



Growth rate and competitive ability of susceptible and multiple-resistant late watergrass (*Echinochloa phyllopogon*) biotypes to rice

Aristeidis Papapanagiotou · Ioannis Vasilakoglou · Kico Dhima · Ilias Eleftherohorinos

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Abstract Late watergrass [*Echinochloa phyllopogon* (Stapf) Koss.] has evolved multiple-resistance (MR) to herbicides in many areas worldwide and scientists wonder if this resistance has imposed fitness cost or advantage on the resistant (MR) biotypes. As multiple-resistance of this weed has also been recorded in Greece, one susceptible (S), one with multiple-resistance to ALS (penoxsulam, bispyribac, imazamox and nicosulfuron + rimsulfuron) and ACCase-cyhalofop (MRAC) and two with multiple-resistance to ALS and synthetic auxin-quinclorac (MRAQ1, MRAQ2) late watergrass biotypes collected from rice fields (northern Greece) were studied for their growth rate and competitive ability (regarding stem number and fresh weight) in the absence and presence of rice. Also, the competition indices relative yield (RY), competitive ratio (CR) and aggressiveness (AI) were evaluated. The S and the

three MR biotypes grown in absence of competition (one plant/pot) showed different shoot number but similar fresh weight growth rates. In contrast, grown under intra-specific competition (one, two, four and six plants/pot), the S late watergrass biotype produced similar fresh weight with that of MRAQ1 but lower than that of the MRAC and MRAQ2 biotypes. However, grown under inter-specific competition (one, two, four and six plants of late watergrass/pot in competition with six rice plants/pot), the MRAQ2 late watergrass biotype produced more shoot number and fresh weight than the other biotypes at all weed densities, but the rice shoot number and fresh weight reduction were not followed the opposite trend of the late watergrass biotypes. Also, the competition indices of the MR biotypes (based on both intra- and inter-specific completion data) were found similar in most cases to that of the S biotype. These findings strongly suggest that there is no clear association between the growth rate and competitive ability of the late watergrass biotypes and their herbicide resistance.

A. Papapanagiotou
Department of Agriculture, University of West Macedonia,
53100 Florina, Greece

I. Vasilakoglou (✉)
Department of Agriculture-Agrotechnology, University
of Thessaly, 41500 Larissa, Greece
e-mail: vasilakoglou@uth.gr

K. Dhima
Department of Agriculture, International Hellenic
University, 57400 Echedoros, Greece

I. Eleftherohorinos
Department of Agriculture, Aristotle University
of Thessaloniki, 54006 Thessaloniki, Greece

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Introduction

Rice (*Oryza sativa* L.) is among the top three most important grain crops globally as it is a staple food for approximately 50% of the world population (Chauhan

et al., 2017). *Echinochloa* species are troublesome and difficult to manage weeds due to their prolific seed production, long seed dormancy, high adaptation to aquatic environment, high phenotypic and genetic variability, high ploidy, great hybridization (although self-pollinated) and production of considerable diverse offspring (Danquah et al., 2002; Kaya et al., 2014; Mascanzoni et al., 2015; Tabacchi et al., 2006). Also, the C₄ pathway of carbon fixation makes *Echinochloa* species more adaptable to stress and allows outcompeting C₃ rice plants (Talbert & Burgos, 2007; Vidotto et al., 2007). *Echinochloa* genus includes approximately 50 species with the allohexaploid barnyard grass [*Echinochloa crus-galli* (L.) Beauv.] and the allotetraploid late watergrass [*Echinochloa phyllopogon* (Stapf.) Koss (*Echinochloa oryzicola* Vasing.)] being the most common across rice fields in various production systems (Iwakami et al., 2015).

Among the *Echinochloa* species, late watergrass is one of the most troublesome weeds in paddy rice fields and poses a significant threat to rice production (Fisher et al., 2000; Rouse et al., 2018). It is strongly adapted to the aquatic environment of water-seeded rice and dominates in continuous cropping rice production systems. This species synchronizes growth requirements with rice and results in significant growth and yield reduction due to higher root development (Boddy et al., 2012; Brim-DeForest et al., 2017; Gibson et al., 2002, 2004). Regarding the competitive ability of *Echinochloa* species against rice is strongly affected by nitrogen and phosphorus fertilization. In particular, Chauhan and Abugho (2013) reported that competitiveness of these weed species increases with increasing nitrogen fertilization, but this is not the case with phosphorus that is partially unavailable for uptake by plants due to its complexes with soil (Weeraratne et al., 2015). However, competitive ability of *Echinochloa* species against rice could be reduced by management practices that delay weed emergence (Awan & Chauhan, 2016; Awan et al., 2021).

Echinochloa species are among the most resistance prone weed species, which is confirmed by the fact that barnyard grass, late watergrass and junglerice [*E. colona* (L.) Link] have evolved resistance to seven herbicide modes of action (Heap, 2023). Regarding late watergrass, biotypes have evolved resistance to acetolactate synthase (ALS) and acetyl CoA carboxylase (ACCase)-inhibiting herbicides, as well as to herbicides that inhibit the very long chain fatty acids synthesis (Boddy et al.,

2012; Brim-DeForest et al., 2017, 2022; Kaloumenos et al., 2013; Yasuor et al., 2009; Yun et al., 2005). Some of the reported late watergrass biotypes were resistant due to altered ALS or ACCase target-site (Heap, 2023; Kaloumenos et al., 2013), while other biotypes had an enhanced ability to detoxify ALS- and ACCase-inhibiting herbicides (Bakkali et al., 2007; Yasuor et al., 2009; Yun et al., 2005). The evolution of these late watergrass resistant biotypes in most cases was mainly attributed to the continuous use of the limited available ALS- and ACCase-inhibiting herbicides in several crops (Nakka et al., 2019).

Herbicide resistance can be studied as an evolved defense mechanism that can alter the ecological fitness, modify evolutionary dynamics and affect fate of resistance alleles in the environment (Lehnhoff et al., 2013). Regarding fitness, it is a measure of survival and ability of a given genotype to produce viable offspring in competition with the wild type biotype or crops (Ashigh & Tardif, 2011). Fitness of herbicide resistant weeds is manifested as changes in various performance traits such as seed germination rate, early plant vigor, above ground and root biomass production, reproductive productivity, and competitive ability (Cousens & Fournier-Level, 2018). More specifically, Boddy et al. (2012) found that the MR late watergrass plants were shorter and had less leaf area and shoot biomass than S plants, while Park et al. (2004) reported that the ALS-resistant downy brome (*Bromus tectorum* L.) germinated earlier than the S biotype at 5 °C but this did not have any effect on other growth characteristics and competitive ability. However, Du et al. (2019) found that the ACCase-resistant American sloughgrass (*Beckmannia syzigachne* Steud.) provide lower aboveground biomass and slightly lower seed production than the S biotype under competition with wheat. These contradictory findings indicate that there is no clear association between the growth response (fitness cost or advantage) and the herbicide resistance of weed biotypes.

In northern Greece, the great ability of late watergrass to establish and flourish in permanent flooded conditions resulted in replacement of barnyard grass in rice paddy fields (Vasilakoglou et al., 2018). As the available data regarding the possible fitness cost or advantage of resistant late watergrass biotypes are limited, the objective of the present work was to investigate the effect of evolved multiple-herbicide resistance in late watergrass biotypes

selected in paddy rice fields of northern Greece on their growth rate and competitive ability against rice.

Materials and methods

Plant material

Late watergrass seeds were collected at the end of the 2019 growing season from survived plants after the repeated application of herbicides for many consecutive years in three water-seeded rice fields located at Halastra (Thessaloniki region, northern Greece). In particular, seeds were collected from one rice field treated with ALS and ACCase inhibitor cyhalofop-butyl (MRAC) and from two rice fields treated with ALS inhibitors and the synthetic auxin-quinclorac (MRAQ1, MRAQ2). Mature seeds were collected by hand from 80 to 90 individual late watergrass plants of each field, pooled together, and characterized as a putative multiple-resistant (MR) population. Also, late watergrass seeds were collected from plants grown at the margins of a rice field located in the same area, which did not receive herbicide applications the previous four years and were effectively controlled by the application of the co-mixture of the ALS-penoxsulam + ACCase-cyhalofop recommended label field rate [were considered as possible susceptible (S) biotype]. During seed collection, care was taken to get a representative sample from each rice field. The collected seeds were transferred to the laboratory, where they were air-dried, air-cleaned to remove non-viable seeds and other plant residues and stored in a refrigerator at 3 to 6 °C. The seed collection of the S and R biotypes from the same area was chosen in order to enhance the similarity of genetic background between biotypes and minimize the effect of other than resistance fitness-determining locus/loci (Keshthkar et al., 2018).

Whole-plant dose–response assays to herbicides

A pot experiment was conducted in Thessaloniki, northern Greece, during 2020. Plants were grown in 20 (diameter) × 17 (depth) cm plastic pots filled with a mixture of silty clay soil:sand (3:1 v/v). Each

pot was seeded with approximately 40 uniform late watergrass seeds of each of the S or three suspected MR biotypes and carefully covered with 1 cm depth of the soil mixture in late March. The late watergrass seeds of each biotype were chemically scarified to break seed dormancy before seeding. In particular, they were treated with concentrated (96%) H₂SO₄ for approximately 4–5 min and afterwards were carefully rinsed with water and subsequently immersed in 1.5% solution of KNO₃ for 2 h. Pots were placed under greenhouse conditions (15/30 °C, night/day), where they were N-fertilized and watered as needed. Five days after emergence, late watergrass was thinned to 6 uniform plants per pot. At the same time, the emerged broad-leaved weeds were hand-removed, while no other grass weeds emerged.

