RESEARCH

Management of penoxsulam- and bispyribac-resistant late watergrass (*Echinochloa phyllopogon*) biotypes and rice sedge (*Cyperus difformis*) in rice

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ABSTRACT

The repeated use of herbicides with the same mode of action maybe has led to buildup of resistant late watergrass (*Echinochloa phyllopogon* (Stapf) Stapf ex Kossenko) populations. Thus, pot experiment was conducted to evaluate the level of penoxsulam- and bispyribac-resistance in seven late watergrass biotypes collected from water-seeded rice fields in Greece. Their susceptibility to imazamox and profoxydim was also studied. Furthermore, the efficacy of various herbicide combinations against the resistant late watergrass biotypes, as well as on rice sedge (*Cyperus difformis* L.) were evaluated in four field experiments. In pot experiment, two biotypes were resistant to both herbicides, while three biotypes were moderately resistant to penoxsulam with one of these simultaneously resistant to bispyribac. Four biotypes were also cross-resistant to imazamox, while all biotypes were susceptible to profoxydim. In field experiments, the double applications of penoxsulam or bispyribac applied at 18 d after seeding (DAS) followed by profoxydim plus halosulfuron at 35 DAS constantly provided the greatest rice yield and satisfactory control of both weeds. Conclusively, late watergrass resistant to penoxsulam and bispyribac biotypes have been built. In particular, rates up to 8 times the recommended rate did not provide acceptable weed control. Effective control of these biotypes and high rice yield can be achieved by double applications of herbicides with different modes of action (penoxsulam or bispyribac until the third leaf of rice [18 DAS] followed by profoxydim at seven-to eight-leaves growth stage [35 DAS]). Simultaneous control of late emerged rice sedge can be achieved by the addition of halosulfuron in the tank-mix with profoxydim.

Key words: Herbicide efficacy, imazamox, profoxydim, resistance management, rice yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in the world (Singh and Khush, 2000). In Greece, it is widely cultivated under flooded conditions in fields in the delta of the rivers Axios, Aliakmon, Nestos, and Sperheios. In rice fields, late watergrass (*Echinochloa phyllopogon* [Stapf] Stapf ex Kossenko or *Echinochloa oryzicola* [Vasinger] Vasinger) is the predominant among *Echinochloa* species, followed by early watergrass (*Echinochloa oryzoides* [Ard.] Fritsch) and barnyard grass (*Echinochloa crus-galli* [L.] P. Beauv.) (Fischer et al., 2000a; Tsoktouridis et al., 2015). It is a polymorphic species (Tsoktouridis et al., 2015), extremely competitive, which can grow well in drilled or water-seeded rice. Generally, *Echinochloa* species are capable to remove 60%-80% of the available N from the soil (Holm et al., 1997; Talbert and Burgos, 2007). According to Fischer et al. (2000a) and Tshewang et al. (2016), barnyard grass or late watergrass infestations in rice can result in detrimental yield losses ranging from 30% to 50% until to complete crop loss. Zhang et al. (2017) also found that barnyard grass interference in canopy light transmission resulted in 12.7%-55.2% rice grain yield losses. Smith (1988) reported that 5-10 barnyard grass plants m^{-2} were estimated to be an adequate threshold infestation level for control practices to prevent yield losses and quality reduction of rice. Rice producers are mainly dependent on chemical weed control, especially in fields where rice is continuously cultivated; however, repeated use of the same herbicides, or herbicides with the same mode of action, has already led to the selection and buildup of resistant late watergrass and barnyard grass populations (Carey et al., 1995; Vasilakoglou et al., 2000; Kaloumenos et al., 2013; Norsworthy et al., 2014; Wilson et al., 2014). To date, late watergrass and barnyard grass resistance to propanil, molinate, thiobencarb, quinclorac, penoxsulam, bispyribac, azimsulfuron, bensulfuron, cyhalofop, and clomazone, as well as imazethapyr and imazamox has been confirmed (Heap, 2017). Although resistance in late watergrass and barnyard grass results in fitness cost (Fischer et al., 2000b; Yang et al., 2017), this potential threat of multiple herbicide resistance in late watergrass and barnyard grass is a major concern for rice producers, while the Clearfield-technology rice, which allows the use of multiple applications of imazethapyr or imazamox, cannot provide effective *Echinochloa* control in all cases (Vasilakoglou and Dhima, 2005).

The use of herbicide combinations that contain different modes of action could be the best short-time management practice to reduce the probability of resistance and preserve the effectiveness of the products labeled in rice, especially in regions where rice is cultivated as continuous monoculture. However, experimental data on effective control of herbicide resistant late watergrass biotypes in rice fields are relatively limited. Therefore, the objectives of this study were: i) to detect the resistance of late watergrass biotypes on penoxsulam or bispyribac in rice fields, ii) to examine the late watergrass control options in rice fields using various herbicide combinations to reduce the potential for resistance or to manage already resistant biotypes, and iii) to examine the simultaneous control of rice sedge.

