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Weed management practices to control herbicide-resistant Echinochloa crus-galli in rice in Mekong Delta, Vietnam

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ABSTRACT

Barnyardgrass (*Echinochloa crus-galli*) is one of most important weeds in rice field in Vietnam. Seventy-eight barnyardgrass seed samples were collected from rice fields in seven provinces in Mekong Delta of Vietnam. The seedlings grown from collected seed samples were evaluated to determine the level of resistance to bispyribac, penoxsulam and quinclorac. Farmers who managed the fields from which samples collected were interviewed about weed management practices and the cost of weed control per season. The average cost for weed management was 1,980,000 VND per hectare, the total cost included 650,000 VND for herbicide and 1,330,000 VND for hand-weeding. *Echinochloa crus-galli* biotypes resistant to bispyribac, penoxsulam and quinclorac were found and biotypes with resistance to multiple herbicides were detected. The average resistance-score of three tested herbicides was 3.4 in the 78 samples collected. There was a correlation between field size, hand-weeding practice, and the herbicide-resistance level, herbicide-resistant weeds were less common in smaller fields where hand-weeding was commonly used. The high cost and limited labour pool for hand-weeding in this region of Vietnam limits the long-term viability of this approach.

Key words: Echinochloa crus-galli, herbicide-resistance, rice, weed management

INTRODUCTION

Mekong Delta of Vietnam covers an area of 40,577 km². There are more than 17 million people that inhabit this region that contain about 4 million hectares of land used for agriculture (Le et al., 2007). Mekong Delta produces more than 50% of food for all of Vietnam and rice export from this region is the most important source of income for Vietnam. About 54% of rice in the Delta is cultivated during the period of May through August, while highest yields are harvested in January to April. Yield is about 21% higher than the average in Vietnam. The average field size per household in the Delta is 1.29 ha compared to 0.44 ha in rest of Vietnam (Nguyen et al., 2017). Land used for agriculture increased from 3,212,000 ha in 2005 to 4,333,400 ha in 2016, while the number of people working in agriculture in the Delta has decreased over the last decade with only 41% of people were involved in framing in 2016 compared to 55% in 2005 (General

Statistical Office of Vietnam, 2017).

The genus Echinochloa includes over 250 species and most of these are considered weeds. Echinochloa species vary in growth habit, distribution and morphology (Barrett and Wilson, 1983; Jaswal et al., 2017). Barnyardgrass (Echinochloa crus-galli) is a C4 monocot (Caton et al., 2010). E. crus-galli is found in 61 countries and considered a serious weed in 36 crops (Bajwa et al., 2015). The weed exhibits adaption to a wide range of photoperiods. Due to the continuous morphological variations among species, the classification of Echinochloa remains a serious problem for weed scientists (Damalas et al., 2008). During the early stage of vegetative growth, it is nearly impossible to distinguish rice plants from barnyardgrass. This can be seen as a result of phenotypic evolution influenced by the selection pressure of hand weeding rice over a very long period. Barnyardgrass (E. crus-galli) is a highly competitive weed in rice because of large

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biomass and seed producing capability, one plant can produce up to 4000 seeds (Gibson *et al.*, 2002). Barnyardgrass can reduce 25% rice yield under high infestation condition (Chin, 2001). The majority of *Echinocloa* species are resistant to most herbicides used in rice and 43 of 127 reported cases of herbicide-resistant grasses were *Echinocloa* species (Heap, 2017).

MATERIALS AND METHODS

Seed Sampling and Weed Management Survey

The seeds of *E. crus-galli* were collected from 76 rice fields in seven provinces of Long An, Tien Giang, Vinh Long, Can Tho, Hau Giang, An Giang and Kiên Giang in the Mekong Delta of Vietnam. Seed sampling was conducted as described by Llewellyn and Powel (2001) and the minimum distance from each location was 10 km to ensure the diversity of sampled seed. A total of 40 to 50 florets of individual plants were collected at each field site, seeds were only collected when there were more than 20 plants bearing seeds within an area of 5000 m². Seeds were collected from plants located no less than 20 m from the border of the field. Seeds were stored at 25 to 28°C for one month before initiating for herbicide resistance determinations in a greenhouse. Seeds were treated with warm water at 45°C for 20 min to break dormancy, seeds were then planted in the greenhouse, the temperature was 26-29°C and day length was 14 h until seedling reached the 3 to 4 leaf stage.

