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Distribution and resistance of barnyardgrass to quinclorac in rice fields in Thailand

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Abstract: Background: In Thailand, *Echinochloa crus-galli* (L.) P. Beauv. (barnyardgrass) is the most problematic weed in rice. Quinclorac was first commercialized in 1997 in Thailand and became the primary herbicide option for barnyardgrass control. The intensive use of quinclorac in rice fields increases the risk of the evolution of resistant barnyardgrass.

Objective: This study was conducted to survey the occurrence of quinclorac-resistant barnyardgrass in Thailand, investigate the levels of quinclorac resistance, and evaluate alternative control measures for quinclorac-resistant barnyardgrass.

Methods: Seeds of barnyardgrass were collected from 165 rice fields, located in 27 provinces of Thailand, and screened for quinclorac resistance. A whole-plant dose-response study was conducted on a susceptible population and three resistant populations to evaluate the resistance level. In addition, effectiveness of alternative herbicides including bispyribac, fenoxaprop, penoxsulam, profoxydim, propanil, and pyribenzoxim were evaluated using the selected populations.

Results: Quinclorac-resistant barnyardgrass was identified in 121 rice fields (73%). Five sites (3%) exhibited developing barnyardgrass resistance to quinclorac whereas barnyardgrass populations in thirty-nine sites (24%) were susceptible to quinclorac. The evaluated resistant populations were at least 93-fold more resistant to quinclorac than the susceptible population. Profoxydim and propanil provided effective control of the two quinclorac-resistant barnyardgrass populations. One population (B56) exhibited no shoot biomass reduction after treatment either with quinclorac or bispyribac, suggesting multiple resistance to auxin mimics and ALS-inhibiting herbicides.

Conclusions: Quinclorac-resistant barnyardgrass cases were confirmed in Thailand. The resistant barnyardgrass populations were broadly distributed on major rice-production areas of Thailand. Multiple-resistance in a quinclorac resistant population requires further investigation.

Keywords: dose-response; *Echinochloa crus-galli*; herbicide resistance; multiple herbicide resistance; weed survey

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1. Introduction

Rice is a staple food and the most important crop in Thailand for more than a millennium. The Central, Northeast, and North regions of Thailand are rice producing zones (Suebpongsang et al., 2020). Thailand is the sixth largest rice producer and the second largest exporter after India (Food and Agriculture Organization, 2021). Rice cultivation in Thailand is categorized into in-season (during the rainy season) and off-season (during the dry season in well irrigated areas) rice farming (Suebpongsang et al., 2020). Weeds are a major problematic pest of rice and compete for light, water, and nutrients, resulting in low rice quality and yield (Singh et al., 2014; Marchesi, Chauhan, 2019).

Barnyardgrass (*Echinochloa crus-galli* (L.) P. Beauv.) is highly competitive compared to the crop and known to be the most problematic among weed species in rice fields (Holm et al., 1977; Rahman et al., 2010; Qiong et al., 2019). Barnyardgrass's morphophysiology as a C_4 photosynthethic mechanism helps increase its competitiveness and infestation levels in paddy fields (Holm et al., 1977). Competition studies of rice and barnyardgrass reveal that rice yield can decrease 30% to 100% with infestations of barnyardgrass (Singh et al., 2014; Marchesi, Chauhan, 2019). Barnyardgrass densities of 5 plants m^{-2} are deleterious to growth and yield of rice (Smith, 1988).

To control broadleaf and annual grass weeds, quinclorac, an auxinic herbicide containing quinolinecarboxylic acid, is used in many crops, such as rice, wheat, barley, canary seed, and canola (Shaner, 2014). It is an option to control *Echinochloa* spp. during the 2-leaf stage. Quinclorac and other synthetic auxin herbicides cause leaf chlorosis on the new leaf blades and subsequent wilting and necrosis of aboveground tissue of susceptible species through the induction of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (Grossmann, Kwiatkowski, 2000). ACC synthase is involved in ethylene synthesis; therefore, the subsequent accumulation of cyanide leads to toxicity (Grossmann, 2010).

