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Mechanisms of Resistance to Bispyribac-Sodium in an *Echinochloa phyllopogon* Accession

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Weeds are a major problem for rice production in California, and late watergrass (*Echinochloa phyllopogon* (Stapf) Koss) is one of the most serious weeds in water-seeded rice. Severe infestations can reduce yields by more than 50%. Flooding only partially controls this weed; thus, farmers rely heavily on herbicides. Resistance to several herbicides, including bispyribac-sodium, an acetolactate synthase (ALS) inhibitor not yet commercially used, has developed in late watergrass populations of California rice. Knowing the mechanisms of bispyribac resistance is relevant to designing herbicide management strategies for delaying resistance development to enhance the successful introduction of this new herbicide. We examined whether an insensitive ALS and cyt P-450-dependent detoxification were possible resistance mechanisms in a bispyribac-sodium-resistant (R) late watergrass population collected in California rice fields, which was previously determined to be resistant to molinate, thiobencarb, and fenoxaprop-ethyl. ALS activity was assayed on leaf extracts from young R and susceptible (S) plants for a range of bispyribac-sodium concentrations, and cross-resistance to another ALS inhibitor, bensulfuron-methyl, was evaluated using whole-plant bioassays. Resistance was not due to reduced ALS sensitivity to bispyribac-sodium in R plants, although the R accession was highly cross-resistant to bensulfuron-methyl. Although S and R plants had similar ALS activity (mg acetoin mg protein^{-1}) without herbicide, more ($P < 0.05$) leaf protein was extracted from R (5.35 mg g^{-1} leaf fresh weight) than from S (3.19 mg g^{-1}) plants, and general ALS activity (mg acetoin g leaf fresh weight^{-1}) for all herbicide concentrations was higher in R than in S plants. The cyt P-450 inhibitors piperonyl butoxide and malathion were used for detection of herbicide degradation by cyt P-450 monooxygenation. The addition of these inhibitors strongly enhanced herbicide phytotoxicity toward R plants, suggesting that metabolic degradation of bispyribac-sodium contributed significantly to the observed resistance.

INTRODUCTION

Weeds have been a major problem for rice production in California (1), and late watergrass (*Echinochloa phyllopogon* (Stapf) Koss) is one of the most serious weeds in water-seeded rice. Severe late watergrass infestations can result in more than 50% rice yield loss (2). Continuous flooding of rice fields only partially controls this weed; thus, farmers rely heavily on the use of herbicides (3).

Repeated use of herbicides that target the same site of action can select for individuals that are genetically endowed to survive herbicide rates that normally would kill or suppress the species (4, 5). The thiocarbamates molinate and thiobencarb and the aryloxyphenoxy-propionate fenoxaprop-ethyl have been used continuously to control grass weeds in California rice, and resistance to these herbicides has developed in late watergrass populations (3). Propanil controls this herbicide-resistant late watergrass, but the repeated use of this herbicide has already resulted in the development of propanil resistance in *Echinochloa* spp. in other rice areas (6–9).

The pyrimidinyl carboxy herbicide bispyribac-sodium (sodium 2,6-bis[(4,6-dimethoxypyrimidine-2-yl)oxy]benzoate) is an acetolactate synthase (ALS; also called acetohydroxyacid synthase or AHAS; EC 4.1.3.18) inhibitor

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(10) that is currently being evaluated in California for *Echinochloa* spp. control in rice. Although commercial-scale applications of this herbicide have not been made to California rice fields, studies have shown resistance to bispyribac-sodium in some late watergrass biotypes also resistant to molinate, thiobencarb, and fenoxaprop-ethyl (3). Ratios of the GR_{50} values of resistant to susceptible (R/S) late watergrass plants ranged from 2.0 to 12.0 for bispyribac-sodium (3). Resistance to another ALS inhibitor, the suffonylurea herbicide bensulfuron-methyl, has developed in several broadleaf and sedge species following its wide use in California for many years (11). The widely used bensulfuron-methyl can also provide from 40 to 80% control of late watergrass; thus, a measure of selection pressure on late watergrass populations can be expected from its repeated use. Resistance to bispyribac-sodium could have developed by previous exposure to bensulfuron-methyl; also, through intensive herbicide use, watergrass may have acquired an enhanced ability to degrade herbicides and survive their action (12, 13).