MATERIALS AND METHODS

Pot resistance experiment

Plant material. Seeds from mature late watergrass plants (at shattering stage) of five morphologically distinct (with differences in panicle size and dense, as well as awn length) biotypes (E1 to E5) were collected at the end of the 2012 growing season. Seed collection had been conducted in the delta of the rivers Axios and Aliakmon, a rice-growing area of northern Greece, from rice fields treated with penoxsulam and/or bispyribac (possible resistant biotypes). Also, seeds of two biotypes (E6 and E7) had been collected from the same area in 2002 growing season, before the commercial use of penoxsulam and bispyribac in Greece (possible susceptible biotypes). All collected seeds were air-dried in the greenhouse, air-cleaned to remove non-viable seeds and other plant residues and stored in a refrigerator at 3 to 6 ºC until used for the experiment.

Biotype dose-response. One pot experiment was conducted at the Technological Educational Institute of Thessaly (Larissa) during 2013. Experiment was carried out using 15 × 25 cm plastic pots filled with a mixture of silty clay soil:sand (2:1 v/v). The physicochemical characteristics of the soil used in the experiment were clay 49%, silt 34%, sand 17%, organic matter 1.2%, pH (1:1 H_2O) 7.5 and 31.2 meq 100 g^{-1} cation exchange capacity (CEC). Seeds of the seven late watergrass biotypes were seeded and covered with 0.5 cm soil in middle March. Before seeding, all seeds were treated with sulfuric acid (96%-98%) for 4 min to break seed dormancy. Pots were placed in the greenhouse and watered as needed. Five days after emergence late watergrass was thinned to 30 plants per pot (replicate) to obtain a uniform population in all pots. At the same time, the emerged broad-leaved weeds were hand-removed, while no other grass weeds emerged. At 3 wk after seeding, 50 kg N ha^{-1} as ammonium nitrate (33.5-0-0) were applied in each pot.

In pots seeded with the biotypes E1 to E5 (possible resistant), herbicide treatments included penoxsulam at ×0, ×1/2, ×1, ×2, ×4, and ×8 of the recommended rate (40 g ha^{-1}) and bispyribac at ×0, ×1/2, ×1, ×2, ×4, and ×8 of the recommended rate (37.5 g ha^{-1}). In biotypes E6 and E7 (possible susceptible) pots, the herbicide treatments included penoxsulam at ×0, ×1/8, ×1/4, ×1/2, ×1, and ×2 of the recommended rate and bispyribac at ×0, ×1/8, ×1/4, ×1/2, ×1, and ×2 of the recommended rate. Also, profoxydim at 200 g ha^{-1} (recommended rate) and imazamox at 50 g ha^{-1} (recommended rate) were included in each late watergrass biotype. All bispyribac, profoxydim, and imazamox treatments were applied in mixture with a non-ionic surfactant (an emulsifiable concentrate containing fatty acid esters alkoxylated alcohols-phosphate esters; Dash HC, BASF, Cheadle, UK) at 1.0 L ha^{-1}. Herbicide application was performed 3 wk after sowing when late watergrass plants had three-to four leaves. All herbicide treatments were applied by an air-pressurized hand-field plot sprayer (AZO-Sprayers, Ede, The Netherlands), with a 2.4 m wide boom fitted with six 8002 flat fan nozzles (Teejet Spray System Co., Wheaton, Illinois, USA), which was calibrated to deliver 300 L ha^{-1} of water at 280 kPa pressure. A completely randomized design with four replicates was used. The experiment was repeated in time, under similar temperature conditions, by using the same biotypes and the same herbicide treatments. Late watergrass control was assessed by counting the shoot numbers per pot and determining the fresh weight of all survived plants in each pot 4 wk after herbicide application. Data were expressed as a percentage of the untreated control.

Rice field efficacy experiments

Experimental site. Four field experiments were conducted in 2012 (Exp1), 2013 (Exp2), 2014 (Exp3), and 2015 (Exp4) to evaluate the efficacy of various herbicide combinations on penoxsulam- and bispyribac-resistant late watergrass biotypes. The experiments were established in four different rice fields, from which resistant late watergrass seeds had been collected. Fields were located at the delta of the rivers Axios and Aliakmon, northern Greece (40º39' to 41' N, 22º43' to 45' E; 1 to 3 m a.s.l.) The soils in the region are mostly silty clay, poorly drained, classified as Typic Xerofluvents under Mediterranean climate conditions. Mean monthly temperature and rainfall data recorded near the experimental area are shown in Figure 1.

Treatments and experimental design. In all years, the previous crop was rice harvested in middle October. Rice straw was baled and removed after harvest. The land was ploughed after harvest and left undisturbed during winter. In middle April, the experimental area was cultivated with a harrow disk to prepare the soil and to incorporate the fertilizers into the soil. The experimental area was naturally infested by late watergrass (60-170 plants m^{-2}) and rice sedge (240-700 plants m^{-2}), as confirmed by visual assessments made during each growing season. In all experiments, N and P at 150 and 60 kg ha^{-1}, respectively, were incorporated before rice seeding.

In all experiments, rice was seeded in the second week of May. The imidazolinone-resistant (Clearfield-technology) varieties Sirio or Luna were seeded in Exp1 and Exp2 or Exp3 and Exp4, respectively, at seeding rate of 200 kg ha^{-1}. Rice was water-seeded by fertilizer spreader, reflecting the common practice in Greek rice fields.

In each experiment, a randomized complete block design was used with four replicates per treatment. Plot size was 2.5 × 6.0 m. All blocks were separated by 2-m buffer zone. The chemical names of herbicides used are listed in the Table 1, while the herbicide treatments are presented in the Tables 3 and 4. Also, one untreated (weedy) control was included to assist yield loss due to late watergrass and rice sedge competition. Most of herbicide treatments were double applications of two or three active ingredients, due to late watergrass resistance, as well as to successive emergence of late watergrass and rice sedge in field conditions. Herbicide treatments varied from year to year in order to achieving

Figure 1. Mean monthly temperature and total monthly rainfall during the experiments.