The emerged plants, at the two to three-leaf growth stage (BBCH code 12–13) (Meier, 2001) were treated with the recommended (1×), 2× and 4× label field rates of the herbicides shown in Table 1. 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. The experiment was repeated in time using a randomized complete block design (RCBD) with three replications for each of the biotype by herbicide by herbicide rate treatment. Late watergrass control was assessed by determining the aboveground fresh weight of surviving plants in each pot at four weeks after herbicide application. Fresh weight data were expressed as a percentage of the untreated control and subjected to analysis of variance (ANOVA). As the ANOVA indicated that one of the putative MR biotypes was multiple resistant to ALS and ACCase inhibitor-cyhalofop (MRAC) and the other two multiple resistant to ALS and synthetic auxin-quinclorac (MRAQ1, MRAQ2), initially collected seeds of these biotypes were further used to produce seeds from survived plants after the application of the penoxsulam + cyhalofop recommended rate on the MRAC biotype and the penoxsulam + quinclorac recommended rate on MRAQ1 and MRAQ2 biotypes. This was made to eliminate possible individual susceptible plants from these biotypes. Since all plants of the S biotype

Table 1 Source of materials for the products used in the whole-plant dose–response experiments against the S and the putative MR late watergrass biotypes

Herbicide ^a	Trade name	Form ^b	Rate	Manufacturer
Penoxsulam	Viper	OD	g ai ha ⁻¹	K&N Efthymiadis SA, Athens, Greece
			40^c	
			80	
Imazamox	Pulsar	SL	160	BASF Hellas, Athens, Greece
			80	
			160	
Bispyribac-Na	Adora	SC	320	Bayer CropScience Hellas, Athens, Greece
			30	
			60	
Nicosulfuron + rimsulfuron	Steadfast Duo	WG	120	Corteva Agriscience Hellas, Athens, Greece
			20.1 + 10.05	
			40.2 + 21.1	
Quinclorac	Facet	SC	80.4 + 40.2	BASF Hellas, Athens, Greece
			300	
			600	
Tembotrione	Laudis	OD	1200	Bayer CropScience Hellas, Athens, Greece
			79.2	
			158.4	
Cyhalofop-butyl	Clincher NEO	EC	316.8	Corteva Agriscience Hellas, Athens, Greece
			300	
			600	
Quizalofop-P-ethyl	Jaguar	EC	1200	Alpha Agricultural Supplies SA, Athens, Greece
			70	
			140	
Profoxydim	Aura	EC	280	BASF Hellas, Athens, Greece
			200	
			400	
Cycloxydim	Focus	EC	800	Corteva Agriscience Hellas, Athens, Greece
			200	
			400	
Flopyrauxifen-benzyl	Loyant	EC	800	
			600	
			1200	
			2400	

^aNicosulfuron + rimsulfuron were applied with the surfactant iodecyl alcohol ethoxylate 90% w/v (Trend® 90 SL, Corteva Agriscience, Athens, Greece) at 0.1% v/v; Imazamox and profoxydim were applied with the 37.5% w/w fatty acid esters + 22.5% w/w alkoxylated alcohols-phosphate esters (Dash HC, BASF Hellas) at 0.4% vol/vol; Bispyribac-Na was applied with the surfactant Bio-power® SL (Bayer CropScience, Athens, Greece) at 0.33% v/v

^bAbbreviations: OD, oil dispersion; SL, soluble liquid; SC, suspension concentrate; WG, water dispersible granule; EC, emulsifiable concentrate

^cThe rates in bold are the label recommended of the herbicides

treated with penoxsulam + cyhalofop or penoxsulam + quinclorac recommended rate were effectively controlled, the seeds used of this biotype in

the following experiments were originated from the initially collected ones.

Growth rate experiment

The experiment was conducted during mid spring–early summer of 2021 growing season, using 20×17 cm plastic pots filled with soil mixture used in the previously described experiments. Uniform scarified seeds of the four late watergrass biotypes, produced according to the previously described single population procedure (Keshtkar et al., 2018), were placed on 9-cm-diameter Petri dishes lined with filter paper, where they were moistened with 10 ml of deionized water and transferred in a greenhouse (15/30 °C, night/day). When the late watergrass seedlings were at approximately one-leaf stage (BBCH code 11), they were transplanted in small jiffy pots and allowed to grow up to two-leaf stage. Afterwards, one two-leaf stage (BBCH code 12) late watergrass seedling of each MR or S biotype was transplanted into the center of each pot and allowed to grow without competition. All pots were placed in the same greenhouse used above for the dose–response experiment and were properly irrigated and fertilized to maintain vigorous growth throughout the duration of the experiment. Pots were rearranged every week to ensure uniform growth conditions for all plants. The late watergrass plants were grown for 63 days after transplanting (BBCH code 79) and plant growth of MR and S late watergrass plants was evaluated in nine subsequent harvest samplings, practiced at 7-day intervals. At each sampling, the late watergrass plants were cut and their total shoot number and fresh weight in each pot (i.e. per plant) were determined. Late watergrass fecundity was not evaluated because of the uncompleted fertilization and seed filling under greenhouse conditions. A randomized complete block design with three replications for each biotype by sampling treatment was used for each of the two repeated experiments.

Intra-specific competition of late watergrass biotypes

A pot experiment was conducted in the same greenhouse and growing season to determine growth parameters of the four previously evaluated late watergrass biotypes in pure stand under four plant densities. One (absence of competition), two, four and six uniform seedlings (intra-specific competition) of each late watergrass biotype at the two-leaf growth stage (BBCH code 12) (originating from the

seedlings prepared for the growth rate experiment) were transplanted in 20×17 cm plastic pots, using a template to achieve a uniform distance (6 cm) among neighbors. The pots were filled with the soil used in the growth rate experiment. All pots were randomly placed in the greenhouse and were rearranged every week to reduce variation due to differences in light regime. Plants were harvested (cut at the soil surface) at 55 days after transplanting (DAT) (BBCH code 71) and above ground vegetative biomass (shoot number and fresh weight) was determined. Each of the two repeated experiments was conducted using a completely randomized design with three replications for each biotype by density treatment.

Late watergrass competition with rice (inter-specific competition)

Simultaneously with the intra-specific competition, a pot experiment was conducted to evaluate the competitive ability of the three MR and one S late watergrass biotypes against the Clearfield rice var. CL33. The experiment was carried in the same greenhouse and time with the intra-specific competition experiment, using 20×17 cm plastic pots filled with the soil mixture used above. The late watergrass seedlings used in this experiment originated from those prepared previously for the growth rate and intra-specific competition experiment. In addition the rice seedlings were grown in the greenhouse at the same time using plastic boxes filled with a standard potting mixture of 50% peatmoss and 50% sand. Late watergrass and rice seedlings approximately at one-leaf stage (BBCH code 11) were transplanted in small jiffy pots and allowed to grow. Uniform two-leaf stage (BBCH code 12) late watergrass and rice seedlings were transplanted in pots to assess the competitive ability of the MR and S late watergrass biotypes against rice. In particular, six rice seedlings were transplanted in two rows spaced 10 cm per each pot, having 3 seedlings spaced 6 cm per row. Then, 0 (weed-free crop control), 1, 2, 4 or 6 late watergrass seedlings, at the two-leaf stage, were transplanted in five rice/weed densities (6:0, 6:1, 6:2, 6:4 or 6:6) using the template shown in Fig. 1. The six rice plants per pot correspond field plant density of 200 plants/m². All pots were placed in the greenhouse and were properly irrigated (watered daily to maintain substrate at

field capacity) and fertilized to maintain vigorous growth throughout the duration of the experiment. Flooded conditions were not used in order to avoid *Echinochloa* growth differences due to rice interference (Chauhan & Abugho, 2013). All pots were rearranged each week in order to ensure uniform growth conditions for all plants. Emerging grass and broad-leaved weeds were carefully removed manually during the experiment to assure absence of competition due to other weeds. Rice and weed shoot number and fresh weight were determined at 55 DAT (BBCH code 71). A randomized complete block design was used in each of the two repeated experiments with three replications for each biotype by weed density treatment. In addition to rice and weed shoot number and fresh weight, the competition indices of late watergrass biotypes grown with rice were calculated by modifying (weed biotypes instead of R-biotype and rice instead of S-biotype) the equations presented by Bagavathiannan et al. (2011). In particular, for each late watergrass biotype, the relative yield (RY) was calculated as $RY_w = P(W_{mix}/W_{mono})$. The RY indicates the possible advantage of a biotype grown in competition with rice and the P is the proportion of a weed biotype in mixture with rice. The W_{mix} and W_{mono} are the shoot number or the fresh weight of each weed biotype grown in mixture with rice and in the absence of rice competition (intra-specific competition), respectively. The competitive ratio (CR) was calculated as $CR_w = [(1-P)/P][RY_w/RY_c]$, while the aggressiveness (AI) as $AI_w = (RY_w/2P) - \{RY_c/[2(1-P)]\}$. The P in AI is the proportion of the weed biotype in mixture with rice, whereas the RY_w and RY_c are the relative yields of weed and rice, respectively. In general, the CR and AI indices indicate the effect of plant density on growth and competitive ability of each biotype.