Because of the extensive use of herbicides in recent decades, barnyardgrass has evolved resistance to herbicides (Délye et al., 2013; Heap, 2021). Quinclorac-resistant barnyardgrass was first observed in 1997 in Southern Spain (Lopez-Martinez et al., 1997), and later in Brazil, China, USA, and Uruguay (Heap, 2021). Quinclorac was first commercialized in Thailand in 1997 and became the primary herbicide option for barnyardgrass control. In 2001, C. Maneechote reported in the International Herbicide-Resistant Weed Database that quinclorac has been effectively used to control cyhalofop-, fenoxaprop-, and quizalofop-resistant barnyardgrass populations in Thailand (Heap, 2021). The intensive use of quinclorac in Thai rice fields has increased the risk of developing resistant barnyardgrass. Multiple herbicide resistance, when the same biotype presents resistance to multiple herbicide modes of action, has been reported in barnyardgrass (Lopez-Martinez et al., 1997; Juliano et al., 2010; Malik et al., 2010; Rahman et al., 2010; Marchesi, Saldain, 2019; Heap, 2021). Multiple herbicide resistance is a serious issue because of the reduction of control measures.

In Thailand, herbicide resistant weeds such as Chinese sprangletop (*Leptochloa chinensis* (L.) Nees) (Olofsdotter et al., 2000; Maneechote et al., 2005; Pornprom et al., 2006; Heap, 2021), gooseweed (*Sphenoclea zeylanica* Gaertn.) (Olofsdotter et al., 2000) and barnyardgrass (Olofsdotter et al., 2000; Heap, 2021), have been reported. However, no studies have surveyed and evaluated quinclorac-resistant barnyardgrass on rice-production areas of Thailand. Therefore, this study was conducted to determine the distribution and level of herbicide resistance for monitoring of resistance development. The objectives of this study were to 1) screen for the occurrence of quinclorac-resistant barnyardgrass in Thai rice fields, 2) investigate the levels of quinclorac-resistant barnyardgrass, and 3) evaluate alternative control measures for quinclorac-resistant barnyardgrass.

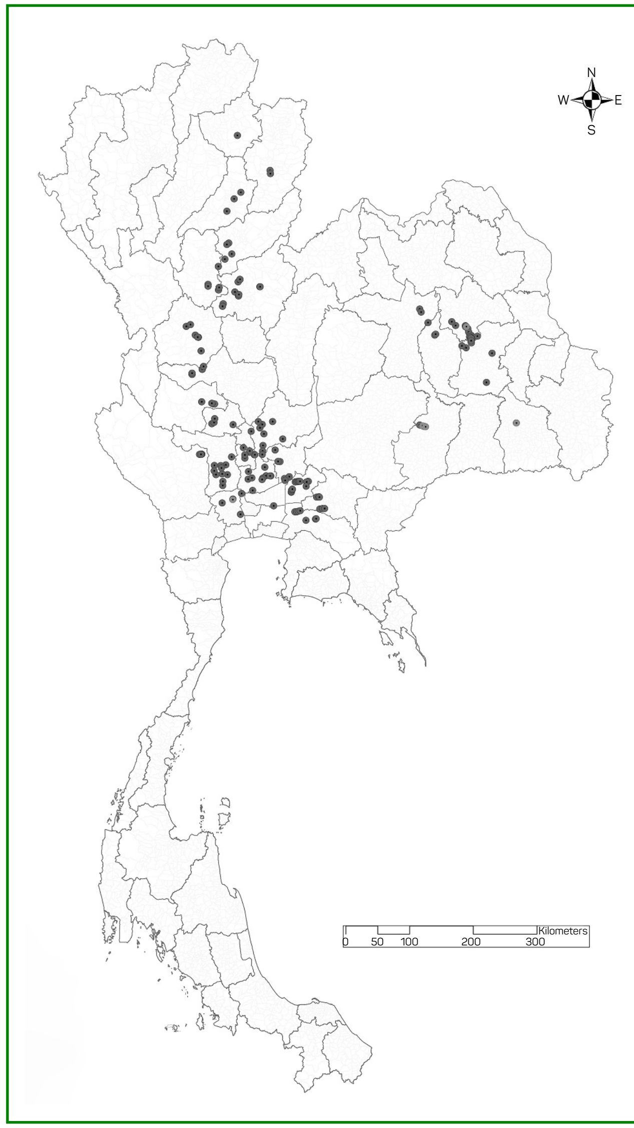

Figure 1 - Sampling locations of barnyardgrass populations collected from rice fields in Thailand in 2017.