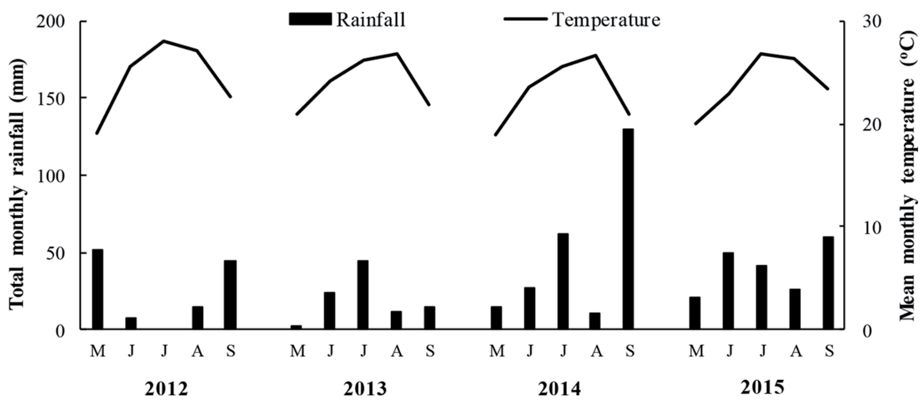


Table 1. Active ingredients, chemical names, commercial names, and companies of the herbicides used in the field experiments.

Active ingredient	Chemical name	Commercial name	Company
Penoxsulam	3-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4] triazolo[1,5-*c*]pyrimidin-2-yl)-α,α,α-trifluorotoluene-2-sulfonamide	Viper	Efthimiadis K&N, Thessaloniki, Greece
Bispyribac	2,6-bis(4,6-dimethoxypyrimidin-2-yloxy)benzoic acid	Adora	Bayer Crop Science Hellas, Athens, Greece
Profoxydim	(5*RS*)-2-{(*EZ*)-1-[(2*RS*)-2-(4-chlorophenoxy) propoxyimino]butyl}-3-hydroxy-5-[(3*RS*)-thian-3-yl] cyclohex-2-en-1-one	Aura	Basf Agro Hellas, Athens, Greece
Imazamox	2-[(*RS*)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid	Pulsar	Basf Agro Hellas, Athens, Greece
Halosulfuron	3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-1-methyl-1*H*-pyrazole-4-carboxylic acid	Permit	Alfa Hellas, Athens, Greece
Penoxsulam plus triclopyr	Penoxsulam + 3,5,6-trichloro-2-pyridyloxyacetic acid	GF2837	Efthimiadis K&N, Thessaloniki, Greece

increased weed control; however the herbicide treatments which revealed increased efficacy were repeated at least for 1 yr. Two days before herbicides application, water was removed from paddy fields; rice fields were re-submerged 2 d after herbicide treatments and were kept flooded until grain physiological maturity. Herbicide applications were made by an air-pressurized hand-field plot sprayer (AZO-Sprayers), with a 2.4 m wide boom fitted with six 8002 double flat fan nozzles (Teejet), which was calibrated to deliver 400 L ha^{-1} of water at 300 kPa pressure. Other common cultural practices were imposed as needed during growing season. In all experiments, the first application of herbicides (18 DAS) was done when rice and late watergrass were at the three- and three to four-leaves growth stage, respectively. The second application of herbicides (35 DAS) was done when rice was at the seven-to eight-leaves growth stage, while the weeds were at various stages due to their successive emergence.

Data collection. In all field trials, rice stand and weed plants present in the center 2 m^2 of each plot were counted at 15 d after seeding (DAS). Rice injury (plant death and reduced growth) and weed control was visually estimated using a scale of 0% (no injury or no control) to 100% (complete plant death) at 17, 28, and 42 d after treatment (DAT). At harvest, carried out at the first week of October each year, late watergrass panicle number and rice sedge umbrella number per plot (15 m^2), as well as rice panicle number and seed yield from the central 1 m^2 of each plot were recorded.

Statistical analyses

The greenhouse (pot) experiment data were analyzed separately for resistant and susceptible late watergrass biotypes, due to different herbicide rates used, using a factorial approach (Repetition time × Biotypes × Herbicide treatment). As the ANOVAs indicated nonsignificant Treatment × Repetition time interaction, means were averaged across the two experiments. Shoot number and fresh weight replicates for penoxsulam or bispyribac treatments were used for regression analysis to determine the 50% growth reduction values (penoxsulam or bispyribac required for 50% inhibition of shoot number or fresh weight [ED_{50}] for the seven late watergrass biotypes). In particular, data were fitted to the four-parameter log-logistic curve (Seefeldt et al., 1995; Ritz, 2010):

$$y = C + \{[D - C]/1 + exp[b(log(x) - log(ED_{50}))]\}$$

where y is the weed shoot number or fresh weight expressed as % of untreated control, x is the herbicide rate (g ha^{-1} + 0.1), D and C are the upper and lower, respectively, values of y, and b is proportional to the slope of the curve around ED_{50}, which is the rate required to halve the shoot number or fresh weight relative to D.