Statistical analyses

The data of the whole-plant dose–response assays were analyzed over the two experiments because the homogeneity of variances checked by Bartlett's test (Snedecor & Cochran, 1989) indicated no significant departure of normality. The combined ANOVA over the two experiments was performed using a $4 \times 11 \times 3$ split-plot approach, where the four weed biotypes were the main plot and the 11 herbicides by three herbicide rates were the subplots.

The combined over two repeated experiments MANOVA (4 biotypes $\times$ 7 growth stages) was performed for the data obtained from the growth rate study. Furthermore, the sigmoidal equation $[y = a / (1 + \exp^{-(x-x_0)/b})]$ was tested for its suitability to describe the relationship between shoot number or fresh weight response and sampling time (Schaedler et al., 2013). In this equation, shoot number or fresh weight is the dependent variable (y), time (days after transplanting) is the independent variable (x), a is the difference between the maximum and minimum asymptote, b is the slope (growth rate) of the curve and x_0 is the days after transplanting that correspond to 50% of the maximum shoot number or fresh weight (dGR_{50}). Also, the confidence interval ($\pm 95\%$) were calculated.

The over two repeated experiments ANOVA was performed for the data obtained from the intra-specific competition of late watergrass using a 4 (biotypes) $\times$ 4 (densities) factorial experiment. As the ANOVAs indicated no significant treatment by experimental repetition time interaction, the presented means are averaged across the two repeated experiments. In addition, the quadratic regression equation ($y = a + bx + cx^2$) was tested for its suitability to describe the relationship between the shoot number and fresh weight of late watergrass plants (y)

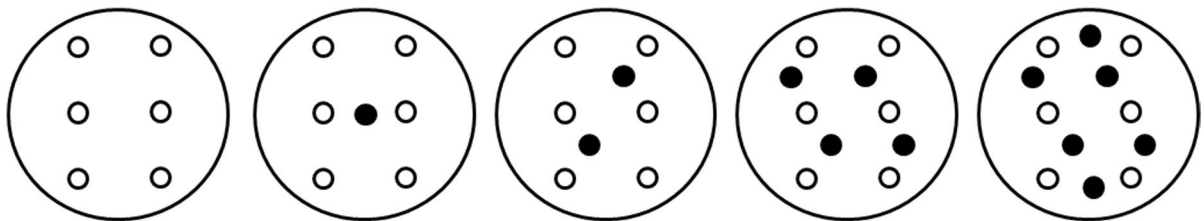


Fig. 1 Schematic presentation of the weed/crop plant density pattern (0:6, 1:6, 2:6, 4:6, 6:6) to assess plant responses of the MR and S late watergrass biotypes grown in competition with rice (rice = open circles vs. R or S late watergrass biotypes = black circles)

and weed density (x), according the results of Awan and Chauhan (2016) who reported that the linear, the quadratic and the exponential equations were best fitted to barnyardgrass growth parameters. Also, the weed density data expressed as one late watergrass plant per pot were also used to test the suitability of linear regression equation to describe the relationship between their shoot number or fresh weight and weed density.

The over two repeated experiments ANOVA was performed for the rice data obtained from the inter-specific (weed-rice) competition data using a 4 (biotypes) $\times$ 5 (densities) factorial experiment. In addition, the over two repeated experiments ANOVA was performed for the weed data obtained using a 4 (biotypes) $\times$ 4 (densities) factorial experiment. As the ANOVAs indicated no significant treatment by experimental repetition time interaction, the presented means are averaged across the two repeated experiments. The inter-specific (weed-rice) competition data were also used for quadratic rational function regression equation [$y = 1 / (a + bx + cx^2)$] to describe the relationship between the rice or late watergrass shoot number and fresh weight (y) and weed density (x). Moreover, for the weed density data expressed as one late watergrass plant per pot, the linear regression equation was tested for its suitability to describe the relationship between the shoot number or fresh weight of each biotype and weed density. This was made to compare the shoot number and fresh weight per plant of the three MR and one S late watergrass biotypes grown in competition with rice (inter-specific competition) with those grown under intra-specific competition. For each growth variable (shoot number or fresh weight), the differences in competition indices of all possible combinations of pairs between the S and MR biotypes were examined using the paired student's t -test ($\alpha = 0.05$). Also, the paired student's t -test ($\alpha = 0.05$) was used to compared the equation parameters for all equation pairs, while the homogeneity of variances was examined with the Bartlett's test (McDonald, 2014).

The SigmaPlot software (version 12.5) was used to estimate the parameters and to test the significance of differences between parameters of the sigmoidal curves (SigmaPlot, 2013). The SPSS software (version 17.0) was used to conduct t -tests (SPSS, 1998), while the MSTAT (MSTAT-C, 1988) program was

also used to conduct ANOVAs. The Fisher's Protected Least Significant Difference test procedures were used to detect and separate mean treatment differences at $p = 0.05$.

Results

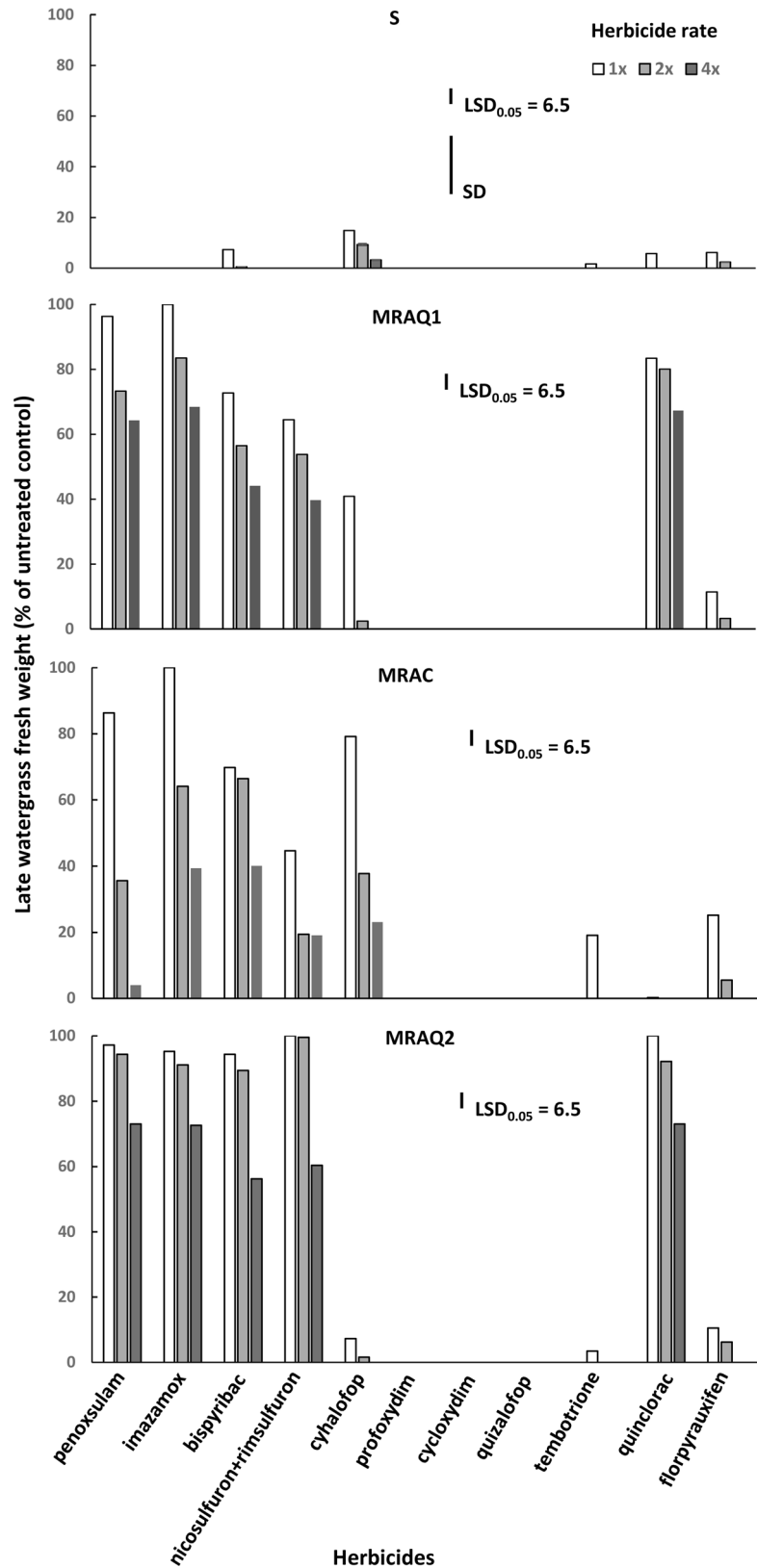
Whole-plant dose–response assays to herbicides

The ANOVA performed indicated that the late watergrass fresh weight was not affected by repetition time or by repetition time $\times$ treatments interaction, but was significantly ($P < 0.001$) affected by biotype $\times$ herbicide, biotype $\times$ application rate, herbicide $\times$ application rate and biotype $\times$ herbicide $\times$ application rate interactions. Therefore, the interaction means of biotype $\times$ herbicide $\times$ application rate are presented in Fig. 2. In particular, the fresh weight of the S biotype was almost completely reduced by all herbicides and rates applied. In addition, the fresh weight of the putative R biotypes was completely reduced by the herbicides profoxydim and quizalofop (ACCase inhibitors), tembotrione (HPPD inhibitor) and florypyrauxifen (synthetic auxin). On the contrary, the fresh weight of the MRAC was not significantly reduced by the ALS inhibitors penoxsulam, bispyribac, imazamox, nicosulfuron + rimsulfuron and the ACCase-cyhalofop, whereas the fresh weight of the MRAQ1 and MRAQ2 was not significantly reduced by the ALS inhibitors and the synthetic auxin-quinclorac. Therefore, based on the overall growth response of the three MR biotypes to herbicide rates, their increasing resistance order is MRAC < MRAQ1 < MRAQ2.

