochrome P450 inhibitor and is a specific synergist for the ALS-inhibiting herbicide chlorsulfuron in *Lolium rigidum*. Inhibitor 1-aminobenzotriazole (ABT) also indicated that herbicide metabolism catalyzed by cytochrome P450 monooxygenases is related to chlortoluron resistance in *Bromus tectorum* biotype (MENENDEZ; BASTIDA; DE PRADO, 2006).

The aim of this study was to determine if P450-mediated enhanced metabolism possibly exists in selected herbicide-resistant *Fimbristylis miliacea* biotypes.

MATERIAL AND METHODS

The experiment was conducted in growth chamber conditions at 35/30 ºC day/night temperature, from November 2010 to March 2011 in the Crop, Soil, and Environmental Science Department at University of Arkansas, Fayetteville, USA. Seeds of two globe fringerush resistant biotypes (FIMMI 10R and FIMMI 12R) to ALS-inhibiting herbicides from Santa Catarina state rice fields , Brazil, were sown in trays and 14 days after, three seedling were transplanted into plastic pots with 10 cm diameter and 9 cm height filled with commercial mixture of substrate (Sunshine Mix, Canada).

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Five days after transplanting, all pots were sprayed with P450 inhibitors malathion (1000 g ai ha^{-1}) and 1-aminobenzotriazole (100 µM ABT) 30 minutes before spraying 1x rate of either pyrazosulfuron-ethyl (17.5 g ai ha^{-1}), penoxsulam (30 g ai ha^{-1}) and bispyribac-sodium (50 g ai ha^{-1}). Each biotype was treated only with the corresponding herbicide it expresses resistance to (SCHAEDLER et al., 2011b), according to Table 1. The experiment was a randomized complete block design with three replications and the experiment was repeated.

Table 1. Treatments tested in resistant (FIMMI 10R and FIMMI 12R) globe fringerush biotypes, University of Arkansas, Fayetteville-AR, 2011.

Biotype	Treatment	Biotype	Treatment
FIMMI 10R	Untreated	FIMMI 12R	Untreated
FIMMI 10R	malathion	FIMMI 12R	malathion
FIMMI 10R	ABT[1]	FIMMI 12R	ABT
FIMMI 10R	pyrazosulfuron	FIMMI 12R	pyrazosulfuron-ethyl
FIMMI 10R	pyrazosulfuron-ethyl+malathion	FIMMI 12R	pyrazosulfuron-ethyl + malathion
FIMMI 10R	pyrazosulfuron-ethyl + ABT	FIMMI 12R	pyrazosulfuron-ethyl + ABT
FIMMI 10R	penoxsulam	FIMMI 12R	penoxsulam
FIMMI 10R	penoxsulam+ malathion	FIMMI 12R	penoxsulam + malathion
FIMMI 10R	penoxsulam + ABT	FIMMI 12R	penoxsulam + ABT
FIMMI 10R	bispyribac-sodium		
FIMMI 10R	bispyribac-sodium + malathion		
FIMMI 10R	bispyribac-sodium + ABT		

[1] 1-aminobenzotriazole

The variables evaluated were globe fringerush biotypes control and dry weight. Control rate (%) was evaluated visually at 28 days after treatment (DAT) The above-ground plant material was cut and oven-dried for at least 72h at 60 ºC and the dry weight was recorded in percentage of the untreated.

Data were pooled across experiment because there was not treatment by experiment interaction. Data were analyzed to combined ANOVA, and means were separated by Fisher's protected LSD test at 5% level of probability.

RESULTS AND DISCUSSION

Control and dry weight from pyrazosulfuron-ethyl, penoxsulam and bispyribac-sodium herbicides were significantly different for FIMMI 10R (Figure 1). Malathion and ABT inhibitors provided lower percentage of control (Figure 1, A, C and E) when compared to the herbicides or combination herbicide+malathion and herbicide+ABT. On the other hand, the dry weight was not significantly different when compared to the untreated.

All herbicides sprayed single or with inhibitors (malathion or ABT), showed higher control of FIMMI 10R biotype when compared with malathion or ABT alone. There were no differences between herbicide control or herbicide+inhibitors, except for the bispyribac-sodium (Figure 1, E). Bisbyribac-sodium showed control rate of 48.5% for FIMMI 10R and was enhanced to 60 and 68% by malathion and ABT, respectively. Moreover, dry weight variable showed significative reduction to bispyribac-sodium herbicide (Figure 1, F). Bispyribac-sodium sprayed single reduced about 56%, and bispyribac-sodium in combination with inhibitors reduced the dry weight 42 and 41% by malathion and ABT, respectively.

FIMMI 12R control was no significant difference (data not showed). In general, FIMMI 12R showed the highest dry weight when sprayed with pyrazosulfuron alone or pyrazosulfuron +inhibitors (Figure 2). On the other hand, penoxsulam did not show significative difference between treatments except to penoxsulam sprayed alone which reduced the dry weight around 18% compared to the untreated (Figure 2). The increase in dry weight observed for FIMMI 12R biotype treated with pyrazosulfuron may be explained as an hormesis effect (CALABRESE, 2008) even with regular rate used in the field.