The Kruskal-Wallis test, a non-parametric analysis comparing means of univariate groups, was performed for the % control data, while the other field experiments data were subjected to a combined over-year ANOVA, separately for the first two and the last two years, due to different treatments included.

The homogeneity of variances was examined with the Bartlett's test. The R software (version 3.4.2; R Foundation for Statistical Computing, Vienna, Austria) was used to estimate the parameters and to test the significance of differences between parameters of the log-logistic curves (Ritz and Streibig, 2005). The STATISTICA (StatSoft, 2013) program was used to conduct Kruskal-Wallis analysis, while the MSTAT (MSTAT-C, 1988) program was used to conduct ANOVA. The Fisher's protected least significant difference test procedures were used to detect and separate mean treatment differences at P = 0.05.

RESULTS AND DISCUSSION

Pot resistance experiment

The ANOVAs performed for the late watergrass data indicated that there were significant Biotype × Herbicide treatment interactions ($P < 0.001$). In particular, the shoot number and fresh weight of the E2 biotype were nonsignificantly reduced by penoxsulam and bispyribac applied at the ×1/2 to ×8 of the recommended application rate (Figures 2 and 3). Penoxsulam and bispyribac applied at the ×1 to ×8 rates provided intermediate reduction of shoot number and fresh weight of E1 biotype. Also, penoxsulam applied at the ×2 to ×8 rates and bispyribac applied at the ×4 to ×8 rates provided great reduction of shoot number and fresh weight of E3, E4, and E5 biotypes. On the contrary, penoxsulam applied at the ×1/4 rate and bispyribac applied at the ×1 rate almost completely reduced shoot number and fresh weight of the susceptible E6 and E7 biotypes. Imazamox applied at the recommended rate provided partial control of the E1, E2, E3, and E4 biotypes; however provided good control (> 80%) of the E5 and excellent control of the E6 and E7 ones (Figure 4). Profoxydim applied at the recommended rate provided very good control (> 90%) of the seven biotypes (Figure 4).

The regression analysis of late watergrass shoot number and fresh weight response to penoxsulam or bispyribac indicated that the four-parameters log-logistic curve provided in most cases good fit of the data (Table 2) with the exception of the E2 for both herbicides and E1 for bispyribac. This poor fit of E1 and E2 biotypes could be attributed to the lower than 50% late watergrass shoot number and fresh weight reduction provided by the highest herbicide rate used. Good fitting for the four-parameters log-logistic curve have been reported by Kaloumenos et al. (2013), and Altop et al.

Figure 2. Shoot number of seven late watergrass biotypes treated with a range of penoxsulam or bispyribac rates at the three-to four-leaves stage. Means are averages over two experiments (see Table 2 for log-logistic curve parameters).