2. Material and Methods

2.1 Seed collection and quinclorac resistance screening

Barnyardgrass surveys and seed collection were conducted in major rice-production areas in 165 rice fields covering 165 sub-districts of 27 provinces of Thailand in 2017 (Figure 1). The districts and provinces represent the local and major regions of Thailand, respectively. Sampling sites were selected based on the presence of barnyardgrass during the collection time. In each rice field, hundreds of barnyardgrass seeds in a mature stage (completely dry) were randomly collected diagonally across the field (European Herbicide Resistance Action Committee, 2017). Seeds were air dried for 2 weeks, rubbed with sandpaper, and kept dry at 4 °C until use. The exact sampling locations are given in Supplementary Table S1. A known susceptible (S) barnyardgrass population was used as a control in the experiment; the seeds were collected from an organic rice field in Ubon Ratchathani Province, Thailand, which has been a herbicide-free rice field for over 10 years.

A greenhouse screening experiment was conducted at the Weed Science and Research Group, Department of Agriculture, Bangkok, Thailand in 2018. The experiment was a randomized complete block design (RCBD) with four replications (n = 25 plants in each replication). Fifty seeds were planted separately for each population in a 20.0 x 30.0 x 17.5 cm plastic tray (width x length x depth) containing commercial potting mix (Free peat, Vriezenveen, Netherlands). The seedlings were thinned to twenty-five plants for each population at the one-leaf growth stage. The greenhouse was kept under 33 °C (day temperature) and 27 °C (night temperature) with a 12-h photoperiod. Quinclorac (Facet®, BASF, Thailand) was sprayed at the two- to three-leaf growth stage at 0.75 kg ai ha^{-1} (recommended label rate) using a knapsack sprayer with an even flat fan nozzle tip (TeeJet 8003EVS, Spraying Systems Co., Wheaton, IL) calibrated to deliver 500 L ha^{-1}. At 15 days after the application, survival plants were recorded for percentage plant survival. Resistance in a population was classified using criteria of Llewellyn and Powles (2001) as resistant (> 20% survivors), developing resistance (1-20% survivors), and susceptible (0% survivors).

2.2 Dose-response of quinclorac-resistant populations

After confirming quinclorac resistant populations, progeny from these resistant populations were subsequently grown in the greenhouse to increase seed quantity for use in this study. These populations were selected on the basis of seed limitations and viability after breaking of dormancy. Quinclorac resistant populations B53, B56, and B102, which had 98%, 100%, and 98% survival, respectively, after exposure to labelled quinclorac rate of 0.75 kg ai ha^{-1} (Supplementary Table S1), were selected for this study. Field history of the locations where the three resistant populations were collected are shown in Table 1. Progeny of B37, which presented 0% survival, was used as a susceptible population. The B37 was collected from an organic paddy field in Changhan District, Roi Et Province, where no herbicide was used since 2006.

The experiment was a RCBD with three replications (n = 10 plants in each replication). Fifty seeds were planted separately for each population. The seedlings were thinned to ten plants for each population at the one-leaf growth stage, under the growth condition as described previously. At the two- to three-leaf growth stage, plants were sprayed with quinclorac at 0.00075, 0.038, 0.075, 0.75, 3, 12, 18 and 24 kg ai ha^{-1} using the same equipment and calibration setting as described previously. Fourteen days after the treatment, shoot biomass was harvested, dried at 60 °C for 72 h, and weighed. The experiment was conducted twice with slightly adjusted rates.