Growth rate experiment

As the plant height of the late watergrass biotypes at each growth stage followed similar trend to that of fresh weight data, these data are not presented. The MANOVA performed for shoot number and fresh weight data of the four weed biotypes grown without competition indicated that they were significantly ($P < 0.001$) affected by biotype, time after transplanting and biotype $\times$ time after transplanting interaction. In addition, the paired student's t -test conducted to compare the equation parameters indicated significant differences between the fitted curves. The late

Fig. 2 Fresh weight of four late watergrass biotypes (MRAQ1, S, MRAC, MRAQ2) as affected by three rates of 12 herbicides. SD, standard deviation



watergrass shoot number and fresh weight increased with increasing sampling time till 49 DAT, but subsequently both growth parameters were slightly increased (Fig. 3). In particular, at 21 DAT, significant differences among the biotypes were not observed, but at 28, 35 and 42 DAT the shoot number and the fresh weight of the S biotype were lower than those of the MR ones. At 49, 56 and 63 DAT, there were no shoot number differences among the biotypes, but the fresh weight of the MRAQ1 biotype was greater than that of the other biotypes. The relationship between shoot number or fresh weight of all biotypes and sampling time was best described by the sigmoidal

equations [high adjusted- R^2 (0.91–0.98)] (Table 2). In addition, the paired student's t-test conducted to compare the equation parameters indicated significant differences between the fitted curves. The increasing order of the estimated slopes (b) for shoot number of the biotypes was MRAQ2 (7.845)=MRAQ1 (8.448)=MRAC (9.445)<S (12.171), while their respective estimated slopes for fresh weight were [MRAC (5.222)=MRAQ1 (5.384)=S (5.389)=MRAQ2 (5.996)]. Based on the estimated days after transplanting to achieve 50% of maximum shoot number (x_0 or dGR_{50}), the increasing order of the biotypes was MRAQ2 (25.064)=MRAQ1

Fig. 3 Shoot number (a) and fresh weight (b) of three MR and one S late watergrass biotypes grown for 63 days after transplanting in absence of competition (pot experiment in a greenhouse). The bars indicate the standard errors

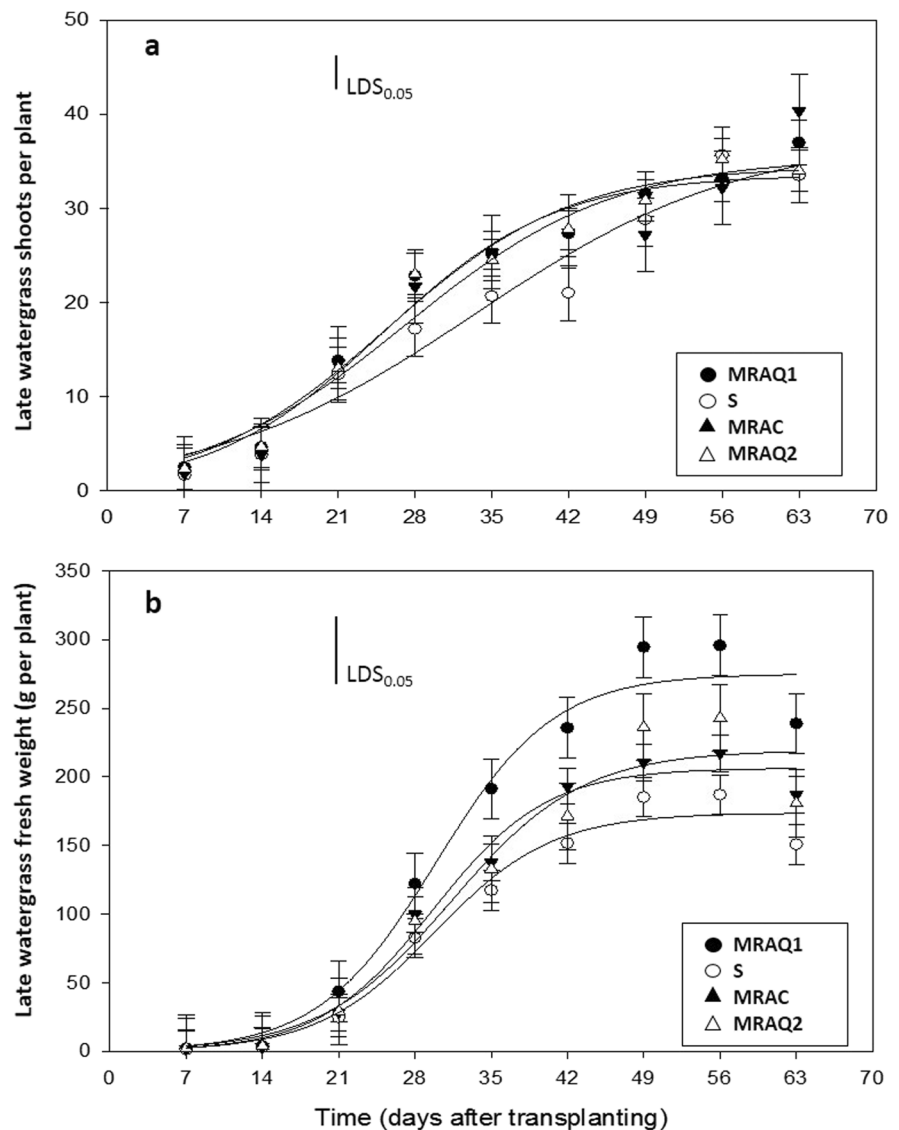


Table 2 Regression parameters of sigmoidal equations [$y = a / (1 + \exp^{-(x-x_0)/b})$] represented the relationship between the shoot number or fresh weight (y) of three R and one S late

watergrass biotypes against time (x, days after transplanted). The data used for regressions are presented in Fig. 3

S	Shoot number					Fresh weight (g)				
	SE	CI (95%)	P	R_a^2		SE	CI (95%)	P	R_a^2	
a	37.593	± 4.958	34.389, 40.797	***	0.94	173.630	± 9.209	167.679, 179.581	***	0.96
b	12.171	± 3.161	10.128, 14.214	**		5.389	± 1.283	4.560, 6.218	**	
dGR ₅₀	33.494	± 4.648	30.490, 36.498	***		29.808	± 1.515	28.829, 30.787	***	
MRAQ1										
a	34.452	± 1.884	33.234, 35.670	***	0.96	275.061	± 14.125	256.933, 284.189	***	0.97
b	8.448	± 1.639	7.389, 9.507	***		5.384	± 1.238	4.584, 6.184	**	
dGR ₅₀	25.447	± 1.892	24.224, 26.670	***		29.930	± 1.463	28.985, 30.875	***	
MRAC										
a	35.354	± 3.569	33.048, 37.660	***	0.91	206.374	± 8.272	201.028, 211.720	***	0.98
b	9.445	± 2.931	7.551, 11.339	*		5.222	± 0.966	4.598, 5.846	**	
dGR ₅₀	27.201	± 3.495	24.942, 29.460	***		29.659	± 1.137	28.924, 30.394	***	
MRAQ2										
a	33.553	± 1.579	32.533, 34.573	***	0.97	219.349	± 17.328	208.151, 230.547	***	0.93
b	7.845	± 1.399	6.941, 8.749	**		5.996	± 1.883	4.779, 7.213	*	
dGR ₅₀	25.064	± 1.612	24.022, 26.106	***		31.366	± 2.257	29.907, 32.825	***	

S, susceptible; MRAC, multiple-resistant to ALS and cyhalofop; MRAQ, multiple resistant to ALS and quinclorac; SE, standard error; CI, confidence interval

a: difference between the maximum and minimum asymptote

b: slope of the curve

d: days after transplanting to achieve 50% of maximum shoot number or fresh weight

*, ** or ***: significant at the 5% level, 1% level or 0.1% level, respectively

(25.447) < MRAC (27.201) < S (33.494), while the respective 50% of maximum fresh weight order was MRAC (29.659) = S (29.808) = MRAQ1 (29.930) = MRAQ2 (31.366). Based on the final shoot number, the increasing order was MRAQ2 (33.553) = MRAQ1 (34.452) = MRAC (35.354) < S (37.593), while the respective order for the final accumulated fresh weight was S (173.630) < MRAC (206.374) < MRAQ2 (219.349) < MRAQ1 (275.061).