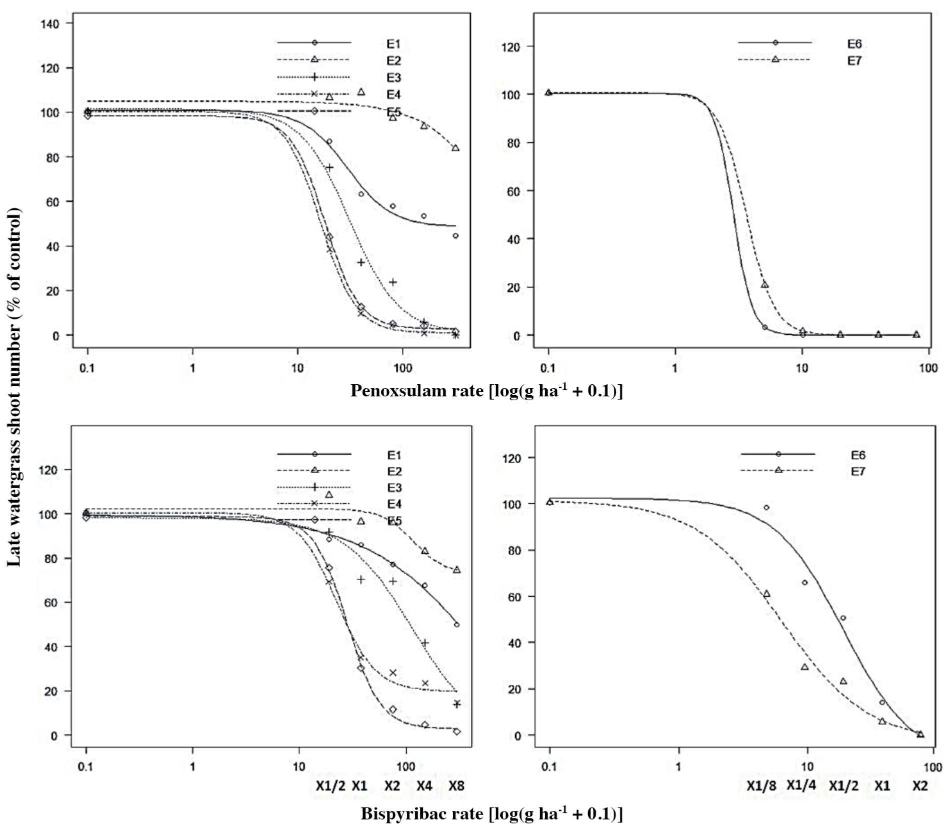
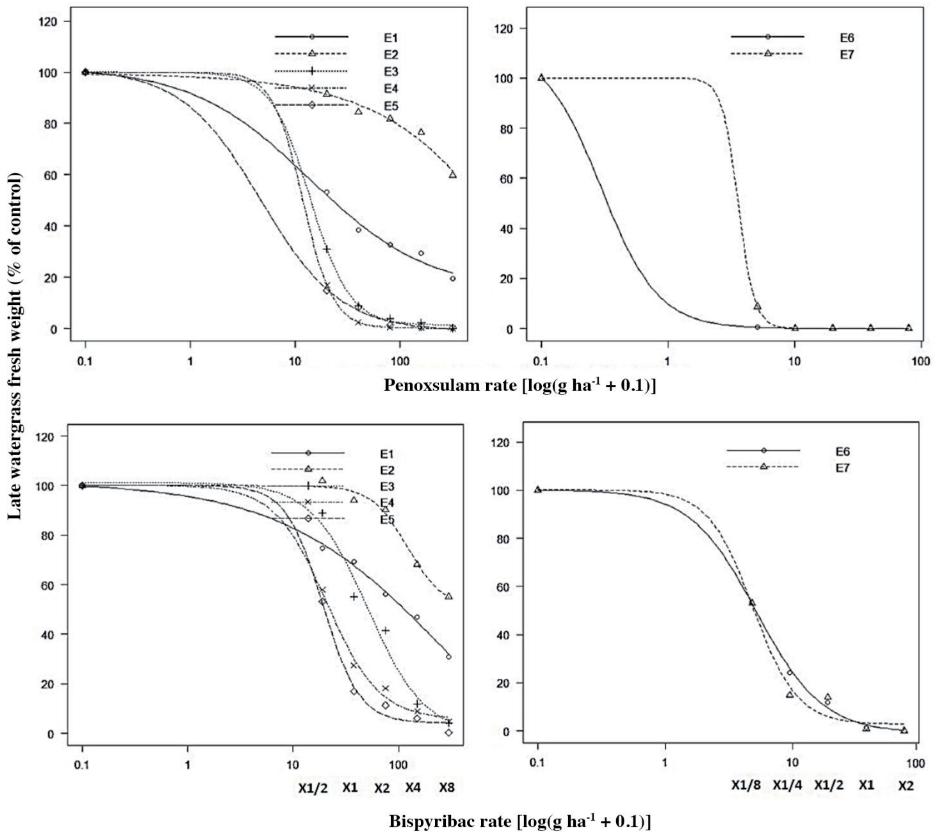
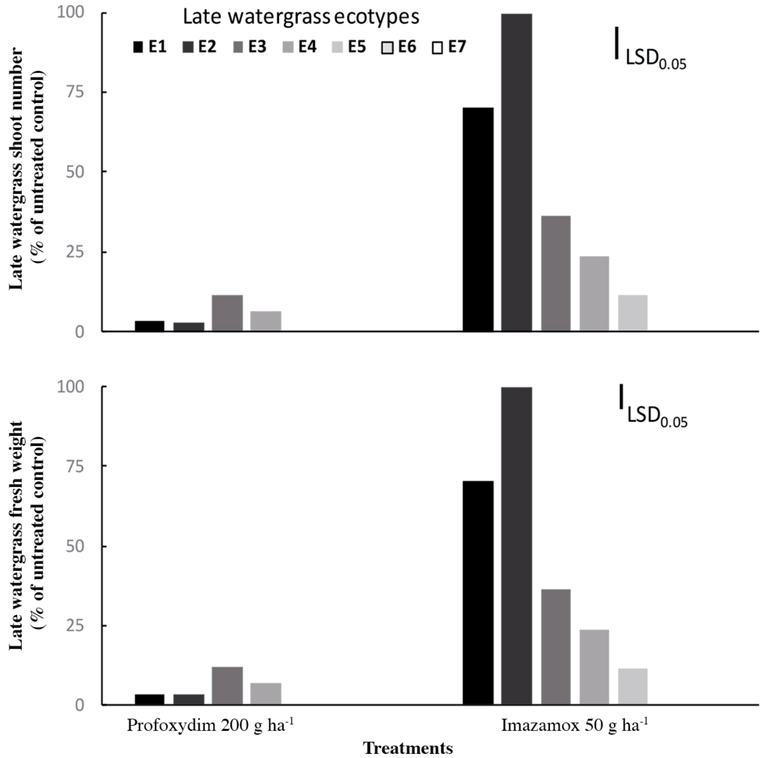


Figure 3. Fresh weight of seven late watergrass biotypes treated with a range of penoxsulam or bispyribac rates at the three-to four-leaves stage. Means are averages over two experiments (see Table 2 for log-logistic curve parameters).