Different mutations in the target ALS enzyme can result in resistance due to reduced affinity for a wide range of ALS inhibitors and in variable degrees and patterns of cross-resistance to ALS inhibitors pertaining to different herbicide classes (14–19). Tolerance to ALS inhibitors can also result from their metabolic alteration in plant tissue (reviewed in 20). Among the more common reactions involved in crop (including rice) tolerance to sulfonylureas are hydroxylation, O-dealkylation, and deesterification (21, 22). Cytochrome P-450 monooxygenase systems (cyt P-450s) have often been implicated in these processes and in the metabolic detoxification of herbicides by plants (23–26). Piperonyl butoxide (PBO) is a cyt P-450 inhibitor (27) that has been used to detect resistance due to metabolism by PBO-sensitive cyt P-450s (26). PBO can suppress the metabolism of several herbicides, including sulfonylureas, inducing injury in resistant biotypes when applied with the herbicide (26, 28). Malathion is also a cyt P-450 inhibitor that has been used to antagonize cyt P-450 monooxygenase-mediated chlorsulfuron and pendimethalin resistance in *Lolium rigidum* Gaud. (29, 30).

Information on resistance to pyrimidinyl carboxys, and for bispyribac in particular, is lacking. Knowledge of resistance mechanisms to bispyribac in late watergrass is relevant to prevention of the development of resistance to this new herbicide in California. Target site resistance would prescribe the use of bispyribac in sequence or in tank mixes with other herbicides also active on the same target weed, but through a different mechanism of action. A degradation enhancement mechanism of resistance would preclude sequences or combinations with herbicides sharing the same degradation pathway as that of bispyribac; the use of synergists to inhibit the degradation mechanism would be helpful if the synergist does not affect selectivity toward the crop. Here we examine whether resistance to bispyribac-sodium in a late watergrass accession with resistance to other herbicides (3) can be due to an insensitive ALS target enzyme, whether cyt P-450-dependent detoxification of bispyribac-sodium also contributes to the observed resistance, and whether this accession is also cross-resistant to bensulfuron-methyl.

MATERIALS AND METHODS

Plant Material

Seeds from the resistant and susceptible late watergrass populations used in the following experiments were collected from California rice fields as reported by Fischer *et al.* (3). The R population had shown resistance to molinate, thiobencarb, fenoxaprop-ethyl, and bispyribac-sodium, whereas the S accession was killed at, or below, the recommended field rates of these herbicides (3).

ALS Assay

For the ALS assays, a modification of Ray's technique (31), which had been successfully used in previous work to identify ALS insensitivity as a mechanism of resistance to bensulfuron-methyl in *Cyperus difformis* L. (D. E.

Bayer and Q. O. Yim, personal communication), was used. This assay detects acetoin production via acetolactate, the ALS enzyme product; a hypothetical direct conversion of acetoin from other sources (32) was not assayed for. R and S plants were grown in a growth chamber at 22°C, and a 15 h daylength was provided by fluorescent and incandescent lights delivering 400 μmol photosynthetic photon flux density (PPFD) m^{-2} s^{-1}. After seedling, emergence pots were flooded to 1–2 cm above the soil surface and complete fertilizer was added as required. Young leaf tissue from plants at the three-leaf growth stage was harvested. Two grams of leaf tissue from each accession was frozen in liquid nitrogen, ground to fine powder, and homogenized in 50 ml cold extraction buffer (K_2HPO_4, 0.1 M, pH 7.5; sodium pyruvate, 0.5 mM; thiamine pyrophosphate, 0.5 mM; $MgCl_2$, 0.5 mM; flavin adenine dinucleotide [FAD],10 μM; and glycerol, 100 ml L^{-1}). The homogenate was filtered through cheese cloth and centrifuged at 20,000*g* for 20 min. The supernatant was saturated to 50% w/v with $(NH_4)_2SO_4$ and the solution centrifuged at 20,000*g* for 20 min. The supernatant was discarded and pellet (extracted crude protein) stored at −80°C. The pellet was resuspended in 2.5 ml resuspension buffer (K_2HPO_4, 0.12 M, pH. 7.0; sodium pyruvate, 20 mM; and $MgCl_2$, 0.5 mM) and protein concentrations were determined using a Sigma protein assay kit, Procedure No. P 5656.[2]

