Penoxsulam Resistance in Califonia's Late Watergrass [*Echinochloa phyllopogon* (Stapf) Koss.]

H. Yasuor[1], M.D. Osuna[1], A Ortiz[2] & A.J. Fischer[1]. [1]**University of California, Davis - Department of Plant Sciences, One Shields Ave., Davis, CA 95616, ajfischer@ucdavis.edu;** [2]**Universidad Central de Venezuela, Maracay, Venezuela**

ABSTRACT

Echinochloa phyllopogon is a major weed of California rice that has evolved multiple-herbicide resistance. Cross-resistance to penoxsulam was evaluated in a resistant (R) population collected in a rice field. Ratios (R/S) of the R to S GR_{50} values of about 5 were observed in whole-plant and seedling dose-response assays. Adding malathion (P450 inhibitor) enhanced herbicide phytotoxicity to R plants, while pre-treatment with thiobencarb (P450 substrate) antagonized penoxsulam. HPLC assays with ^{14}C-penoxsulam showed higher clomazone meatabolism in R plants; malathion inhibited penoxsulam metabolism and accumulation of parent compound in R plants was similar to S plants treated with penoxsulam alone. ALS activity assays were similar for R and S plants. These results suggest *E. phyllopogon* resistance to penoxsulam is due to P450-mediated enhanced metabolism and not due to reduced ALS sensitivity.

Keywords: *Echinochloa phyllopogon*, herbicide resistance, cytochrome P450, malathion, acetolactate-synthase.

RESUMEN – Resistencia a Penoxsulam en [*Echinochloa phyllopogon* (Stapf) Koss.] de Arrozales de Califonia

Echinochloa phyllopogon es una de las principales malezas del arroz californiano que ha evolucionado resistencia a múltiples herbicidas. Se evaluó la resistencia cruzada a penoxsulam en una población resistente (R) colectada a campo. La relación entre las GR_{50} (R/S) fue aproximadamente 5 en experimentos de respuesta a dosis en plantas. Agregando malatión a la mezcla incrementó la toxicidad de penoxsulam sobre plantas R, mientras que tratamiento previo con tiobencarbo (sustrato P450) tuvo efecto antagonístico. Ensayos HPLC usando ^{14}C-penoxsulam mostraron mayor metabolismo de penoxsulam en plantas R; malatión inhibió el metabolismo de penoxsulam resultando en igual acumulación del compuesto parental en plantas R que en plantas S tratadas con penoxsulam solamente. La actividad de la enzima ALS fue igual en plantas R y S. Estos

resultados sugieren que la Resistencia de *Echinochloa phyllopogon* a penoxsulam se debe a un elevado metabolismo P450 y no a baja sensibilidad del sitio activo.
Palabras-clave: *Echinochloa phyllopogon*, resistencia a herbicidas, cytocromo P450, malatión, acetolactato-sintasa.

INTRODUCTION

Penoxsulam is a new triazolopirimidine sulfonamide herbicide that controls annual grasses, sedges and broad leaf weeds in rice; it inhibits the acetolactate synthase enzyme (ALS) in plants. *Echinochloa phyllopogon* is a major weed of rice in California, where many populations exhibit resistance to multiple herbicides with different modes of action. Resistance involves enhanced cytochrome P450 (P450) metabolism (Yun et al. 2005). Penoxulam failure to control this weed has already been detected in California rice fields. Our objectives were: 1 to quantify penoxsulam resistance in a resistant (R) *E. phyllopogon* biotype; 2 to assess the effects of malathion (P450 inhibitor) and thiobencarb (P450 substrate), and to examine metabolic profiles to investigate if resistance is due to enhanced P450 metabolism; and (3) conduct ALS activity assays to detect possible target-site resistance.

MATERIALS AND METHODS

Plant material. Susceptible (S) and R *E. phyllopogon* populations were collected in California rice fields; the R biotype had shown resistance to molinate and thiobencarb (thiocarbamates), cyhalofop and fenoxaprop (aryloxyphenoxy), byspiribac sodium (pyrimidinyl benzoate), bensulfuron methyl (sulfonylurea), (Fischer *et al.*, 2000) and clomazone.

Dose-response experiments. In whole-plant experiments, penoxsulam (0, 1.25, 2.5, 5, 10, 20 and 40 g ai ha^{-1}) was applied to 3-leaf plants; also, one set of R plants was treated with 1000 g ai ha^{-1} malathion 4 hr before applying penoxsulam. Aboveground fresh weight per pot was determined 15 days after spraying. Treatments were arranged in a completely randomized design with four replications and each experiment was repeated twice.