(2014) studied the resistance of late watergrass and early watergrass, respectively, to rice herbicides. The lack-of-fit test performed to compare pairs of regression lines showed that, in most cases, late watergrass data could not be described by a single regression and, consequently, predicted ED_{50} values pertained to statistically different data populations. The ED_{50} values presented on Table 2 suggest that the E7 biotype was susceptible to both penoxsulam (with ED_{50} values equal to 3.6 g ha^{-1}, which is rate lower than the ×1/10 of the recommended rate) and bispyribac (ED_{50} values 4.9 and 6.2 g ha^{-1}, which are rates lower than the ×1/5 of the recommended rate). The E6 biotype was susceptible to penoxsulam (ED_{50} values 0.3 and 2.9 g ha^{-1}, which are rates lower than the ×1/10 of the recommended rate) and moderately susceptible to bispyribac (ED_{50} values 5.2 and 18.9 g ha^{-1}, which are rates lower than the ×1/2 of the recommended rate). The biotypes E4 and E5 were moderately resistant to both herbicides (ED_{50} values ranged from 4.7 to 18.2 g ha^{-1} for penoxsulam, which are rates lower than the ×1/2 of the recommended rate and from 19.1 to 2.79 g ha^{-1} for bispyribac, which are rates lower than the recommended rate). The E3 biotype was resistant to bispyribac (ED_{50} values 51.2 and 113.9 g ha^{-1} [which are rates greater than the recommended rate]) and moderately resistant to penoxsulam (ED_{50} values 13.9 and 31.4 g ha^{-1}, which are rates lower than the recommended rate). The E1 and E2 biotypes were resistant to both herbicides. Regarding the E1 biotype, the ED_{50} values were 13.8 and 29.9 g ha^{-1} (which are rates lower than the recommended rate) for penoxsulam. However, for the E1 and bispyribac, as well as for the E2 biotype and both herbicides, the log-logistic curve did not provide good fitting and so the ED_{50} values could not be calculated, because the highest rates of both herbicides (320 and 300 g ha^{-1} for penoxsulam and bispyribac, respectively) provided shoot number and fresh weight reduction lower than 50%. All biotypes were susceptible to profoxidim; however, E1 and E2 or E3 and E4 biotypes were cross-resistant or moderately cross-resistant, respectively, to imazamox (Figure 4). Similarly, Fischer et al. (2000b) and Yasuor et al. (2009) found that late watergrass was resistant to bispyribac and penoxsulam, respectively. Norsworthy et al. (2014) found that

Figure 4. Shoot number and fresh weight of seven late watergrass biotypes treated with profoxydim or imazamox at the three-to four-leaves stage. Means are averages over two experiments.



one barnyard grass biotype was resistant to penoxsulam, bispyribac and imazethapyr. In the same experiment, the corresponding ED_{50} values for the resistant biotype were 254, 49, and 170 g ha^{-1}, while for the susceptible biotype were 10, 6, and 12 g ha^{-1}. Chen et al. (2016) found that the ED_{50} values of penoxsulam-resistant barnyard grass biotypes in

Table 2. Log-logistic curve parameters and predicted ED_{50} values for the relationship between shoot number (% of control) or fresh weight (% of control) of seven late watergrass biotypes and penoxsulam or bispyribac application rate [log(g ha^{-1} + 0.1)].

	Shoot number				Fresh weight			
Late watergrass biotype	Upper limit	Lower limit	*b* ± SE	ED_{50} ± SE	Upper limit	Lower limit	*b* ± SE	ED_{50} ± SE
Penoxsulam				g ha^{-1}				g ha^{-1}
E1	100.9	48.5	2.06 ± 1.64	29.9 ± 9.4	102.0	14.4	0.77 ± 0.82	13.8 ± 6.6
E2	-	-	-	> 320	-	-	-	> 320
E3	101.5	1.0	1.86 ± 0.69	31.4 ± 5.7	100.0	1.3	2.32 ± 1.45	13.9 ± 3.7
E4	100.2	1.0	2.56 ± 1.59	16.5 ± 3.1	100.0	0.1	3.03 ± 3.9	11.8 ± 6.0
E5	98.3	2.8	2.70 ± 1.42	18.2 ± 2.6	101.2	-1.1	1.15 ± 1.45	4.7 ± 3.4
E6	100.4	0.0	5.88 ± 4.10	2.9 ± 1.4	112.3	0.0	1.93 ± 1.27	0.3 ± 0.2
E7	100.7	0.0	3.94 ± 2.62	3.6 ± 0.8	100.0	0.0	6.69 ± 3.71	3.6 ± 2.9
Bispyribac								
E1	-	-	-	> 300	-	-	-	> 300
E2	-	-	-	> 300	-	-	-	> 300
E3	98.1	-1.5	1.34 ± 0.45	113.9 ± 25.3	101.2	-0.7	1.61 ± 0.43	51.2 ± 11.8
E4	100.5	19.6	2.39 ± 1.21	22.4 ± 3.7	100.2	5.6	1.75 ± 0.77	20.8 ± 3.7
E5	98.7	2.7	2.79 ± 0.89	27.9 ± 3.6	100.1	4.1	2.50 ± 1.16	19.1 ± 2.5
E6	102.5	-11.5	1.62 ± 0.62	18.9 ± 6.4	100.2	-0.9	1.68 ± 0.73	5.2 ± 1.0
E7	101.4	-2.8	1.30 ± 0.81	6.2 ± 2.1	100.2	2.7	2.49 ± 1.34	4.9 ± 0.7

The dose-response regression equation is $y = C + \{D - C) / 1 + \exp[b(\log(x) - \log(ED_{50}))]\}$, where y is shoot number or fresh weight expressed as percent of the untreated mean control, b is the slope of the curve around the ED_{50}, C denotes the lower limit of the response when the rate x approaches infinity, D is the upper limit when the rate approaches zero, and x is the herbicide rate in g ai ha^{-1} + 0.1.
ED_{50}: Herbicide application rate (g ha^{-1}) required for 50% late watergrass shoot number or fresh weight inhibition.