Acetolactate synthase activity was assayed by adding 0.1 ml of enzyme preparation to 0.5 ml reaction mixture (K_2HPO_4, 20 mM, pH 7.0; sodium pyruvate, 20 mM; thiamine pyrophosphate, 0.5 mM; $MgCl_2$, 0.5 mM; and FAD, 10 μM) containing increasing concentrations of technical-grade bispyribac-sodium and incubated at 30°C for 2 h. The reaction was terminated by adding 0.05 ml H_2SO_4 (6 N). Then 0.5 ml creatine (5 g L^{-1} in water) was added, and the solutions were incubated at 60°C for 15 min. The added sulfuric acid terminated the ALS reaction and decarboxylated the enzyme product acetolactate to acetoin. Acetoin was detected as a colored complex ($A_{525\ nm}$) formed after adding 0.5 ml ∝-naphthol (50 g L^{-1} freshly prepared in 2.5 N NaOH) and incubating at 60°C for 15 min. A standard curve with commercial acetoin was used to quantify enzyme reaction products.

Based on the results of an initial experiment with bispyribac-sodium concentrations ranging from 0 to 500 nM, two other experiments were conducted with bispyribac-sodium concentrations ranging from 0 to 25 nM. Each experiment was conducted on separate tissue extracts from different plant material, with three replicate samples at each herbicide concentration for each experiment. Data are presented as means ± SE for common concentrations in all experiments. Also, R and S means at each concentration were separated by an analysis of variance *F* test with $P = 0.05$.

Cyt P-450 Monooxygenase Inhibitors

PBO study. A preliminary study (Study 1) was conducted in the greenhouse with germinated watergrass plants of the R accession seeded into 6.5.-cm^2 pots. After establishment, plants were thinned to three per pot. Treatments consisted of an untreated check, PBO at 600 and 1200 g ai ha^{-1}, bispyribac-sodium at 49.38 g ai ha^{-1}, a sequential application of PBO (600 g ai ha^{-1}) followed by bispyribac-sodium (49.38 g ai ha^{-1}), and a sequential application of PBO (1200 g ai ha−1) followed by bispyribac-sodium (49.38 g ai ha^{-1}) (Table 1). The rate of bispyribac-sodium used had proven lethal to the S accession in previous experiments (3). All applications were foliar and made at the four- to five-leaf stage of growth. Bispyribac-sodium was sprayed using a cabinet track sprayer with an even spray 8002E fan nozzle[3] delivering a spray volume of 140 L ha^{-1} under 207 kPa pressure. A silicone–polyester copolymer[4] was added to the bispyribac-sodium solution. PBO was

[2] Sigma Diagnostic, P. O. Box 14508, St. Louis, MO 63178.

[3] Spraying Systems Co., North Avenue, Wheaton, IL 60188.

[4] Osi Specialties Inc., 777 Old Saw Mill River Road, Tarrytown, NY 10591-6728.

applied using a chromatograph atomizer delivering 377 L spray volume ha^{-1}. After spraying PBO at 10–12 am, plants were left indoors overnight; bispyribac was applied approximately 24 h later, and plants were placed in the greenhouse when dry 4 h later. Sixteen days after seeding (four-leaf-stage plants), a complete soluble fertilizer solution was added to all pots. Pots were maintained flooded with water up to 2 cm above the soil surface. Plants were grown at an average temperature of 30°C and a 16 h daylength. Natural sunlight was supplemented by 400 μmol m^{-2} s^{-1} PPFD from high-pressure sodium lamps. Each treatment was replicated twice. Aboveground fresh weight per pot was determined 25 days after spraying. Data were analyzed as means ± SE.