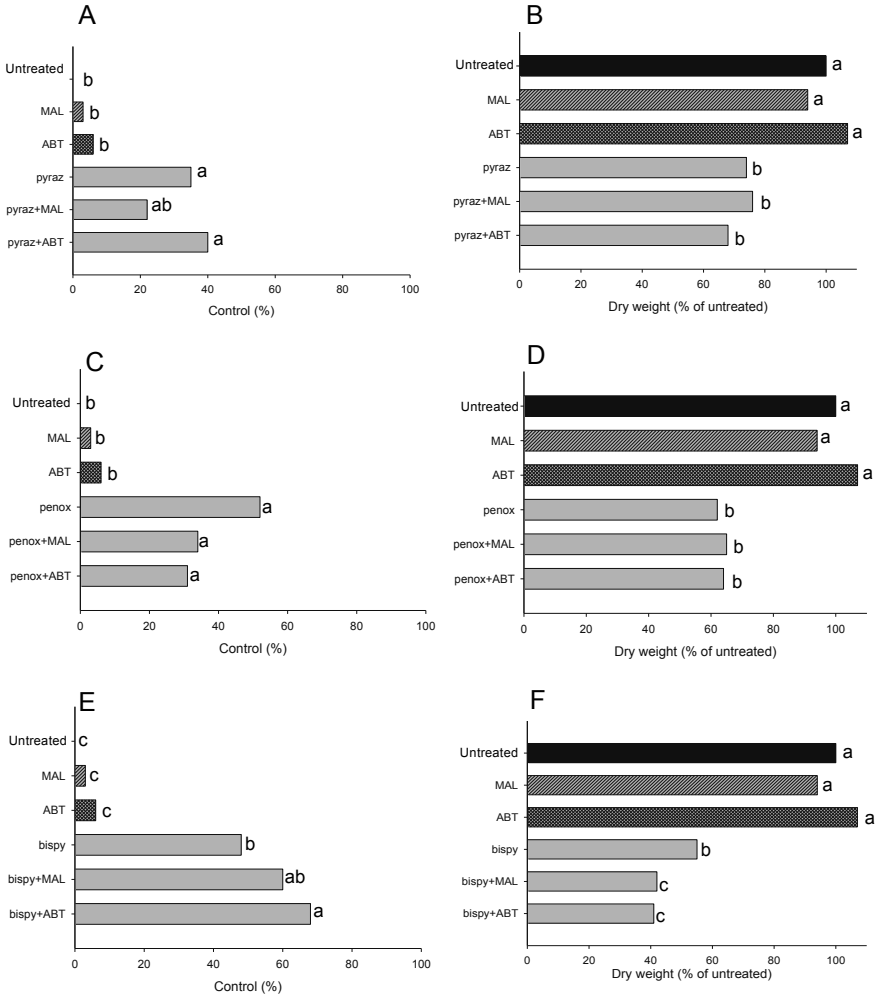

Figure 1. Control (A, C and E) and dry weight (B, D and F) 28 days after treatment in *Fimbristylis miliacea* (FIMMI 10R) resistant to ALS-inhibitors herbicides pyrazosulfuron-ethyl, penoxsulam and bispyribac-sodium, University of Arkansas, Fayetteville-AR, 2011. Graphs A and B – pyrazosulfuron-ethyl; C and D – penoxsulam; E and F – bispyribac-sodium. (MAL – only malathion; ABT – only 1-aminobenzotriazole; pyraz. – pyrazosulfuron-ethyl; penox – penoxsulam; bispy – bispyribac-sodium). Bars with different lowercase are significantly different ($p \leq 0.05$) according to "Fischer`s test".

In a specific case, ABT inhibitor was very effective in improving bispyribac-sodium activity to FIMMI 10R. The increased activity, when it occurred, did not completely overcome the resistance to any herbicide, indicating that P450-mediated metabolism is partially responsible for resistance in some cases. In many cases, metabolism based resistance may not be involved at all. Alternatively, herbicide metabolism may still be a factor, but with other monooxygenases or enzyme family.

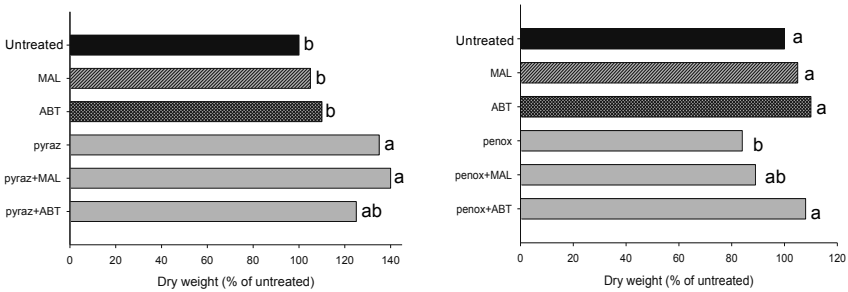

Figure 2. Dry weight 28 days after treatment with pyrazosulfuron-ethyl and penoxsulam in *Fimbristylis miliacea* (FIMMI 12R) resistant to ALS-inhibitors, University of Arkansas, Fayetteville-AR, 2011. (MAL – only malathion; ABT – only 1-aminobenzotriazole; pyraz. – pyrazosulfuron-ethyl; penox – penoxsulam). Bars with different lowercase are significantly different ($p \leq 0.05$) according to "Fischer`s test".

CONCLUSION

The control of FIMMI 10R biotype by bispyribac-sodium is increased with the addition of P-450 ABT inhibitor. This experiment provides direction for follow-up research on herbicide-resistant globe fringerush biotypes and help generates more information on weed resistance management.

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