2.3 Alternative control measures for quinclorac-resistant barnyardgrass

The populations used in the dose-response experiment were used in this study, except the B53. Resistant population B53 was collected from the same province and had similar herbicide history as B56. The experiment was a RCBD with three replications (n = 10 plants in each replication). Fifty seeds of progeny of resistant and susceptible populations were planted separately for each population. The seedlings were thinned to ten plants for each population at the one-leaf growth stage, under the growth condition as described previously. Plants at the two- to three-leaf growth stage were sprayed with herbicides from four different modes of action: acetyl-CoA carboxylase (ACCase) inhibitors, acetolactate synthase (ALS) inhibitors, photosystem II (PSII) Serine 264 binders inhibitors, and auxin mimics (Table 2), using the same equipment and calibration setting as described previously. An untreated control for each population was included for treatment comparisons. Fourteen days after the treatment, shoot biomass was harvested, dried at 60 °C for 72 h, and weighed.

Table 1 - Herbicide use records of the fields where the three quinclorac-resistant barnyardgrass populations (B53, B56, and B102) were collected

Population	Location (District, Province)	Year[1]	Planting seasons per year[2]	Herbicide applied
B53	Bang Nam Priao, Chachoengsao	2000	2	2,4-D + propanil
		2005	2	pyrazosulfuron
		2007	2	pyribenzoxim, bispyribac, penoxsulam
		2015	2	quinclorac
		2016	2	quinclorac + clomazone
B56	Phanom Sarakham, Chachoengsao	2000	1	2,4-D + propanil
		2005	1	pyrazosulfuron
		2007	1	pyribenzoxim, bispyribac, penoxsulam
		2015	1	quinclorac
		2016	1	quinclorac + clomazone
B102	Phra Phutthabat, Saraburi	2000	2	2,4-D + propanil
		2004	2	pyrazosulfuron
		2007	2	pyribenzoxim + bispyribac
		2014	2	quinclorac
		2016	2	quinclorac + clomazone

[1] Years which available herbicide records. [2] Monoculture rice production.

Table 2 - Modes of action and chemical groups of herbicides evaluated in the alternative control measures for quinclorac-resistant barnyardgrass experiment

Mode of action	Chemical group	Active ingredient	Sources of Materials	Dose (kg ai ha^{-1})
Inhibition of Acetyl CoA Carboxylase (ACCase Inhibitors)	Aryloxphenoxy-propionates (FOPs)	Fenoxaprop-P-ethyl	Ricestar®, Bayer, Thailand	0.069
	Cyclohexanediones (DIMs)	Profoxydim	Tetris®, BASF, Thailand	0.122
Inhibition of Acetolactate Synthase (ALS Inhibitors)	Pyrimidinyl benzoates	Bispyribac-sodium	Nominee Gold®, TJC Chemical, Japan	0.050
		Pyribenzoxim	Pyanchor®, Sharpformulators, Thailand	0.031
	Triazolopyrimidines – type 2	Penoxsulam	Rainbow 25 OD®, Dow AgroSciences, Thailand	0.038
Inhibition of Photosynthesis at PS II - D1 Serine 264 Binders	Amides	Propanil	Propa®, P.Chemitech, Thailand	2.025
Auxin Mimics	Quinoline-carboxylates	Quinclorac	Facet®, BASF, Thailand	0.750

2.4 Statistical analysis

ANOVA was used to determine significant differences between the R and S barnyardgrass populations in the whole-plant and between the shoot biomass responses to herbicides in the alternative control assays. Least significant difference (LSD) at $p<0.05$ was used to separated means. Dose-response curves were constructed using the four-parameter log-logistic model (LL.4) equation:

$$y = C + \frac{D - C}{1 + (x/GR_{50})^{b}} \tag{1}$$

Where y represents the percentage of untreated control of shoot biomass; C is the mean response at very high herbicide rate (lower limit); D is the mean response when the herbicide rate is zero (upper limit); x is the herbicide rate (kg ai ha^{-1}); b is the slope of the curve at GR_{50}; and GR_{50} is the herbicide rate which provided 50% shoot biomass reduction. For each barnyardgrass population, the regression parameters and approximate standard error were obtained using the package drc (Ritz et al., 2015). The resistance level was calculated by the ratio of the GR_{50} of the R population to that of the S population. All statistical analyses were performed using the program R v. 4.1.1 (R Core Team, 2021).