Two other greenhouse experiments were conducted in Study 2 with the same bispyribac-sodium-resistant late watergrass accession. Treatments consisted of an untreated check, PBO at 1046 g ai ha^{-1}, PBO at 1569 g ai ha^{-1}, bispyribac-sodium at 66.16 g ai ha^{-1}, a sequential application of PBO (1046 g ai ha^{-1}) followed by bispyribac-sodium (33.08 g ai ha^{-1}), and a sequential application of PBO (1569 g ai ha^{-1}) followed by bispyribac-sodium (33.08 g ai ha^{-1}) (Table 1). As in the preliminary experiment, the rates of bispyribac-sodium are within the GR_{50} values observed for this accession in previous work (3). Growing conditions and chemical application procedures were the same as in the preliminary study. However, in these experiments the volume of application for bispyribac-sodium was 309 L ha^{-1} under 207 kPa pressure. Four plants were grown in each pot, and treatments were arranged in a completely randomized design with four (first experiment) or five (second experiment) replications. Aboveground fresh weight per pot was determined 21 days after spraying. Data from the two experiments were pooled, normalized to percentage of the untreated control, and analyzed by ANOVA. Treatment means were separated using Fisher's protected LSD ($P = 0.05$).

Malathion study. A preliminary study (Study 1) with the R accession was conducted using the following treatments: untreated check, malathion at 300 and 1000 g ai ha^{-1}, bispyribac-sodium at 49.38 g ai ha^{-1}, a sequential application of malathion (300 g ai ha^{-1}) followed by bispyribac-sodium (49.38 g ai ha^{-1}), and a sequential application of malathion (1000 g ai ha^{-1}) followed by bispyribac-sodium (49.38 g ai ha^{-1}) (Table 2). All other procedures were the same as in the preliminary PBO experiment above.

Two other experiments, comprising Study 2, were conducted with the following treatments: an untreated check, malathion at 347 g ai ha^{-1}, bispyribac-sodium at 33.08 g ai ha^{-1}, and a sequential application of malathion (347 g ai ha^{-1}) followed by bispyribac-sodium (33.08 g ai ha^{-1}) (Table 2). Experimental design, growing conditions, and all other procedures were identical as in the two replicated PBO experiments above. A commercial formulation of malathion[5] was used in all three experiments.

Cross-Resistance Experiment

Cross-resistance to bensulfuron-methyl was determined by conducting whole plant bioassays in the greenhouse. The experiments were conducted at an average daily temperature of 28°C and a 16-h daylength. Natural sunlight was supplemented by 400 μmol PPFD m^{-2} s^{-1} from high-pressure sodium lamps. Germinated seeds were planted in 16-cm^2 pots filled with a Yolo clay loam (fine-silty, mixed, nonacid, thermic Typic Xerorthents, 1.7% organic matter). After emergence seedlings were thinned to four plants per pot. Pots were fertilized shortly after thinning with a complete fertilizer as required. At the 1.5- to 2-leaf stage of growth bensulfuron-methyl was applied at rates of 0, 17.3, 34.5, 69.0, 138, and 276 g ai ha^{-1} using a cabinet track sprayer delivering a spray volume of 140 L ha^{-1} at a pressure of 276 kPa with an even-spray 8001E fan nozzle. Bensulfuron-methyl rates correspond to approximately 0, 0.25, 0.5, 1, 2, and 4 times the recommended field rate

[5] Malathion 50, dilutable concentrate; Chemisco, Division of United Industries Corporation, P.O. Box 15842, St. Louis, MO 63114-0842.

used in California rice (33). Treatments were arranged in a completely randomized design with four replications. Immediately after spraying, pots were flooded to 1 cm above the soil surface, and within 72 h after spraying, water level was raised to 10 cm above the soil. Twenty days after herbicide treatment, the aboveground shoots were harvested and fresh weight was determined. Fresh weight data were expressed as percentage of the untreated control and fitted to the log-logistic regression model proposed by Streibig *et al.* (34). Data from two experiments were pooled and regression analysis of all data points was conducted using the SigmaPlot version 4.0 (1997) statistical software. Herbicide rates to inhibit growth by 50% (GR_{50}) were calculated using the regression equations. The R/S ratio was calculated by dividing the GR_{50} of the resistant accession by the GR_{50} of the susceptible accession.