China ranged from 28.2 to 80.7 g ha^{-1}, while some biotypes were simultaneously resistant to other rice herbicides such as bispyribac, quinclorac, cyhalofop, oxadiazon, and pretilachlor. Also, Eberhardt et al. (2016) reported one barnyard grass biotype in Brazil with multiple resistance to penoxsulam, cyhalofop, and quiclorac, but susceptible to propanil.

For both shoot number and fresh weight data, the ratios (R/S) of the resistant or moderately-resistant to susceptible ED_{50} values were greater than 5.7 and 3.6 for penoxsulam and bispyribac, respectively. Similarly, Yasuor et al. (2009) found R/S ratio which ranged from 5 to 9 in penoxsulam resistant late watergrass, while Altop et al. (2014) found that early watergrass accessions had 100% survival at 6 times the recommended application rate.

Differences in susceptibility to penoxsulam and bispyribac among the seven late watergrass biotypes could be associated with differences in herbicide metabolism via P450 monooxidation (Fischer et al., 2000b; Yasuor et al., 2009). However, Kaloumenos et al. (2013) found that the late watergrass resistance to penoxsulam and bispyribac was mostly due to mutation in ALS enzyme. The same researchers (Kaloumenos et al., 2013) found that two late watergrass biotypes were cross-resistant to penoxsulam, bispyribac, imazamox, foramsulfuron, nicosulfuron, and rimsulfuron, whereas all biotypes tested were susceptible to profoxydim.

Rice field efficacy experiments

The herbicide treatments, evaluated during the 4-yr experiment, had as target to manage both resistant late watergrass biotypes and rice sedge, taking into consideration that rice cropping is continuous in this area and both weeds have a successive emergence in watered-rice field conditions. So, the purpose of the experimental trials was to evaluate as many treatments as possible to find the most effective simple or double applications of two or three active ingredients. However, the herbicide treatments provided high efficacy were repeated at least for 1 yr.

The ANOVA performed for the treatments of the first two years indicated no significant Year × Treatments interaction. So, the means presented in the Table 3 are averaged across year. In particular, the double application of penoxsulam/profoxydim and penoxsulam/imazamox provided 90.0% and 91.6%, respectively, late watergrass control at 42 DAT (Table 3). The simple application of profoxydim provided lower late watergrass control. At harvest, the double applications of penoxsulam/profoxydim and penoxsulam/imazamox provided acceptable reduction of late watergrass panicles. However, rice sedge had not been successfully controlled and, at harvest, significant rice sedge umbrellas were recorded in all treatments. Especially, profoxydim applied at 25 DAS totally failed to control rice sedge. The greatest rice yield (9.36 t ha^{-1}) was achieved by the double application of penoxsulam/imazamox.

In Exp3, most of the evaluated treatments were applications of two or three herbicides due to great efficacy against the two main rice weeds, as it had been seen in the previous years. At 42 DAT, most of treatments provided very good control of late watergrass (95.8%-98.0%) and rice sedge (88.5%-95.0%) (Table 4). However, poor late watergrass control was achieved by the application of penoxsulam/imazamox+halosulfuron. Also, poor rice sedge control was achieved by the application of profoxydim+halosulfuron and profoxydim+penoxsulam+triclopyr. The greatest rice yields (9.40 and 10.20 t ha^{-1}) was achieved by the simple application of profoxydim+penoxsulam+triclopyr and the double application of bispyribac/profoxydim+halosulfuron.

Among simple applications in Exp4, profoxydim+halosulfuron applied at 30 DAS provided partial control of late watergrass (50.0%) and rice sedge (42.5%), as well as intermediate rice yield (7.35 t ha^{-1}) (Table 4). The simple

Table 3. Effectiveness of herbicide treatments on resistant late watergrass biotypes and rice sedge, as well as rice yield during the two first years of the study. Means are averaged across the two years (2012-2013).

			Late watergrass		Rice sedge		Rice	
Herbicide treatments	Rate	Timming	% control	Panicles m^{-2}	% control	Umbrellas m^{-2}	Panicles	Seed yield
	g ha^{-1}	DAS	42 DAT	Harvest	42 DAT	Harvest	nr m^{-2}	t ha^{-1}
Untreated control[l]	-	-	0.0c	80a	0.0c	27ab	38d	0.53d
Profoxydima	200	25	77.4b	8b	0.0c	32a	359c	3.78c
Penoxsulam/profoxydim[a]	41/200	18/35	90.0a	3b	46.9b	25ab	546b	7.94b
Penoxulam/imazamox[a]	41/60	18/35	91.6a	2b	75.0a	19b	694a	9.36a
CV, %			12.0	23.4	21.5	20.6	12.8	17.2

DAS: Days after seeding; DAT: days after the first treatment.
[a]Plus Dash at 1.0 L ha^{-1}.
Means in each column followed by the same letter did nonsignificantly differ according the protected LSD test at P = 0.05.

Table 4. Effectiveness of herbicide treatments on resistant late watergrass biotypes and rice sedge, as well as rice yield during the two last years of the study (2014-2015).