3. Results and Discussion

3.1 Quinclorac resistance screening

To screen for the occurrence of quinclorac resistance at 0.75 kg ai ha^{-1} of the 165 barnyardgrass populations evaluated, approximately 76% and 24% exhibited resistance and susceptibility, respectively (Table 3; Figure 2a). Across the surveyed areas, 73% and 3% of the fields contained a barnyardgrass population classified as resistant (> 20% survival) and developing resistant (1-20% survival) to quinclorac, respectively. The cluster of quinclorac-resistant populations was observed in the lower North (Figure 2b) and Central (Figure 2c) regions of Thailand. The lower North and Central areas are the main areas for off-season rice production (commercial production) with at least 2 seasons rice monoculture per year. The upper North and Northeast regions produce only in-season rice, and some parts of these regions rely on organic rice production and use local varieties. The populations that were developing resistance to quinclorac occurred in 3% of the fields located in the Central (Figure 2c) and Northeast regions (Figure 2d). Although barnyardgrass is highly self-fertilizing (Tsuji et al., 2003), gene flow ranged from 5.6% to 12.5% at the distance of 0.25 to 0 m, respectively (Bagavathiannan, Norsworthy, 2014), which raises the concern of resistant invasion due to the possible distribution of gene flow.

Table 3 - Resistance classification and plant survival of 165 barnyardgrass populations collected from rice fields in Thailand after exposure to quinclorac at 0.75 kg ai ha^{-1}

Resistance classification[1]	Plant survival[2] (%)	Number of populations	% of populations
Resistant	81+	52	32
	61-80	55	33
	41-60	9	5
	21-40	5	3
Subtotal	-	121	73
Developing resistance	1-20	5	3
Susceptible	0	39	24
Total	-	165	-

[1] Resistant (> 20% survivors), developing resistance (1 - 20% survivors), and susceptible (0% survivors). [2] Plant survival was assessed 15 days after exposure to quinclorac at 0.75 kg ai ha^{-1} (recommended label rate).

3.2 Dose-response of quinclorac-resistant populations

To estimate the severity of resistance in the situation related to the real environment, whole-plant dose-response study was assessed on three quinclorac resistant populations