RESULTS AND DISCUSSION

ALS Activity

The amount of extracted leaf protein in R samples (5.35 mg g^{-1} leaf fresh weight $\pm$ 0.60, averaged across three experiments) was higher ($P < 0.05$) than that in S samples (3.19 mg $g^{-1} \pm 0.43$). ALS activity in mg acetoin g^{-1} leaf fresh weight was higher in the R biotype for all herbicide concentrations according to single ANOVA tests with $P = 0.05$ at each concentration (Fig. 1A). However, ALS activities per mg protein for the S and R biotype (16.8 $\pm$ 0.61 and 20.9 $\pm$ 2.2 mg acetoin mg^{-1} protein, respectively) were not statistically different (ANOVA, $P = 0.05$). ALS general activity (mg acetoin g^{-1} leaf fresh weight) was also assayed in the presence of 100 and 500 nM bispyribac-sodium in one of the three experiments conducted (data not shown). ALS general activity at 100 nM bispyribac-sodium was 5.2 mg $g^{-1} \pm 2.8$ (S) and 19.9 mg $g^{-1} \pm 2.9$ (R) ($P < 0.023$), and that at 500 nM was 2.5 mg $g^{-1} \pm 2.1$ (S) and 17.4 $\pm$ 5.2 (R) mg g^{-1} ($P < 0.056$).

The ALS enzyme of the R accession was more sensitive to bispyribac-sodium than that of the S accession (Fig. 1B). Therefore, resistance to bispyribac-sodium cannot be attributed to an altered herbicide binding site on ALS of the R accession. Despite the high tolerance of rice to bispyribac, Shimizu (10) found no differences in the sensitivity to bispyribac in ALS enzymes of rice and target weeds, suggesting that bispyribac selectivity toward rice may be due to metabolic detoxification and herbicide uptake. It has also been observed that sulfonylurea resistance in a *Lolium rigidum* population occurred despite a sulfonylurea-sensitive ALS (29). The similar ALS activity mg $protein^{-1}$ in both accessions, in the absence of herbicide, would suggest that the enzyme is not overexpressed in the R accession (20). However, as suggested by (35), gene

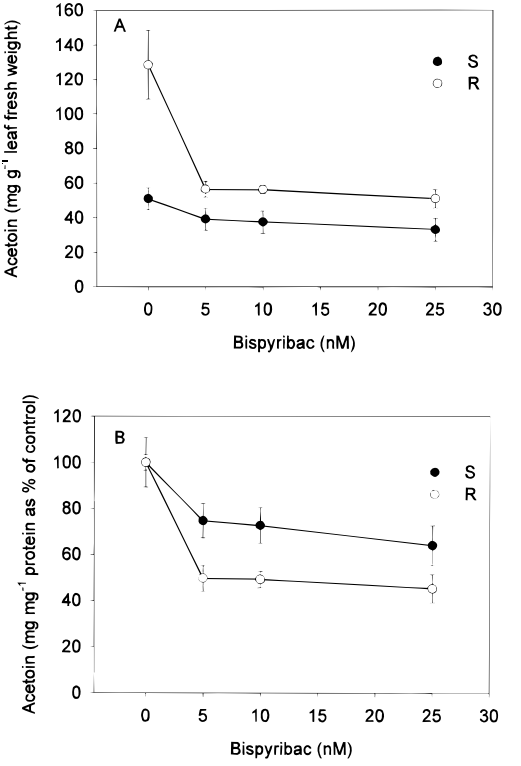

FIG. 1. ALS activity measured as the amount of acetoin produced per unit of leaf tissue (A) or per unit extracted leaf protein (B) after leaf extracts were incubated with a range of bispyribac-sodium concentrations. Each point is the mean $\pm$ SE of nine observations from three experiments with three replicates each.

amplification might be involved in the differential tolerance of these accessions in which protein levels were higher in the R type. It has been postulated that increasing use of ALS-inhibiting herbicides might select for plants with increased levels of ALS enzyme in weed populations (20, 35).

Cyt P-450 Monooxygenase Inhibitors

The contribution of herbicide metabolism to bispyribac-sodium resistance in the R accession was evaluated using the cyt P-450 inhibitor PBO. We also tested the potential of a commercial formulation of malathion, also a cyt P-450 inhibitor, to enhance bispyribac-sodium control of the same accession.

A preliminary study (Study 1) suggested that control by bispyribac, at a rate within the range of previously observed GR_{50} values (3), could be synergized with a nonphytotoxic rate of PBO (Table 1, Study 1). An inspection of the means in Table 1 (Study 1) using Colby's approach (36) suggested strong synergistic effects, even at the high PBO rate, which reduced watergrass growth by about 13% (data not shown). In the fully replicated experiments of Study 2, neither rate of PBO alone reduced watergrass growth, but when plants were treated with PBO and bispyribac-sodium, their growth was suppressed by 88 to 97% (Table 1, Study 2). The growth suppression resulting from the combined chemical treatment far exceeded ($P < 0.05$) the 53% suppression obtained with the herbicide alone.