We contacted the rice field owners to determine herbicide application history over the past 12 months. Information collected included field size, number of seasons per year rice was planted, water management, number of herbicide applications per season, handweeding practice, cost of herbicide and handweeding, The information was used to better understand possible factors that might explain herbicide resistance occurrence.

Herbicide Screening and Herbicideresistance Evaluation

The barnyardgrass seedlings at 3 to 4 leaf stage were treated with bispyribac-sodium (10% SC formulation) at 25 g a. i./ha,

penoxsulam (2.5% OD formulation) at 12.5 g a. i./ha and quinclorac (25% SC formulation) at 250 g a. i./ha. The experiment was designed as a randomized complete block with four replications, one pot per replication and 10 plants per pot. Herbicides were applied to the foliage using a spray application chamber (Research track sprayer SB-8, Devries Manufacturing), spray pressure was calibrated to deliver 300 l/ha at 140 kPa. At 14 days after treatment, per cent weed mortality was assessed. The herbicide-resistance level rating was based on per cent weed mortality in comparison to mortality of herbicidesusceptible (S) biotypes was 81-100% and R² was 72-80%, RR was 36-71% and RRR was 0-35% (Moss et al., 2007).

RESULTS AND DISCUSSION

Herbicide Application Practice and Weed Management in Rice Field of Mekong Delta

Rice cultivation and management practice: Weed samples were collected in wide area within seven provinces of Mekong Delta in intensive rice cultivation areas (Table 1) with an average of 2.7 crops season per year. An Giang averaged 2.3 crops per season as compared to three crops per season in Kiên Giang, Long An, Tien Giang and Vinh Long provinces. Mekong River water level during the flooding season (from August to November in Solar calendar or July to October in Lunar calendar) and levee or flood wall systems were the main factors affecting the number of rice crops per season. Provinces with large areas covered by levee systems were able to grow more than two rice crops per season, while areas affected by flooding were often limited to two or less rice crops per season. The average size of rice field in this study was 1.5 ha, and cultivated by one farmer and a uniform weed management practice was applied across the field. There were differences among field sizes in the provinces. Farmers in Long An and Kiên Giang had larger farm sizes than those in Vinh Long and Can Tho. The average farm size in Kiên Giang and Long An was 2.3 to 2.9 ha per field, while in Vinh Long and Can Tho the farm size was 0.7 to 0.9 ha. Field size was an important factor affecting weed management practices.

Herbicides provide an effective solution

Province	Season per year	Field size (ha)	Herbicide application season	Water management
An Giang	2.3c	1.6bc	2.16ab	2.5
Can Tho	2.5bc	0.9c	2.15ab	1.9
Hau Giang	2.7abc	1.1c	2.5ab	2.2
Kiên Giang	3.0a	2.3ab	2.5ab	2.2
Long An	3.0a	2.9a	2.7a	2.1
Tien Giang	3.0a	1.3c	2.0b	2.8
Vinh Long	3.0a	0.7c	2.1ab	2.2
Average	2.7	1.5	2.3	2.2
F	*	*	*	NS
C. V. (%)	14.5	46.4	25.4	29.7

Table 1. Herbicide management practice in rice field at Mekong Delta

NS: Not Significant. Water management score rating: 1: Easy, good land levelling and water supply is good, farmer can actively control the water level in the field; 2: Moderate, good land levelling and water supply, but soil texture is not good for standing water and 3: Difficult, uneven field surface, difficult to control water level in the field. Means followed by the same letter are not significantly different at P=0.05 (Tukey-Kramer multiple comparison procedure).

for weed management in Mekong Delta. On an average farmers apply herbicides 2.3 times per season with a range from 1 to 4 applications per season. Farmers in Long An do average 2.7 herbicide applications per season. The lowest herbicide application incidence was 2.0 times per season in Tien Giang. The most popular herbicide application included pre-emergent herbicide at 0-3 days after seeding (DAS) followed by post-emergent herbicide at 7-20 DAS and a late-post-emergent treatment at 20-30 days after seeding sometime used. In general, weed management by herbicide application at two times appeared to be most popular approach according to farmer interviews.