ALS activity studies. ALS activity was measured *in-vitro* on 3-4 leaf R and S plants following a standard procedure (Osuna *et al*., 2002). Three experiments were conducted and each treatment was assayed in triplicate.

Effect of thiobencarb in penoxsulam toxicity. Dose-response studies were conducted with six rates of penoxsulam (0, 15, 30, 45, 60 and 75 g ai ha^{-1}) each applied alone or in mixture with 1120 g ai ha^{-1} thiobencarb.

^{14}C-penoxsulam metabolism. Three-leaf R and S plants were treated hydroponically with a solution containing 1.87 kBq ^{14}C-penoxsulam plus technical grade herbicide to a total penoxsulam concentration corresponding to a field rate of 49.3 g a.i. ha^{-1} applied to a flooded field. Half of the plants received also 33 mg a.i. L^{-1} malathion in the growth medium. Plant extracts were assayed by HPLC using a gradient mobile phase of 0.01% acetic acid:acetonitrile.

Statistical analysis. Fresh weight and ALS activity data as percentage of the untreated control were fitted log-logistic or quadratic regressions to calculate herbicide rates to inhibit 50% plant growth (GR_{50}) or enzyme activity (I_{50}) and R/S ratios; data from repeated experiments were pooled.

RESULTS & DISCUSSION

Resistance ratios (R/S) of about 5 (Table 1) confirmed resistance in R *E. phylllopogon* to penoxsulam Malathion synergized penoxsulam against R *E. phylllopogon* (Table 1). In previous studies, R *E. phylllopogon* resistance to bispyribac-sodium was abolished by additing the P-450 inhibitors piperonyl-butoxide (PBO) or malathion (Fischer *et al*. 2000b), P450-endowed resistance to other ALS-inhibitors had been similarly detected by malathion in previous studies (Osuna et al., 2002).

Thiobencarb (1120 g ha^{-1}) had no effect on R *E. phylllopogon* growth but reduced penoxsulam toxicity (Fig. 1) causing a 3X increase in its GR_{50} (not shown). The mechanism of this antagonism is not clear, but thiocarbamates can be substrates of P-450 and may induce monooxigenase activity thus hastening herbicide degradation by plants (Devine *et al*. 1993). These results suggest P450 involvement in regulating penoxsulam toxicity to R *E. phylllopogon*, and contribution of P450-mediated metabolism to penoxsulam resistance in this biotype. The ALS was inhibited by the herbicide in both S and R plants (Fig. 3), thus resistance was not due to a penoxsulam-insensitive target site in R plants (Table 2). This agrees with previous studies with on bispyribac-sodium and

bensulfuron-methyl resistance by this R biotype (Osuna *et al*., 2002). In the metabolism assays using ^{14}C-penoxsulam, HPLC elution profiles of extracts from R and S treated plants showed higher proportion of the active parent herbicide and less polar metabolites in S vs. R plants; addition of malathion reduced clomazone metabolism and increased the proportion of parent compound in R plants to a level comparable to that in S plants (Fig. 2). These results explain the synergistic effects of malathion on penoxsulam-treated R plants and the idea that penoxsulam resistance in *E. phyllopogon* is due to enhanced herbicide metabolism.

We conclude from this study that an *E. phyllopogon* biotype that was selected for resistance to multiple herbicides by decades of repeated thiocarbamate (thiobencarb and molinate) use in California rice, is also cross-resistant to penoxsulam. This explains control failures observed in the field. The mechanism of resistance is an enhanced ability of R plants to detoxify the herbicide through malathion-sensitive P450 monoaxidases. Resistance to penoxsulam does not involve an altered target site. This is consistent with previous studies on this and similar *E. phyllopogon* biotypes from California for which P450-mediated metabolism mediated resistance to other ALS- inhibiting herbicides (Osuna *et al*., 2002).

Table 1. GR_{50} and R/S ratios for penoxsulam-treated R and S *E. phyllopogon* plants, and effect of applying malathion to R plants before spraying penoxsulam; values were calculated from regression curves presented in Figure 1.

	GR_{50} (g ha^{-1})	95 % CI	R/S
S	1.87	1.56 – 2.17	-
R + malathion	4.11	3.58 – 4.64	2.20
R	9.02	7.11 – 12.09	4.83

Table 2. IR_{50} and R/S ratio obtained in ALS activity assays of extracrts from penoxsulam-treated R and S *E. phyllopogon* plants; values were calculated from regression curves presented in Figure 3.