Results from experiments with the commercial formulation of malathion were similar to those with PBO (Table 2). Even when a high malathion rate in the preliminary experiment (Study 1) somewhat inhibited R watergrass plants, inspection of the means in Table 2 (Study 1) using Colby's approach (36) still suggested synergism by malathion on bispyribac-sodium. The malathion rate used in the formally replicated experiments of Study 2 did not injure the R watergrass accession, and the combined treatment of malathion followed by bispyribac-sodium increased control of the R watergrass accession by 31% above that obtained with the herbicide alone (Table 2, Study 2). Thus, 347.7 g ai ha^{-1} of the cyt P-450 monooxygenase inhibitor malathion from a commercial formulation synergized a sublethal rate of bispyribac-sodium

TABLE 1
Response of the Resistant Accession (Aboveground Fresh Weight as Percentage of the Untreated Control) to Bispyribac-Sodium and Piperonyl Butoxide (PBO)

Treatments	Rate (g ai ha^{-1})	Study 1[a] (%)	Study 2[b] (%)
Untreated check		100 ± 7.31	100 ± 5.21
PBO	600	97.16 ± 10.63	—
PBO	1200	86.68 ± 6.44	—
PBO	1046	—	108.65 ± 9.08
PBO	1569	—	125.33 ± 11.53
Bispyribac-sodium	49.38	50.66 ± 6.47	—
Bispyribac-sodium	33.08	—	47.08 ± 7.34
PBO fb[c] bispyribac-sodium	600 fb 49.38	14.37 ± 2.40	—
PBO fb bispyribac-sodium	1200 fb 49.38	5.99 ± 0.60	—
PBO fb bispyribac-sodium	1046 fb 33.08	—	2.59 ± 1.39
PBO fb bispyribac-sodium	1569 fb 33.08	—	12.10 ± 4.59
LSD (0.05)		—[d]	20.73

[a] Values in this column are means ± SE of two observations.
[b] Values in this column are means ± SE of nine observations; data pooled from two experiments.
[c] fb, followed by.
[d] LSD not computed.

TABLE 2
Response of the Resistant Accession (Aboveground Fresh Weight as Percentage of the Untreated Control) to Bispyribac-Sodium and Malathion

Treatments	Rate (g ai ha^{-1})	Study 1[a] (%)	Study 2[b] (%)
Untreated check		100 ± 5.25	100 ± 13.03
Malathion	300	96.64 ± 7.85	—
Malathion	1000	79.68 ± 4.85	—
Malathion	347.1	—	102.17 ± 14.59
Bispyribac-sodium	49.38	45.10 ± 5.46	—
Bispyribac-sodium	33.08	—	48.45 ± 4.82
Malathion fb[c] bispyribac-sodium	300 fb 49.38	8.42 ± 2.02	—
Malathion fb bispyribac-sodium	1000 fb 49.38	4.98 ± 1.21	—
Malathion fb bispyribac-sodium	347.1 fb 33.08	—	16.63 ± 4.42
LSD (0.05)		—[d]	29.60

[a] Values in this column are means ± SE of two observations.
[b] Values in this columm are means ± SE of nine observations; data pooled from two experiments.
[c] fb, followed by.
[d] LSD not computed.

to strongly suppress the bispyribac-resistant late watergrass accession.

Since cyt P-450 inhibitors substantially abolished resistance, results from these experiments suggest that herbicide degradation involving cyt P-450 activity contributed to bispyribac resistance in the accession studied. The cyt P-450 system serves as a mechanism of selectivity between crop and weed species and has been implicated in selected weed herbicide resistance, including sulfonylureas (37). The enhancement of primisulfuron and nicosulfuron activity on corn and soybean with the addition of PBO was attributed to inhibition of metabolic herbicide degradation (38). The contribution of a PBO-sensitive mixed-function oxidase to rice tolerance of bensulfuron-methyl (28) and to primisulfuron and thifensulfuron resistance in a *Kochia scoparia* accession (26) were similarly demonstrated.