Weed management through water management was rated from 1 to 3 score (Table 1). The average score among farmers in the seven provinces was 2.2, which means water management in Mekong Delta was commonly used for weed management. The field in Tien Giang province got highest score (2.8) in water management, while the score for water management in Cân Tho was 1.9.

Removal of weeds by labourers at 20-30 days after seeding was assessed during interviews with farmers. About 80% of 61 farmers controlled weeds by having them removed by hand after the last herbicide application. The weeds were removed by field owners or by people from local villages. This manual removal was used to control all emerged weeds, no specific weed species were mentioned. All farmers interviewed in Vinh Long (15% of total 61 respondents) removed weeds by hand in their rice crops, while more

than 40% of farmers in Long An did not remove weeds by hand after herbicide application (Fig. 1).

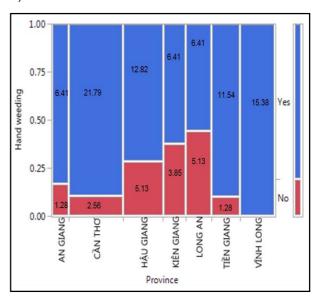


Fig. 1. Mosaic plot diagram of hand-weeding practice after herbicide application in 61 survey fields in Mekong Delta (Number in cell presented % of each segment of the total. In figure, red colour means there was no hand-weeding after herbicide application, blue colour means there was hand-weeding after herbicide application).

Weed Management Cost

The average cost for weed management in Mekong Delta was 2,740,000 VND per season (Table 2), which included 990,000 VND for herbicide use and 1,750,000 VND for removing weeds by hand. Weed management cost was 654,000 VND and 1,330,000 VND/ha for

Province	Cost for weed	Cost/ ha	Cost for herbicide/	Cost for herbicide/ha	Cost for hand-	Cost for hand-
	management	Па	season	nerbicide/ na	weeding/ season	weeding/ ha
Can Tho	2.05b	2.27ab	0.66bc	0.73ab	1.38	1.53ab
Hau Giang	1.93b	1.8b	0.89abc	0.82a	1.03	0.98b
Kiên Giang	2.99ab	1.34b	1.34ab	0.59ab	1.65	0.75b
Long An	4.11a	1.41b	1.39a	0.48b	2.72	0.93b
Tien Giang	3.36ab	2.94a	1.06abc	0.715ab	2.29	2.23a
Vinh Long	1.83b	2.35ab	0.51c	0.64ab	1.34	1.71ab
Average	2.74	1.99	0.99	0.65	1.75	1.33
F	*	*	*	*	NS	*
C. V. (%)	16.5	11.3	17.8	5.4	9.6	9.2

Table 2. The cost for weed management in Mekong Delta

Number presented as 1,000,000 VND. NS: Not Significant. Means followed by the same letter are not significantly different at P=0.05 (Tukey-Kramer multiple comparison procedure).

herbicides and removing weeds by hand, respectively. The cost for hand-weeding/ha was higher than the cost for the herbicide. The cost for hand-weeding in Tien Giang was 2,230,000 VND/ha, which was higher than in Kiên Giang province where the hand-weeding cost was 750,000 VND/ha. Availability of labour force in rural areas was an important factor that impacts the cost of hand-weeding. Most farmers in Tien Giang province mentioned the labour shortage in the local area, while the same issue was not mentioned commonly in Kiên Giang province. In addition, the difference in weed pressure, labour cost and tolerance level of farmer to weed infestations could explain differences. Farmers in Tien Giang were more aggressive in weed control compared to farmers in other locations. Complete control or acheiving a "weed-free field" was a popular expectation in this province, while some escaped weeds were acceptable in other provinces.

All interviewed farmers understood the importance of weed management. Farmer investment in weed control depended on level of weed infestation and family budget. They might accept weed control at different levels, but at least one herbicide application and one hand-weeding were minimum practice in provinces. Even when the cost for weed control management was high, most farmers would pay the cost. All farmers agreed that uncontrolled weed would cause a significant reduction in rice yield and the accumualtion of weed seed in the soil would cause more problems the next cropping season.