Intra-specific competition of late watergrass biotypes

The combined over two repeated experiments ANOVA indicated significant ($P < 0.001$) late watergrass biotype and late watergrass biotype x density interaction. In general, the MRAC and MRAQ2 biotypes produced more shoots at all densities than the other two biotypes (Fig. 4a, b), while all biotypes produced the greater fresh weight only at one and two plants per pot. In addition, the increasing shoot number order of the four biotypes

(averaged across the two repeated experiments and weed density) was S (27.1) = MRAQ1 (27.8) < MRAC (31.7) = MRAQ2 (33.6), while the respective order for fresh weight was S (195.8) < MRAQ1 (202.8) = MRAC (207.3) < MRAQ2 (230.6) (Table 3). Moreover, the quadratic regression used to describe the relationship between the above non-proportional increase of shoot number or fresh weight of late watergrass and weed density indicated best fit as confirmed by the high R^2 , while the paired student's t-tests performed showed significant differences among curve parameters. Based on the fitted equations, the order of the estimated slopes for shoot number of the four biotypes was MRAQ1 (1.046) < S (2.225) = MRAQ2 (2.898) < MRAC (17.934) (Fig. 4a), while the respective order for fresh weight slopes was S (30.921) < MRAQ1 (36.611) < MRAQ2 (40.078) < MRAC (66.554) (Fig. 4b).

Based on the calculated growth parameters of one weed plant per pot, the MRAQ2 biotype produced greater shoot number and fresh weight at one plant

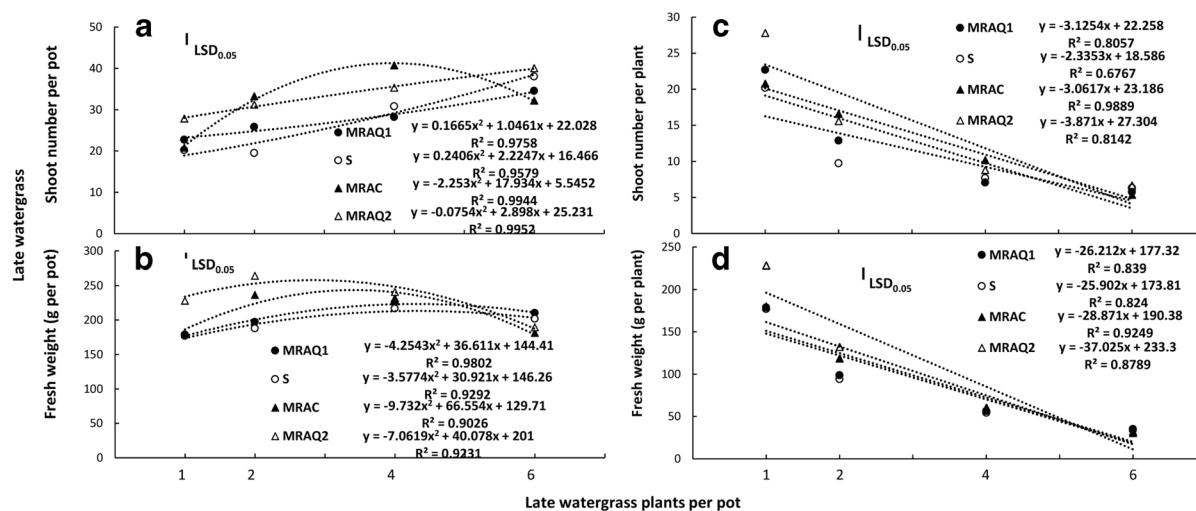


Fig. 4 Quadratic regression equations fitted on shoot number (a) and fresh weight (b) of three MR and one S late watergrass biotypes grown in pure stand under four densities (intra-spe-

cific competition), along with the linear regression equations fitted on their shoot number (c) or fresh weight (d) per plant

per pot, but this was not the case at the other densities (Fig. 4c, d). The relationship between the two growth parameters of one late watergrass plant and weed density was best described by the linear regression (high R^2). The order of the estimated slopes for the shoot number of the four biotypes was MRAQ2 (-3.871) < MRAQ1 (-3.125) = MRAC (-3.062) < S (-2.335) (Fig. 4c), while the respective fresh weight order was MRAQ2 (-37.025) < MRAC (-28.871) < MRAQ1 (-26.212) = S (-25.902) (Fig. 4d).

Late watergrass competition with rice

As the ANOVA of late watergrass data indicated significant ($P < 0.001$) biotype and biotype $\times$ weed density interaction only, the late watergrass biotype means

(averaged across repetition time and density) and the biotype $\times$ weed density interaction means (averaged across repetition time) are presented. In particular, the increasing shoot number (averaged across repetition time and weed density) order of biotypes grown in competition with rice was MRAQ1 (18.5) < S (25.3) < MRAC (28.7) < MRAQ2 (34.9), while the respective order of fresh weight was S (139.4) < MRAC (148.5) = MRAQ1 (151.5) < MRAQ2 (181.0) (Table 4).

The relationship between the growth parameters of late watergrass and weed density per plot was best described by the nonlinear regression (high R^2). The estimated shoot number slope order of late watergrass biotypes was MRAQ1 (2.930) < S (4.602) < MRAQ2 (10.448) < MRAC (13.855) (Fig. 5a), while the respective order for the fresh weight slopes was MRAQ1

Table 3 Shoot number and fresh weight per pot of three MR and one S late watergrass biotypes (averaged across repeated experiments and weed density) grown in pure stand

	Late watergrass (LW) biotypes			
	S	MRAQ1	MRAC	MRAQ2
LW pure stand (per pot)				
Shoot number	27.1	c*	27.8	bc
Fresh weight	195.8	c	202.8	b

*In each row, means followed by the same letter did not differ according Fisher's Protected Least Significant Difference test at $p = 0.05$

Table 4 Shoot number and fresh weight per pot of three MR and one S late watergrass biotypes (averaged across repeated experiments and weed density) grown in competition with rice.

Also, shoot number and fresh weight of rice (averaged across weed density and repeated experiments) grown in competition with each of the late watergrass biotypes

	Late watergrass (LW) biotypes							
	S		MRAQ1		MRAC		MRAQ2	
LW in rice (per pot)								
Shoot number	25.3	c*	18.5	d	28.7	b	34.9	a
Fresh weight	139.4	c	151.5	b	148.5	b	181.0	a
Rice (per pot)								
Shoot number	13.9	ab	12.2	b	14.2	a	12.6	ab
Fresh weight	29.5	a	25.2	b	29.5	a	28.3	a

*In each row, means followed by the same letter did not differ according Fisher's Protected Least Significant Difference test at $p=0.05$

(57.201) < MRAQ2 (65.742) < S (74.605) < MRAC (80.318) (Fig. 5b). In contrast to the above nonlinear regression findings, the relationship between the shoot number and fresh weight of the calculated one late watergrass plant per pot of all biotypes grown under different weed densities in competition with rice (intra- and inter-specific competition) was best described by the linear regression (high R^2). The increasing order of the estimated slopes for the shoot number of the four biotypes was MRAQ2 (-2.933) = MRAC (-2.379) = S (-2.269) < MRAQ1 (-1.393) (Fig. 5c), while the respective one for the fresh weight was MRAQ2 (-22.300) < MRAQ1 (-16.870) = MRAC (-15.484) = S (-13.338) (Fig. 5d).

The ANOVA of rice data grown in competition with different densities of the four weed biotypes indicated that rice growth parameters were significantly affected ($P < 0.001$) by late watergrass biotype, weed density, and biotype $\times$ weed density interaction. In general, the rice shoot number and fresh weight were significantly reduced due to late watergrass biotype competition, but their reduction was not proportional with the increasing weed density (Fig. 5e). In particular, the competition of 1, 2, 4 or 6 MR late watergrass plants per pot (averaged across the three MR biotypes) reduced the rice shoot number by 59.3%, 70.9%, 79.8% or 80.6%, whereas the respective reduction caused by the S plants was 49.2%, 63.8%, 79.9% or 80.6%. In addition, the rice fresh weight reduction due to the presence of 1, 2, 4 or 6 MR late watergrass plants per pot was 65.5%, 71.5%, 84.3% or 87.9% (averaged across the three MR biotypes), while the respective reduction

resulted from the S plants was 59.2%, 66.2%, 82.3% or 84.4%.

The growth response of rice due to competition of one plant per pot of the S, MRAC, MRAQ1 and MRAQ2 biotypes indicated that the rice shoot number was reduced by 15.7%, 15.8%, 10.2% and 11.7%, respectively, (Fig. 5e), while the respective reduction due to competition of two S and MRAC, MRAQ1 and MRAQ2 plants was 11.2%, 11.2%, 6.8% and 9.0%. However, at four and six late watergrass plants per pot, all biotypes resulted in similar shoot number reduction trend. In agreement with the shoot number findings, fresh weight of rice grown in competition with one plant of S, MRAC, MRAQ1 and MRAQ2 was reduced by 29.2%, 20.7%, 18.8% and 16.8%, while the respective reduction at two weed plants was 31.8%, 24.2%, 23.5% and 23.8. Again, at four and six plants per pot, all biotypes affected similarly rice fresh weight (Fig. 5f). The relationship between the rice shoot number or fresh weight and weed density was best described by the quadratic rational function regression (high R^2). Based on these findings, the shoot number slope order of rice reduction due to weed biotypes was S (0.0332) = MRAC (0.033) < MRAQ2 (0.054) < MRAQ1 (0.073) (Fig. 5e), while the respective fresh weight order was S (0.018) = MRAC (0.018) < MRAQ2 (0.024) < MRAQ1 (0.037) (Fig. 5f).