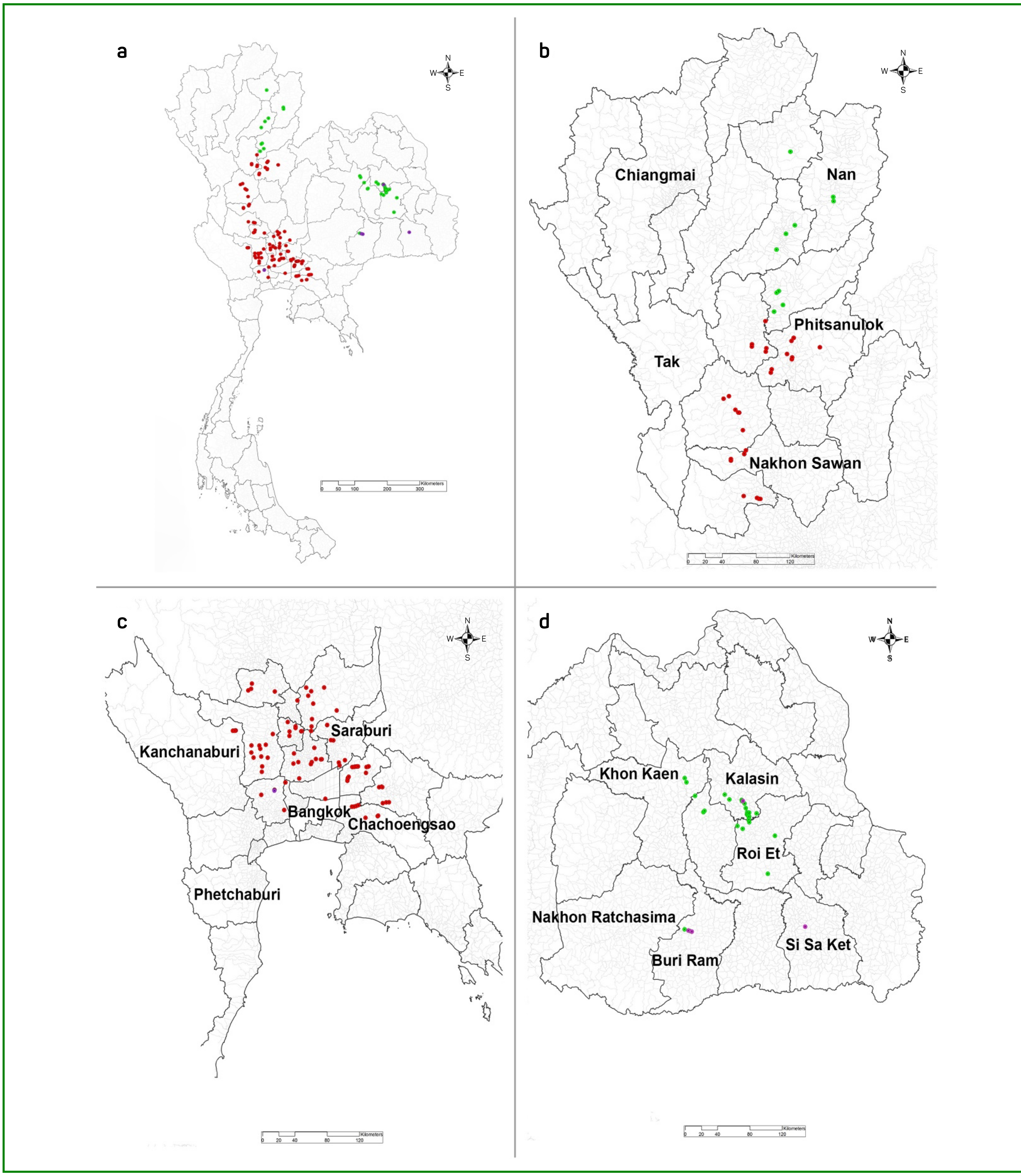

Figure 2 - Distribution and resistance classification of 165 barnyardgrass populations collected from major rice fields in Thailand after exposure to quinclorac at 0.75 kg ai ha^{-1} (a), North region (b), Central region (c), and Northeast region (d). Green, purple, and red circles represent susceptible, developing resistance, and resistant populations, respectively

(B53, B56, and B102) and the susceptible population (B37). The GR_{50} for quinclorac in the resistant populations ranged from 93- to 193-fold greater than for the susceptible population, confirming quinclorac resistance (Table 4; Figure 3).

Based on the herbicide use records, quinclorac has been used from 2014 to 2016 (Table 1). Within 2-3 years, these barnyardgrass populations might have evolved quinclorac resistance possible due to the selection pressure imposed by

Table 4 - The GR_{50} values (kg ai ha^{-1}) and resistance ratios (R:S) of three quinclorac-resistant (B53, B56, and B102) and one susceptible (B37) barnyardgrass populations evaluated in the dose-response experiment 14 days after treatment with quinclorac

Population	GR_{50}[1]	Resistance ratio (R:S)	P-value[2]
B53	6.327	137.5	0.013
B56	4.298	93.4	0.013
B102	8.874	192.9	0.009
B37	0.046	-	-

[1]The model fitted corresponded to shoot biomass (% of untreated control) = C + (D-C) / [1+(x / GR_{50})^b]; Abbreviations: C, lower limit; D, upper limit; b, slope of the curve; x, herbicide rate; GR_{50}, herbicide rate provided 50% shoot biomass reduction. [2]P-value for testing the null hypothesis that R:S=1.