Herbicide-resistant Echinochloa crus-galli in Mekong Delta

The three herbicides of bispyribac, penoxsulam and quinclorac were most common herbicides for barnyardgrass control in the survey, asked farmers were rarely do the tankmixing for herbicide application, multiple applications at different timings were major practice. The R Rating system (S, R2, RR and RRR) described by Moss et al. (2007) was adopted for rating the herbicide-resistance level to bispyribac, penoxsulam and quinclorac in collected barnyardgrass samples (Table 3). Bispyribac-resistant E. crus-galli was found in 6 of 7 provinces, except in An Giang, where 100% population samples were susceptible to bispyribac. About 46% of collected barnyardgrass was classified as bispyribacresistant (RR) in Vinh Long. Bispyribacresistant populations were found in Kiên Giang and Can Tho where 30 and 35% of the sampled populations were RR. Similar to bispyribac, penoxsulam is an ALS inhibitor herbicide. Penoxsulam-resistant *E. crus-galli* was found. Several penoxulam-resistant (73%) samples were found in Hau Giang province. Penoxsulam-resistant barnyardgrass was reported in other provinces. About 23 to 36% of the barnyardgrass population samples were determined to be resistant to penoxsulm across all locations.

To evaluate herbicide-resistance level, we converted the "R Rating Scale" to "resistance-score" for calculating the herbicide-resistance level of bispyribac, penoxsulam and

Province Per cent of herbicide-resistant barnyardgrass population (RR and RRR) An Can Hau Kiên Long Tian Vinh Giang Tho Giang Giang An Giang Long 25.0 Bispyribac 0.0 12.5 5.8 0.00 14.2 16.7 Penoxulam 16.6 6.2 17.6 20.0 14.3 16.7 0.0 Quinclorac 33.3 31.2 11.7 20.0 14.3 16.7 0.0 Bis-Pen 16.6 0.0 35.2 0.00 0.0 16.7 50.0 Bis-Ouin 16.6 18.7 5.8 20.0 28.5 0.0 25.0 Pen-Quin 16.6 6.2 17.6 20.0 0.0 0.0 0.0 Bis-Pen-Quin 0.0 25.0 5.8 20.0 28.6 33.3 0.00 17.0 12 16.0 5.0 7.0 6.0 4.0 Per cent total sample 50 87.5 89.4 80.0 87.5 60.0 33.3

Table 3. Percentage of barnyardgrass population resistant to bispyribac, penoxsulam and quinclorac in different provinces

R: Rating scale (Moss *et al.*, 2007) based on control efficacy at label dose, where control efficacy of susceptible (S) was 81-100%; R² was 72-80%, RR was 36-71% and RRR was 0-35%.

quinclorac in sampled barnyardgrass populations. For resistance-score, the suspected-resistance population (R²) was equal to 1, confirmed-resistance (RR) equal to 2, and high-resistance (RRR) was equal to 3 (Table 4). The mean resistance-score for bispyribac, penoxsulam and quinclorac was not different among provinces in Mekong Delta. The average resistance-score was 3.4 for the herbicides in 78 samples of seven provinces.

Table 4. Herbicide resistance-score of bispyribac, penoxsulam and quinclorac in 78 barnyardgrass populations

Province	N	Resistance-score	Mean
An Giang	6	14d	2.3
Can Tho	19	72a	3.9
Hau Giang	14	54b	3.8
Kiên Giang	8	27c	3.4
Long An	9	41bc	4.7
Tien Giang	10	24c	2.3
Vinh Long	12	40bc	3.3
Total	78	272	-
F	_	*	NS
C. V. (%)	-	-	38.7

The resistance-score was calculated from total R in "R Rating Scale" results of all three herbicides, where S was 0, R^2 was 1, RR was 2, RRR was 3.

NS: Not Significant.

Impact of the Field Size and Water Management on Herbicide Resistance-score

The impact of field size on resistance-score of barnyardgrass was low under good and moderate water management condition, the correlation between two observations was unclear with R^2 ranging from 0.09 to 0.18 (Fig. 2); however, the correlation was stronger under poor water management with R^2 =0.21. Larger

fields under difficult water management condition were infested by more herbicideresistant *E. crus-galli*, because it was difficult for weed management under poor water mangement, and the large field size made it more of a challenge to control barnyardgrass. In general, good water coverage after herbicide application was an important factor for successful weed management program in rice field could help to suppress weed seed germination after herbicide treatment (Ranju *et al.*, 2014), therefore, reduced risk of herbicide-resistant barnyardgrass escape.