Malathion has been used to demonstrate that cyt P-450 monooxigenase activity was responsible for the resistance of maize (39) and *L. rigidum* (29) to select sulfonylurea herbicides and to synergize pendimethalin to suppress a pendimethalin-resistant *L. rigidum* population with multiple resistance to other herbicides (30).

Cross-Resistance Study

Growth of S watergrass was suppressed by about 70% with 69 g a.i. bensulfuron ha^{-1}, which is a representative field rate for broadleaf and sedge control in California (33), but the R accession was highly cross-resistant to bensulfuron-methyl (Fig. 2). According to the R/S ratio estimated by the regression equations in Fig. 2, the bensulfuron-methyl rate required to reduce the growth of the bispyribac-resistant accession by 50% was more than 15 times higher than that for an equivalent effect on the S accession. Substantial watergrass growth was still present at four times the recommended field rate of bensulfuron-methyl (Fig. 2). Moderate to high tolerance to bensulfuron-methyl had been reported for certain barnyardgrass and green sprangletop accessions in Japan (22).

In summary, bispyribac resistance in this resistant late watergrass accession most probably does not involve an insensitive ALS, although resistant plants exhibited higher ALS activity per unit leaf fresh weight than S plants. Although the mechanism of the observed cross-resistance to bensulfuron-methyl still needs to be established, previous repeated use of bensulfuron-methyl could have selected for plants with higher

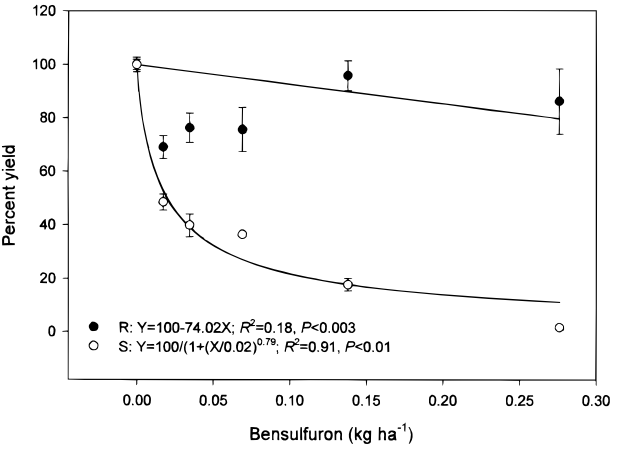

FIG. 2. Dose response of susceptible (S) and resistant (R) *E. phyllopogon* accessions to bensulfuron-methyl. Each point is the mean ± SE of eight observations from two experiments with four replicates each.

ALS activity per unit fresh weight, as suggested by Saari *et al.* (20).

The strong suppression achieved when cyt P-450 inhibitors were added to the herbicide suggests that metabolic degradation of bispyribac-sodium contributed significantly to the observed resistance. The R population had also shown resistance to other herbicides including molinate (3), which has been the herbicide most frequently used by California rice farmers for almost 30 years (2). Molinate is a thiocarbamate herbicide, which is detoxified by rice and *E. phyllopogon* through oxidation to sulfoxide and sulfone and to hydroxy derivatives (40–42). Detoxification of the thiocarbamate herbicide EPTC via oxidation to sulfoxide and sulfone is inhibited by the monooxygenase inhibitor PBO (43). Oxidative metabolism of molinate in both rats and humans is mediated by cyt P-450 monooxygenation (44). Further research should establish whether a common degradation pathway developed by the repeated use of molinate can account for the observed cross-resistance to molinate, bispyribac-sodium, and possibly other herbicides in the R population of *E. phyllopogon*. The existence of such a common degradation pathway would explain why there was resistance to bispyribac even before it becomes commercially available to farmers. Cyt P-450 oxygenase activity is the basis of herbicide selectivity between many crops and their associated weeds, and enhanced cyt P-450 activity has been implicated as a mechanism of simultaneous resistance to herbicides of different structure and mode of action (12, 13). Therefore, relying solely on the use of alternative selective herbicides to manage herbicide-resistant watergrass in California rice would be a risky strategy. The need for an integrated weed management approach must be stressed, whereby weeds are also subjected to nonchemical weed control practices. Bispyribac provides good control of watergrass in California rice (45). Tank mixtures with propanil, for which resistance has not been detected in California, the use of deep (18-cm) flooding, and a uniform stand of rice should help delay the development of resistance.

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