The calculated competition indices of the MR biotypes, based on both intra- and inter-specific completion data, were found similar in most cases to that of the S biotype (Table 5). In particular, based on shoot number of one plant per pot, the MRAQ1 biotype showed lower relative yield and

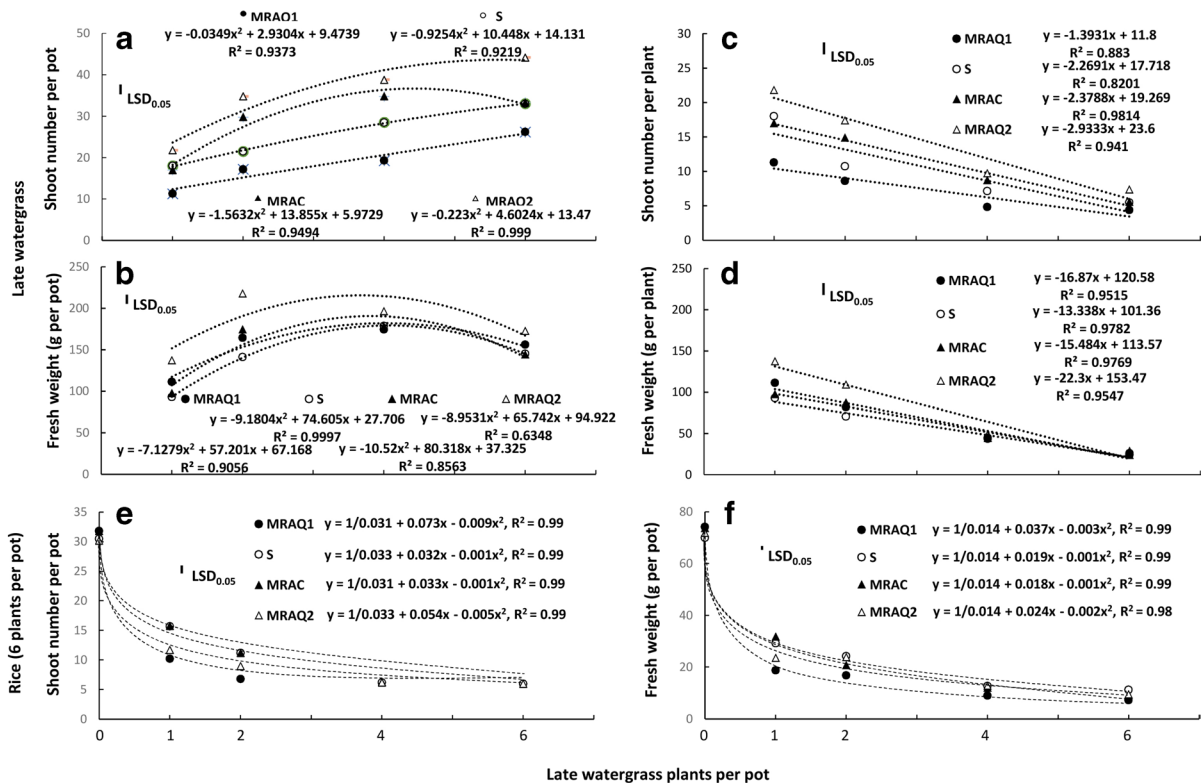


Fig. 5 Quadratic regression equations fitted on shoot number (a) and fresh weight (b) of three MR and one S late watergrass biotypes grown in competition with rice, along with the linear regression equations fitted on their shoot number (c) or fresh

weight (d) per plant. The quadratic rational function regression equations fitted on shoot number and fresh weight of rice grown in competition with the four weed biotypes are shown in Fig (e) and Fig (f), respectively

greater aggressiveness than the other biotypes. However, regarding fresh weight of one plant per pot, the MRAQ1 biotype showed greater competitiveness than the other biotypes, while the MRAQ2 biotype showed greater relative yield than the other biotypes.

Discussion

Whole-plant dose–response assays to herbicides

The reduced efficacy of the ALS-inhibiting herbicides penoxsulam, bispyribac, imazamox, nicosulfuron + rimsulfuron and the ACCase-cyhalofop against the MRAC along with the reduced efficacy of the previously mentioned ALS herbicides and the synthetic auxin-quinclorac against MRAQ1 and MRAQ2 supports the evidence of multiple resistance evolution in these three late

watergrass biotypes. Similar results for ALS-inhibiting herbicides resistance were reported by Kaloumenos et al. (2013) and Vasilakoglou et al. (2018), who found that late watergrass biotypes originating from the same area in Greece were cross-resistant to rice ALS-herbicides penoxsulam, bispyribac and imazamox, and to corn ALS-herbicides foramsulfuron, nicosulfuron and rimsulfuron. Although the mechanisms of the field-selected multiple-resistance was not determined in this study, the results by Fisher et al. (2000), Iwakami et al., (20142019), Yasuor et al. (2009) and Yun et al. (2005) showed that field-evolved herbicide cross-resistance and multiple-resistance of late watergrass to ALS and ACCase inhibitors is either conferred by enhanced metabolism due to the induction of cytochrome P450 monooxygenases or reduced target-enzyme sensitivity due to mutations in the *ALS* gene (Amaro-Blanco et al., 2021; Dimaano et al., 2022; Liu et al., 2019; Panozzo et al., 2021; Yasuor et al., 2010). Regarding target-site

Table 5 Relative yield (RY), competitiveness (CR) and aggressiveness (AI) for shoot number and fresh weight of three R and one S late watergrass biotypes grown with rice

RY: relative yield, CR: competitive ratio, AI: aggressiveness
* indicates differences between the susceptible (S) index mean and the resistant-biotypes index means, according the paired samples t-test ($\alpha=0.05$)

	Shoot number				Fresh weight			
	1:6	2:6	4:6	6:6	1:6	2:6	4:6	6:6
RY in rice								
S	0.12	0.28	0.37	0.43	0.07	0.19	0.33	0.36
MRAQ1	0.07*	0.17	0.27	0.38	0.09	0.21	0.31	0.37
MRAC	0.11	0.22	0.34	0.52	0.08	0.18	0.31	0.40
MRAQ2	0.11	0.28	0.44	0.55	0.08	0.21	0.33	0.46
CR in rice								
S	1.73	3.00	4.55	4.41	1.26	2.17	4.55	4.49
MRAQ1	1.55	3.12	3.51	4.02	2.46*	3.68	6.39	7.65
MRAC	1.64	2.55	4.32	5.48	1.25	2.62	4.72	6.38
MRAQ2	2.02*	3.74	5.35	5.56	1.84	2.49	4.58	6.88
AI in rice								
S	-0.18	-0.07	0.04	0.08	-0.15	-0.07	0.03	0.07
MRAQ1	-0.11*	-0.04	0.02	0.07	-0.09	-0.04	0.04	0.08
MRAC	-0.18	-0.07	0.03	0.11	-0.15	-0.06	0.03	0.08
MRAQ2	-0.14	-0.05	0.05	0.11	-0.12	-0.07	0.03	0.10

resistance, Amaro-Blanco et al. (2021) found that the Pro197Ser, Pro197Thr and Ser653Asn substitutions in the *ALS* gene and the Ile1781Leu substitution in the *ACCase* gene were responsible for the late watergrass multiple-resistant biotypes to ALS- and ACCase-inhibiting herbicides. Kaloumenos et al. (2013) and Panozzo et al. (2021) also reported that cross-resistance of late watergrass biotypes to ALS-herbicides were due to Trp574Leu substitution in the *ALS* gene, while Liu et al. (2019) found that the Pro197Ser mutation in a penoxsulam-resistant late watergrass biotype resulted in reduced binding affinity to herbicide but increased ALS-enzyme activity. However, Bakkali et al. (2007) reported that the higher rate of glutathione conjugation catalyzed by glutathione-S-transferase was the major mechanism in the fenoxaprop-resistant late watergrass biotype.

Growth rate experiment

The fact that shoot number slope of the S biotype was higher than the MRAC, MRAQ1, and RAQ2 biotypes, along with the similar respective fresh weight slopes of S and MR biotypes, strongly suggest lack of association between growth rate of MR biotypes and their multiple-resistance. Similar results were reported by Bagavathiannan et al. (2011), who found that the barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] biotypes with multiple

resistance to propanil and clomazone provided greater shoot number than the S biotype, while differences were not observed among R and S biotypes regarding the total dry weight. In addition, Boddy et al. (2012) reported that a multiple-resistant late watergrass biotype to the ALS-ACCcase-clomazone herbicides provided lower leaf area, shoot biomass and seeds than the S biotype, while Brim-DeForest et al. (2022) found that a multiple-resistant late watergrass biotype to the ALS-ACCcase-molinate herbicides showed a shorter time to achieve maximum growth rate than the S biotype. In contrast to these findings, Bonow et al. (2018) did not find any growth penalty on plant height, leaf area, shoot and root dry weight in an ALS-imazapyr + imazapic-cross-resistant barnyardgrass biotype.