	I_{50} (nM)	95 % CI	R/S
S	11.78	3.00 – 54.00	-
R	4.67	2.80 – 8.50	0.40

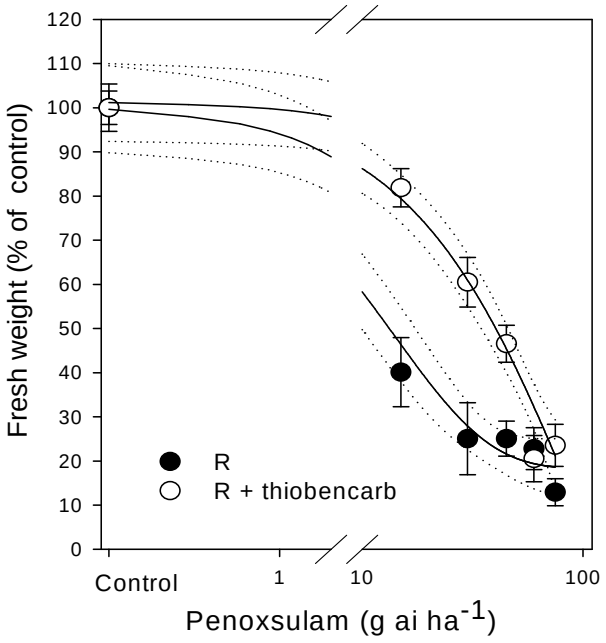

Figure 1. Fresh weight of R and S *E. phyllpogon* biotypes treated with different doses of penoxsulam applied alone or in a mixture with thiobencarb (1120 g ha^{-1}).

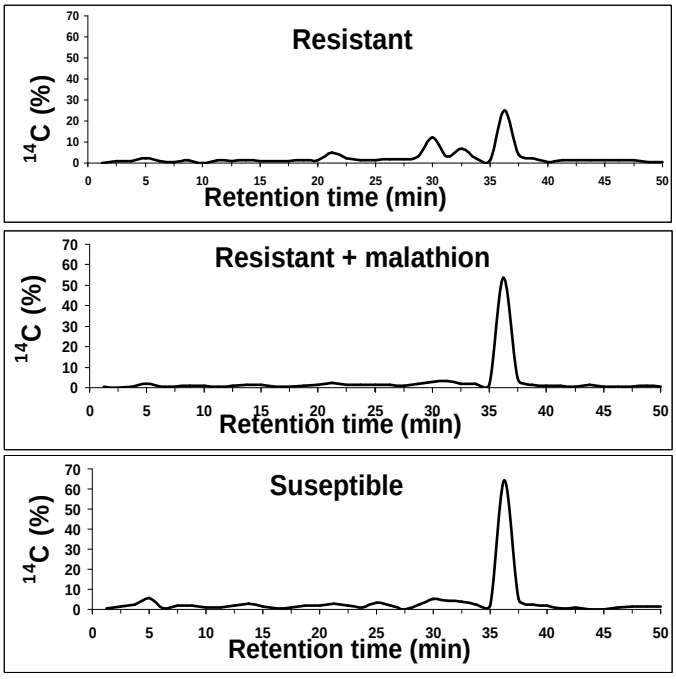

Figure 2. HPLC elution profiles of ^{14}C-labeled compounds extracted from R and S late watergrass plants following a 48-h exposure of plant roots to ^{14}C-penoxsulam (XDE-638-Het-2-^{14}C) with and with malathion (M) added to the herbicide mix.

CITED LITERATURE

DEVINE, M..; DUKE, S.O.; FEDTKE, C. Physiology of Herbicide Action. Englewood Cliffs, NJ, Prentice Hall, 1993. p. 95–112; 376–394.

FISCHER, A.J.; ATEH, C.M.; BAYER, D.E.; HILL, J.E. Herbicide-resistant *Echinochloa oryzoides* and *E. phyllopogon* in California *Oryza sativa* fields. Weed Sci., v.48, p.225-230, 2000.

FISCHER. A.J.; BAYER, D.E.; CARRIERE, M.D.; ATEH, C.M.; YIM K.O. Mechanisms of resistance to bispyribac-sodium in an *Echinochloa phyllopogon* accession. Pestic. Biochem. Physiol., v.68, p.156–165, 2000b.

OSUNA, M.D.; VIDOTTO, F.; FISCHER, A.J.; BAYER, D.E.; DE PRADO, R.; FERRERO, A. Cross-resistance to bispyribac-sodium and bensulfuron-methyl in *Echinochloa phyllopogon* and *Cyperus difformis*. Pestic. Biochem. Physiol., v. 73, p.9-17, 2002.

YUN, M.S.; YOGO, Y.; MIURA, R.; YAMASUE, Y.; FISCHER, A.J. Cytochrome P-450 monooxygenase activity in herbicide-resistant and –susceptible late watergrass (*Echinochloa phyllopogon*). Pestic. Biochem. Physiol., v.83, p.107-114, 2005