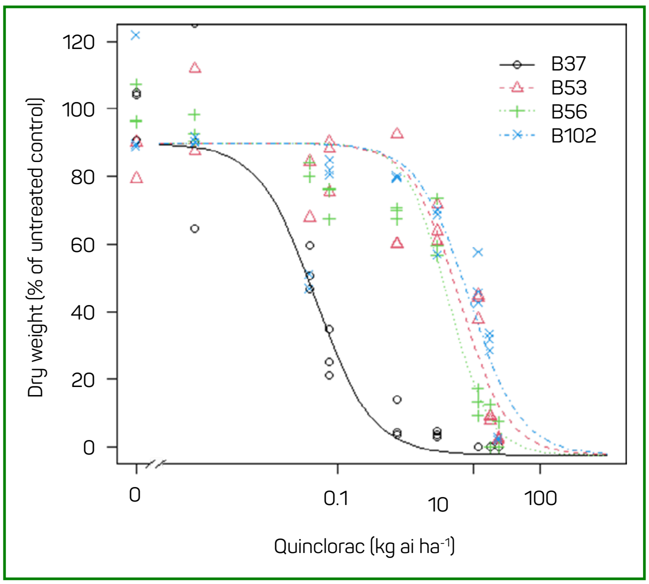

Figure 3 - Shoot biomass of three resistant (B53, B56, and B102) and one susceptible (B37) barnyardgrass populations as affected by quinclorac. Symbols and lines represent actual and predicted growth response, respectively

repeated uses. Similar results were found for intensive use of a tank-mix with propanil, quinclorac, and clomazone for more than a decade which led to the evolution of quinclorac resistance (Marchesi, Saldain, 2019). Malik et al. (2010) reported the propanil-resistant and quinclorac-resistant barnyardgrass within 6 years after exposure to both herbicides due to high selection pressure. However, a study reported resistance to quinclorac in *Echinochloa* spp. despite the fact that quinclorac had never been used in the field (Yasuor et al., 2012).

3.3 Alternative control measures for quinclorac-resistant barnyardgrass

Plants from quinclorac resistant populations were treated with various post-emergent (POST) herbicides, alternatives to quinclorac for barnyardgrass control, which included ALS inhibitors (bispyribac, penoxsulam, and pyribenzoxim), ACCase inhibitors (fenoxaprop and profoxydim), and a PSII inhibitor (propanil). The shoot biomass of the susceptible population (B37) differed from the untreated control after exposure to each herbicide evaluated, including quinclorac ($p<0.05$; Table 5). For B56 and B102, shoot biomass after treating with quinclorac was not different from the untreated control, confirming quinclorac resistance. For B56, shoot biomass was not different between quinclorac and bispyribac, indicating that B56 was not effectively controlled with bispyribac. The application of penoxsulam and pyribenzoxim were marginally effective for B56 control, with 50% and 42% shoot biomass reduction when compared with untreated control, respectively. Profoxydim, propanil, and fenoxaprop provided the best B56 control, with shoot biomass reduction of at 85%, 68%, and 59% when compared with untreated control, respectively. For B102, shoot biomass after treatment with bispyribac and pyribenzoxim was different from untreated control but was not different from quinclorac. This result suggests that the application of bispyribac and pyribenzoxim were marginally effective for B102 control. Moreover, fenoxaprop and penoxsulam treatments were marginally effective with shoot biomass reduction of 47% and 58% compared with untreated control, respectively. Propanil and profoxydim provided the best B102 control with shoot biomass reduction of 91% and 67% compared with untreated control, respectively.

Herbicide resistance has evolved either from target-site resistance (TSR) or nontarget-site resistance (NTSR) mechanisms. While the NTSR mechanisms include reduced uptake and/or translocation, increased sequestration, or metabolism to nontoxic compounds, the TSR mechanisms involve mutation in genes at the target-site, gene amplification, and overexpression (Gaines et al., 2020; Rigon et al., 2020). Quinclorac resistance in grass weed species evolved through NTSR mechanisms (Gaines et al., 2020). The mechanisms responsible for quinclorac resistance include the alteration along the normal auxin reception-signal transduction pathway that can lower ACC synthase activity, while the β-cyanoalanine synthase (β-CAS), which is responsible for detoxification of the cyanide generated upon ethylene synthesis, is enhanced (Yasuor et al., 2012; Gaines et al., 2020). Enhanced herbicide metabolism can affect almost all herbicide families and is known as the major threat among NTSR mechanisms (Rigon et al., 2020).