Intra-specific competition of late watergrass biotypes

As both growth parameters of S and MR biotypes were not proportionally increased with increasing density, the relationship between the shoot number or fresh weight of all biotypes and weed density was best described by the nonlinear regression (high R^2). The fact that shoot number slope of the S biotype was higher than the MRAQ1 but similar and higher with the respective MRAQ2 and MRAC biotypes, along with its lower fresh weight slope than the ones of the MR biotypes indicates lack of association

between growth response of MR biotypes and their multiple-resistance. However, as both growth parameters of one calculated plant per pot of all biotypes were proportionally decreased with increasing density, the relationship between the shoot number or fresh weight of each biotype and weed density was best described by the linear regression (high R^2). The above reduced growth parameters of each plant with increasing density are attributed to intra-specific competition of all biotypes. The higher shoot number slope of the S biotype than the respective slopes of the MR biotypes, along with its similar fresh weight slope with MRAC and higher than the ones of MRAQ2 and MRAQ1 confirms again the lack of association between intra-specific competition of MR biotypes and their multiple-resistance. Similar results were reported by Awan and Chauhan (2016), who found that the linear, the quadratic and the exponential equations were the best fit to describe barnyardgrass growth parameters against density. Boddy et al. (2012) also reported different growth response between R and S weed biotypes grown under different densities.

Late watergrass competition with rice

As shoot number and fresh weight of all biotypes grown in competition with rice were not proportionally increased with increasing weed density, the relationship between these two growth parameters of late watergrass and weed density per plot was best described by the nonlinear regression (high R^2). The higher shoot number slope of the S biotype than the MRAQ1 and the lower than the MRAQ2 and MRAC ones, along with the respective higher fresh weight slope of the S biotype than the MRAQ1 and MRAQ2 but lower than the MRAC illustrates again lack of association between growth response of the MR biotypes and their multiple-resistance. In addition, the fact that the shoot number and fresh weight slopes of one late watergrass plant per pot indicated differences between the S and MR biotypes grown under different weed densities with rice confirmed again that there is no any association between competitive ability of MR biotypes and their multiple-resistance, which agree with the results reported previously for the same biotypes grown under intra-specific competition. In addition, the similarity in most cases between the calculated competition indices (relative yield, competitive

ratio, aggressiveness) of the S and MR biotypes indicates that the interpretation of the results obtained from such kind of competition studies depends on the statistical procedure applied for the analysis of data. Similar results were reported by Boddy et al. (2012), who found that the S and the multiple-resistant late watergrass biotypes had similar inter-specific interference ability against rice. Also, Bagavathiannan et al. (2011) found similar competition indices in most cases between the propanil- or clomazone-resistant barnyardgrass biotypes and S one.

The significant reduction of rice shoot number and fresh weight due to MR late watergrass biotype competition confirms their recorded significant threat to rice production by Fischer et al. (2000) and Rouse et al. (2018), and additionally explains their great ability to replace the barnyardgrass in rice paddy fields of northern Greece (Vasilakoglou et al., 2018). Moreover, the fact that the order of rice growth reduction due to four weed biotypes was $S = MRAC < MRAQ2 < MRAQ1$ strongly suggests that the competitive ability of the MR late watergrass biotypes is not associated with their multiple-resistance. However, other factors such as nitrogen fertilization, water regime, rice density, and time of weed emergence, affect the competitiveness of *Echinochloa* against rice. More specifically, Chauhan and Abugho (2013) found that the competitive ability of barnyardgrass against rice was affected by nitrogen fertilization, water regime and rice density. In addition, Awan and Chauhan (2016) and Awan et al. (2021) found that the competitiveness of barnyardgrass against rice was affected by the time of weed emergence, while Chauhan and Johnson (2010) found that barnyardgrass had the ability to reduce rice interference by increasing leaf weight.

The approach to control genetic background was not applied in the resistance fitness study because it creates a group of plants having the same genotype, which does not exist naturally and takes into consideration that fitness differences in biotypes originating from different environments-fields is strongly correlated with the resistance gene (Papanagiotou et al., 2015; Vila-Aiub et al., 2011). In addition, the genetic control approach does not help to determine the importance of the resistance genes in influencing the fitness of the naturally occurring resistant (homozygous or heterozygous) biotypes in comparison with the natural susceptible biotypes.

Therefore, the naturally occurring R and S biotypes were used for the fitness study.

Conclusively, the whole-plant dose–response results of this study showed high levels of cross-resistance in three late watergrass biotypes to the ALS-inhibiting herbicides penoxsulam, bispyribac, imazamox and nicosulfuron + rimsulfuron, while in one and two of them multiple-resistance to the ACCase inhibitor-cyhalofop and to the synthetic auxin-quinclorac were also found, respectively. The growth rate, intra-specific competition (four plant densities in the absence of rice) and inter-specific competition (five late watergrass densities in the presence of rice) parameters indicated unclear association between the growth response of the MR populations and their resistance. These fitness results illustrate clearly that variation in fitness of MR biotypes are not linked with the resistance alleles but it could be attributed to the biology and evolutionary origin of the biotypes, the polymorphisms at non-resistance alleles, the inbreeding depression, the non-random mating, the linkage disequilibrium processes and the genotype by environment interaction (Délye et al., 2013; Papapanagiotou et al., 2015; Vila-Aiub et al., 2011).

Author contributions All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by A. Papanagiotou, I. Vasilakoglou and K. Dhima. The first draft of the manuscript was written by Ioannis Vasilakoglou, while the validation, reviewing and final editing was made by I.G. Eleftherohorinos. All authors read, commented and approved the final manuscript.

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Data Availability The raw data are available on any possible demand.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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References

- Amaro-Blanco, I., Romano, Y., Palmerin, J. A., Gordo, R., Palma-Bautista, C., De Prado, R., & Osuna, M. D. (2021). Different mutations providing target site resistance to ALS- and ACCase-inhibiting herbicides in *Echinochloa* spp. from rice fields. *Agriculture*, 11(5), 382. <https://doi.org/10.3390/agriculture11050382>
- Ashigh, J., & Tardif, F. J. (2011). Water and temperature stress impact fitness of acetohydroxyacid synthase-inhibiting herbicide-resistant populations of eastern black nightshade (*Solanum ptycanthum*). *Weed Science*, 59, 341–348.
- Awan, T. H., & Chauhan, B. S. (2016). Effect of emergence time, inter- and intra-specific competition on growth and fecundity of *Echinochloa crus-galli* in dry-seeded rice. *Crop Protection*, 87, 98–107.
- Awan, T. H., Cruz, P. C. S., & Chauhan, B. S. (2021). Influence of *Echinochloa crus-galli* density and emergence time on growth, productivity and critical period of competition with dry-seeded rice. *International Journal of Pest Management*, 67, 1969469.
- Bagavathiannan, M. V., Norsworthy, J. K., Jha, P., & Smith, K. (2011). Does resistance to propanil or clomazone alter the growth and competitive abilities of barnyardgrass (*Echinochloa crus-galli*)? *Weed Science*, 59, 353–358.
- Bakkali, Y., Ruiz-Santaella, J. P., Osuna, M. D., Wagner, J., Fischer, A. J., & De Prado, R. (2007). Late Watergrass (*Echinochloa phyllopogon*): Mechanisms involved in the resistance to fenoxaprop-p-ethyl. *Journal of Agricultural Food Chemistry*, 55, 4052–4058.
- Boddy, L. G., Streibig, J. C., Yamasue, Y., & Fischer, A. J. (2012). Biomass, fecundity, and interference ability of multiple herbicide-resistant and -susceptible late watergrass (*Echinochloa phyllopogon*). *Weed Science*, 60, 401–410.
- Bonow, J. F. L., Andres, A., Nogueira, E., & Lamego, F. P. (2018). Fitness of *Echinochloa crus-galli* var. *mitis* biotypes susceptible and resistant to imazapyr + imazapic herbicide. *Comunicata Scientiae*, 9, 737–747.
- Brim-DeForest, W. B., Al-Khatib, K., & Fischer, A. J. (2022). Emergence and early growth of multiple herbicide-resistant and -susceptible late watergrass (*Echinochloa phyllopogon*). *Weed Technology*, 36, 101–109.
- Brim-DeForest, W. B., Al-Khatib, K., Linquist, B. A., & Fischer, A. J. (2017). Weed community dynamics and system productivity in alternative irrigation systems in California rice. *Weed Science*, 65, 177–188.
- Chauhan, B. S., & Abugho, S. B. (2013). Effects of water regime, nitrogen fertilization, and rice plant density on growth and reproduction of lowland weed *Echinochloa crus-galli*. *Crop Protection*, 54, 142–147.
- Chauhan, B. S., Jabran, K., & Mahajan, G. (2017). *Rice production worldwide*. Springer International Publishing AG, Switzerland. <https://doi.org/10.1007/978-3-319-47516-5>