Correlation between Field Size and Handweeding Practice

Hand-weeding is an important practice in integrated herbicide management program (Herbicide Resistance Action Committee 2017). In our research, field size appeared to impact hand-weeding by farmers in the Mekong delta. Farmers with larger fields tended to do less hand-weeding compared to farmers with smaller fields (Fig. 3).

Thirty-one out of 76 farmers had fields greater than 1.5 ha. About 68% of those farmers would not do hand-weeding after the last herbicide application. The labour cost for handweeding larger fields appeared to be the main factor impacting the decision to hand-weed. The average expense for hand-weeding was 1,330,000 VND/ha over 650,000 VND for herbicide cost (Table 2). In addition, the handweeding cost for larger fields would be increased, and result in a decreased return by end of season.

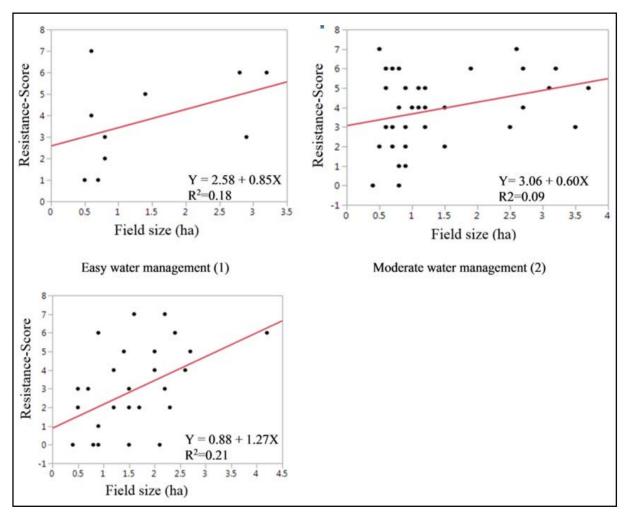


Fig. 2. Correlation between resistance-score and field size under different water management conditions.

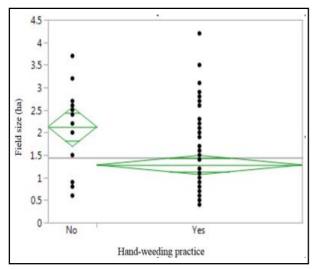


Fig. 3. One-way ANOVA t-test for field size and handweeding practice (The means diamond illustrates the mean with 95% confidence interval).

Impact of Hand-weeding on Herbicideresistance of Echinochloa crus-galli

The resistance-score for a field without hand-weeding was higher than a field with hand-weeding (Fig. 4). On an average resistance-score was 4.8 for 15 fields without hand-weeding compared to resistance-score of 3.1 for hand-weeded fields. Hand-weeding decreased the resistance-score of fields. Field size and hand-weeding were two factors impacting herbicide resistance-score (Figs. 3 and 4). More hand-weeding was applied in rice fields less than 1.5 ha in size. Herbicide-resistant barnyardgrass was also less common in small fields.

Hand-weeding after herbicide application of farmer was an important factor contributing to decrease or delay the herbicide resistance in rice fields. However, the cost for

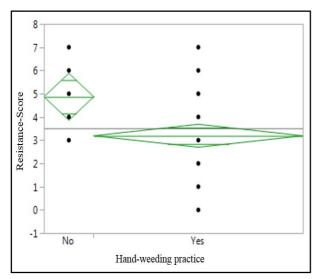


Fig. 4. One-way ANOVA t-test for the impact of hand-weeding to herbicide resistance in rice field (The means diamond illustrates the mean with 95% confidence interval).

this weed management practice was higher than the cost of herbicides and the labour cost increased with the decline in labour supply in rural areas. This phenomenon can be partially explained by the trend of younger people moving to urban areas in recent years (Vo et al., 2010; Duong and Nguyen, 2014). In study of Kieloch et al. (2017), the farm size was also identified as a factor affecting weediness decision, however, the small maize farm was infested with more weed than larger farm. It is opposite to the occurrence in the rice farm of this study. The difference may be due to the uneffective weed surpressing practices, especially the low-dose herbicide used in maize farm.

CONCLUSION

The trend of labour shortage in rice growing areas should be considered weed management program in the Mekong Delta, not only for *E. crus-galli* management but also for control of other weed species in rice fields. Weed management programmes that integrate herbicide products, mechanical methods and crop rotation system need to be implemented to better mangement evolution of herbicide resistance in rice cropping regions.

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