Ineffective control by using bispyribac for B56 (Table 5; Supplementary Table S2) could possibly suggest the multiple resistance to ALS inhibitors. In quinclorac-resistant barnyardgrass, multiple resistance has been reported to PSII inhibitors (atrazine and propanil) (Lopez-Martinez et al., 1997; Malik et al., 2010; Rahman et al., 2010; Heap, 2021); to ALS inhibitors (imazethapyr, bispyribac-sodium, penoxsulam, thifensulfuron-methyl, metsulfuron-methyl, triasulfuron, sulfometuron-methyl, and pyrazosulfuron)

Table 5 - Shoot biomass of two quinclorac-resistant (B56 and B102) and one susceptible (B37) barnyardgrass populations evaluated in the alternative control measures experiment.

Treatment	Dose[1] (kg ai ha^{-1})	Shoot biomass[2] (g)					
		B37		B56		B102	
untreated control	0.000	0.217	a	0.237	a	0.238	a
bispyribac	0.050	0.078	b	0.171	ab	0.159	bc
fenoxaprop	0.069	0.066	b	0.098	bcd	0.126	cd
penoxsulam	0.038	0.021	cd	0.119	bc	0.101	cd
profoxydim	0.122	0.022	cd	0.035	d	0.079	de
propanil	2.025	0.023	cd	0.076	cd	0.021	e
pyribenzoxim	0.031	0.054	bc	0.137	bc	0.162	bc
quinclorac	0.750	0.002	d	0.230	a	0.216	ab
p-value	-	<0.001		<0.001		<0.001	

[1] Recommended label rate for use in rice. [2] Shoot biomass harvested 14 days after treatment with herbicides. Means followed by the same letter within a column do not significantly differ by the least significant difference (LSD) test at $p<0.05$.

(Qiong et al. 2019; Heap, 2021); and to ACCase inhibitors (cyhalofop-butyl) (Rahman et al., 2010). Hwang et al. (2022) reported that the NTSR mechanisms (decreased absorption and translocation, and enhanced metabolism) were involved in barnyardgrass multiple resistance to florpyrauxifen-benzyl and cyhalofop-butyl (auxinic mimics and ACCase inhibitors, respectively). Multiple herbicide resistance reduces herbicide options for controlling barnyardgrass in rice fields.

4. Conclusions

In this study, the survey suggested a broad distribution of quinclorac-resistant barnyardgrass in rice fields in Thailand. Of the 165 randomly collected barnyardgrass populations evaluated for quinclorac resistance at 0.75 kg ai ha^{-1}, approximately 76% exhibited resistance and 24% were susceptible. Three quinclorac-resistant barnyardgrass populations (B53, B56, and B102) were at least 93-fold higher than the susceptible population (B37). One population (B56) exhibited no shoot biomass reduction after treatment either with quinclorac or bispyribac, suggesting multiple resistance to auxin mimics and ALS inhibitors. This implies that diversified weed management programs are necessary for quinclorac-resistant barnyardgrass management. Sequential herbicide applications and herbicide mixtures as an effective alternative for quinclorac-resistant barnyardgrass control warrants further investigation. In addition to POST, combination of types and application timing of conventional herbicide practices can be diversified by using pre-emergence (PRE), delayed PRE, or pre-flood applications for barnyardgrass control. Alternative weed management methods such as tillage, crop rotations, flooding, and preventing weeds from producing seeds should be considered to delay the evolution of barnyardgrass resistance to herbicides in rice.

Author's contributions

All authors read and agreed to the published version of the manuscript. JP and SI: conceptualization of the manuscript and development of the methodology; data analysis; supervision; writing the original draft of the manuscript. AC: data collection and curation. SI: data interpretation. JP: project administration. JP, AC, and SI: writing, review, and editing.

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