- Chauhan, B. S., & Johnson, D. E. (2010). Responses of rice flatsedge (*Cyperus iria*) and barnyardgrass (*Echinochloa crus-galli*) to rice interference. *Weed Science*, 58, 204–208.
- Cousens, R. D., & Fournier-Level, A. (2018). Herbicide resistance costs: What are we actually measuring and why? *Pest Management Science*, 74, 1539–1546.
- Danquah, E. Y., Johnson, D. E., Riches, C., Arnold, G. M., & Karp, A. (2002). Genetic diversity in *Echinochloa* spp. collected from different geographic origins and within rice fields in Cote d'Ivoire. *Weed Research*, 42, 394–405.
- Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trend in Genetics*, 29, 649–658.
- Dimaano, N. G., Tominaga, T., & Iwakami, S. (2022). Thiobencarb resistance mechanism is distinct from CYP81A-based cross-resistance in late watergrass (*Echinochloa phyllopogon*). *Weed Science*, 70, 160–166.
- Du, L., Qu, M., Jiang, X., Li, X., Ju, Q., Lu, X., & Wang, J. (2019). Fitness costs associated with acetyl-coenzyme A carboxylase mutations endowing herbicide resistance in American sloughgrass (*Beckmannia syzigachne* Steud.). *Ecology and Evolution*, 9, 2220–2230.
- Fisher, A. J., Bayer, D. E., Carriere, M. D., Ateh, C. M., & Yim, K. O. (2000). Mechanisms of resistance to bispyribac-sodium in an *Echinochloa phyllopogon*. *Pesticide Biochemistry and Physiology*, 68, 156–165.
- Gibson, K. D., Fischer, A. J., & Foin, T. C. (2004). Compensatory responses of late watergrass (*Echinochloa phyllopogon*) and rice to resource limitations. *Weed Science*, 52, 271–280.
- Gibson, K. D., Fischer, A. J., Foin, T. C., & Hill, J. E. (2002). Implications of delayed *Echinochloa* spp. germination and duration of competition for integrated weed management in water-seeded rice. *Weed Research*, 42, 351–358.
- Heap, I. (2023). *The international survey of herbicide resistant weeds*. <https://www.weedscience.org>. Accessed 20 Feb 2023
- Iwakami, S., Hashimoto, M., Matsushima, K.-I., Watanabe, H., Hamamura, K., & Uchino, A. (2015). Multiple-herbicide resistance in *Echinochloa crus-galli* var. *formosensis*, an allohexaploid weed species, in dry-seeded rice. *Pesticide Biochemistry and Physiology*, 119, 1–8.
- Iwakami, S., Kamidate, Y., Yamaguchi, T., Ishizaka, M., Endo, M., Suda, H., Nagai, K., Sunohara, Y., Toki, S., Uchino, A., Tominaga, T., & Matsumoto, H. (2019). CYP81A P450s are involved in concomitant cross-resistance to acetolactate synthase and acetyl-CoA carboxylase herbicides in *Echinochloa phyllopogon*. *New Phytologist*, 221, 2112–2122.
- Iwakami, S., Uchino, A., Kataoka, Y., Shibaike, H., Watanabe, H., & Inamura, T. (2014). Cytochrome P450 genes induced by bispyribac-sodium treatment in amultiple-herbicide-resistant biotype of *Echinochloa phyllopogon*. *Pest Management Science*, 70, 549–558.
- Kaloumenos, N. S., Chatzilarzidou, S. L., Mylona, Ph. V., Polidoros, A. N., & Eleftherohorinos, I. G. (2013). Target-site mutation associated with cross-resistance to ALS-inhibiting herbicides in late watergrass (*Echinochloa oryzicola* Vasing.). *Pest Management Science*, 69, 865–873.
- Kaya, H. B., Demirci, M., & Tanyolac, B. (2014). Genetic structure and diversity analysis revealed by AFLP on different *Echinochloa* spp. from northwest Turkey. *Plant Systematics and Evolution*, 300, 1337–1347.
- Keshthkar, E., Abdolshahi, R., Sasanfar, H., Zand, E., Beffa, R., Dayan, F. E., & Kudsk, P. (2018). Assessing fitness cost from a herbicide-resistance management perspective: A review and insight. *Weed Science*, 67, 137–148.
- Lehnhoff, E. A., Keith, B. K., Dyer, W. E., Peterson, R. K., & Menalled, F. (2013). Multiple herbicide resistance in wild oat and impacts on physiology, germinability, and seed germination. *Agronomy Journal*, 105, 854–862.
- Liu, J., Fang, J., He, Z., Li, J., & Dong, L. (2019). Target site-based resistance to penoxsulam in late watergrass (*Echinochloa phyllopogon*) from China. *Weed Science*, 67, 380–388.
- McDonald, J. H. (2014). *Handbook of biological statistics* (3rd ed.). Sparky House Publishing.
- Mascanzoni, E., Perego, A., Marchi, N., Scarabel, L., Panozzo, S., Ferrero, A., Acutis, M., & Sattin, M. (2015). *Epidemiology and agronomic predictors of herbicide resistance in rice at a large scale*. HAL-02399206. <https://hal.archives-ouvertes.fr/hal-02399206>. Accessed 15 Sept 2022
- Meier, U. (2001). *Growth stages of mono- and dicotyledonous plants*. BBCH monograph. Berlin and Braunschweig: Federal Biological Research Centre for Agriculture and Forestry, p. 158.
- MSTAT-C. (1988). *MSTAT-C, A microcomputer program for design, management and analysis of agronomic research experiments*. East Lansing: Crop and Soil Sciences Department, Michigan State University, p. 496.
- Nakka, S., Jugulam, M., Peterson, D., & Asif, M. (2019). Herbicide resistance: Development of wheat production systems and current status of resistant weeds in wheat cropping systems. *Crop Journal*, 7, 750–760.
- Panozzo, S., Mascanzoni, E., Scarabel, L., Milani, A., Dalazen, G., Merotto, A. J., Tranel, P. J., & Sattin, M. (2021). Target-site mutations and expression of *ALS* gene copies vary according to *Echinochloa* species. *Genes*, 12, 1841. <https://doi.org/10.3390/genes12111841>
- Papapanagiotou, A. P., Paresidou, M. I., Kaloumenos, N. S., & Eleftherohorinos, I. G. (2015). ACCase mutations in *Avena sterilis* populations and their impact on plant fitness. *Pesticide Biochemistry and Physiology*, 123, 40–48.
- Park, K. W., Mallory-Smith, C. A., Ball, D. A., & Mueller-Warrant, G. W. (2004). Ecological fitness of acetolactate synthase inhibitor-resistant and -susceptible downy brome (*Bromus tectorum*) biotypes. *Weed Science*, 52, 768–773.
- Rouse, C. E., Burgos, N. R., Norsworthy, J. K., Tseng, T.-M., Starkey, C. E., & Scott, R. C. (2018). *Echinochloa* resistance to herbicides continues to increase in Arkansas rice fields. *Weed Technology*, 32, 34–44.
- Schaedler, C. E., Noldin, J. A., Agostinetto, D., Dal Magro, T., & Fontana, L. C. (2013). Germination and growth of *Fimbristylis miliacea* biotypes resistant and susceptible to acetolactate synthase-inhibiting herbicides. *Planta Daninha*, 31, 687–694.
- SigmaPlot. (2013). *SigmaPlot for windows* (ver. 12.5). San Jose, CA: Systat Software Inc, p. 443.
- Snedecor, G. W., & Cochran, W. G. (1989). *Statistical Methods* (8th ed.). Iowa State University Press.
- Statistical Package for the Social Sciences (SPSS). (1998). *SPSS Base 8.0 user's guide and SPSS applications guide*. Chicago, IL: SPSS, Prentice Hall, p. 372.

- Tabacchi, M., Mantegazza, R., Spada, A., & Ferrero, A. (2006). Morphological traits and molecular markers for classification of *Echinochloa* species from Italian rice fields. *Weed Science*, 54, 1086–1093.
- Talbert, R. E., & Burgos, N. R. (2007). History and management of herbicide-resistant barnyardgrass (*Echinochloa crus-galli*) in Arkansas rice. *Weed Technology*, 21, 324–331.
- Vasilakoglou, I., Dhima, K., & Gitsopoulos, Th. (2018). Management of penoxsulam- and bispyribac-resistant late watergrass (*Echinochloa phyllopogon*) biotypes and rice sedge (*Cyperus difformis*) in rice. *Chilean Journal of Agricultural Research*, 78, 276–286.
- Vidotto, F., Tesio, F., Tabacchi, M., & Ferrero, A. (2007). Herbicide sensitivity of *Echinochloa* spp. accessions in Italian rice fields. *Crop Protection*, 26, 285–293.
- Vila-Aiub, M. M., Neve, P., & Roux, F. (2011). A unified approach to the estimation and interpretation of resistance costs in plants. *Heredity*, 107, 386–394.
- Weeraratne, L. V. Y., Suriyagoda, L. D. B., & Marambe, B. (2015). Barnyard grass (*Echinochloa crus-galli* (L.) P.Beauv) in less competitive on rice (*Oryza sativa* L.) when phosphorus (P) is applied to deeper layers in P-deficient and moisture-limited soils. *Plant and Soil*, 391, 1–17.
- Yasuor, H., Osuna, M. D., Ortiz, A., Saldain, N. E., Eckert, J. W., & Fischer, A. J. (2009). Mechanism of resistance to penoxsulam in late watergrass [*Echinochloa phyllopogon* (Stapf) Koss.]. *Journal of Agricultural Food Chemistry*, 57, 3653–3660.
- Yasuor, H., Zou, W., Tolstikov, V. V., Tjeerdema, R. S., & Fischer, A. J. (2010). Differential oxidative metabolism and 5-ketoclozoxazole accumulation are involved in *Echinochloa phyllopogon* resistance to clomazone. *Plant Physiology*, 153, 319–326.
- Yun, M.-S., Yogo, Y., Miura, R., Yamasue, Y., & Fischer, A. J. (2005). Cytochrome P-450 monooxygenase activity in herbicide-resistant and -susceptible late watergrass (*Echinochloa phyllopogon*). *Pesticide Biochemistry and Physiology*, 83, 107–114.

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