			Late watergrass		Rice sedge		Rice	
Herbicide treatments	Rate	Timming	% control	Panicles m^{-2}	% control	Umbrellas m^{-2}	Panicles	Seed yield
	g ha^{-1}	DAS	42 DAT	Harvest	42 DAT	Harvest	Nr m^{-2}	t ha^{-1}
Exp3 (2014)								
Untreated control	-	-	0.0f	56a	0.0e	5c	400e	3.71d
Penoxsulam/profoxydim +halosulfuron[a]	41/50+38	18/35	96.5a	2f	95.0a	2def	830bc	7.92c
Bispyribac[a]/profoxydim+ halosulfuron[a]	38/200+38	18/35	95.8a	1f	88.5ab	1fg	977ab	10.20a
Penoxsulam/imazamox+ halosulfuron[a]	41/60+53	18/35	31.5d	25c	91.8a	1fg	413e	3.55d
Profoxydim+halosulfuron[a]	200+53	30	98.0a	2f	44.3cd	2de	817bc	8.53bc
Profoxydim+penoxsulam+ triclopyr	200+48+360	30	98.0a	1f	45.0cd	2defg	907abc	9.40ab
Bispyribac[a]/profoxydim+ penoxsulam+triclopyr	38/200+48+360	18/35	96.8a	1f	91.8a	1g	1017a	8.45bc
Exp4 (2015)								
Untreated control	-	-	0.0f	36b	0.0e	5bc	23f	0.18f
Penoxsulam/profoxydim+ halosulfuron[a]	41/50+38	18/35	95.0a	1f	87.5ab	1efg	785c	10.57a
Bispyribac[a]/profoxydim+ halosulfuron[a]	38/200+38	18/35	94.3a	1f	81.3b	3d	775c	9.68ab
Penoxsulam/imazamox+ halosulfuron[a]	41/60+53	18/35	13.8e	33b	53.8c	2de	118f	1.59e
Profoxydim+halosulfuron[a]	200+53	30	50.0c	9e	42.5d	6b	600d	7.35c
Profoxydim+penoxsulam+ triclopyr	200+48+360	30	62.5b	16d	35.0d	10a	413e	4.59d
Bispyribac[a]/profoxydim+ penoxsulam+triclopyr	38/200+48+360	18/35	95.0a	2f	85.0ab	2de	768c	10.48a
CV, %			9.5	22.7	11.8	22.6	17.8	14.2

DAS: Days after seeding; DAT: days after the first treatment.
[a]Plus Dash at 1.0 L ha^{-1}.
Means in each column followed by the same letter did nonsignificantly differ according the protected LSD test at P = 0.05.

application of profoxydim+penoxsulam+triclopyr provided lower control of late watergrass and rice sedge, as well as lower rice yield than those provided in Exp3. This fact could be attributed to greater temperature recorded during May in 2015 than in 2014 (Figure 1) resulting in greater growth stage of both weeds at the application time. Among the double applications, penoxsulam/profoxydim+halosulfuron, bispyribac/profoxydim+halosulfuron, and bispyribac/profoxydim+penoxsulam+triclopyr provided the greatest rice yield (9.68 to 10.57 t ha^{-1}), as well as very good control of late watergrass (about 95%) and rice sedge (> 80%).

In both Exp3 and Exp4, the late watergrass control achieved by imazamox+halosulfuron at 35 DAS (following the application of penoxsulam at 18 DAS) was poor, maybe due to antagonism between imazamox and halosulfuron in the tank mixture. Similarly, Matzenbacher et al. (2015) found that the mixture of acetyl-CoA carboxylase (ACCase)-inhibitors herbicides with acetolactate synthase (ALS)-inhibitors, quinclorac, clomazone+propanil, or thiobencarb resulted in antagonism. However, the same researchers (Matzenbacher et al., 2015) found that mixtures of profoxydim with cyhalofop resulted in synergism. The same was recorded in mixtures of clomazone or quinclorac with imazapyr+imazapic, bispyribac, or cyhalofop.

Generally, the greatest control of both late watergrass and rice sedge was achieved by the double applications of herbicides with different mode of action, while most of the simple applications did not provide satisfactory weeds control and rice yield. During the last 3 yr of field experiments, the treatments of penoxsulam or bispyribac applied at 18 DAS followed by profoxydim+halosulfuron applied at 35 DAS provided very good control of late watergrass and rice sedge emerged simultaneously with rice and later during rice growth. This very good efficacy had as result the great rice yield. Wilson et al. (2014) found that imazethapyr alone failed to control an ALS-resistant barnyard grass biotype. However, when imazethapyr combined with fenoxaprop, barnyard grass control improved to 78%. Also, excellent control was achieved when imazethaptyr combined with clomazone, quinclorac, thiobencarb, or pendimethalin. In addition, Matzenbacher et al. (2013) found that profoxydim and cyhalofop effectively controlled penoxsulam- or bispyribac-resistant barnyard grass biotypes.

CONCLUSIONS

The results of this study suggest that late watergrass resistant to penoxsulam and bispyribac biotypes have been built. Also, imazamox did not provide satisfactory control of most of these biotypes. Short-time effective control of these biotypes and high rice yield can be achieved by double applications of herbicides with different modes of action (the first application with penoxsulam or bispyribac until the third leaf of rice [18 DAS] and the second with profoxydim at 35 DAS). Control of late emerged rice sedge can be simultaneously achieved by the addition of halosulfuron in the second application. However, the level of late watergrass resistance indicates a very high risk of relying upon continuous rice cropping in this area with an inevitable shift in weed flora to more resistant biotypes. Consequently, the herbicide rotations and/or the herbicide mixtures should be combined with crop rotation in order to weed resistance be restricted.

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