Australian Journal of Crop Science

AJCS

AJCS 4(2):107-115(2010) ISSN:1835-2707

Study on resistant biotypes of *Echinochloa crus-galli* in Malaysia

***M. Motior Rahman[1], Ismail Bin Sahid[2] and Abdul Shukor Juraimi[3]**

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

[2]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

[3]Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor DE, Malaysia

***Corresponding author: mmotiorrahman@yahoo.com**

Abstract

Present study was carried out to identify resistant biotypes of *Echinochloa crus-galli* and to determine their control measures by application of herbicides. Propanil, quinclorac and cyhalofop-butyl was tested against 10 populations of *Echinochloa crus-galli* which was collected from rice fields of Kedah, Malaysia. Weed populations such as KPT, SB1, SB2, KPE, SY, TD, DB, KB1 and KB2 was identified as resistant biotypes and only KP population recorded as susceptible biotype to propanil. Out of 10 populations, only KB1 was found resistant biotypes and all others identified as susceptible to quinclorac. Weed populations of KP, KPT, SB1, KPE, SY and KB2 was found susceptible while SB2, TD and KB1 identified as resistant biotypes to Cyhalofop-butyl. Among 10 tested populations, only KB1 was found resistant biotype to propanil, quinclorac and cyhalofop-butyl. The ED_{50} values from the dose-response experiments indicated that the resistant biotype was >four times resistant to propanil, >10 times resistant to quinclorac and >17 times resistant to cyhalofop-butyl, respectively than susceptible biotype. Resistant biotype KB1 was controlled by combined application of quinclorac and propanil at rates of 0.30 and 5.50 and kg a.i. ha^{-1} or quinclorac and cyhalofop-butyl at rates of 0.30 and 0.80 kg a.i. ha^{-1}, respectively.

Keywords: *Echinochloa crus-galli*, propanil, quinclorac, cyhalofop-butyl, barnyardgrass Malaysia.

Introduction

Rice (*Oryza* spp.) is an important staple food and is consumed by nearly one-half of the global population (Fageria and Baligar, 2003). Transplanting of rice seedlings is the traditional way of rice cultivation in many Asian countries. Increasing labor costs, scarcity of irrigation water and more recently developed agricultural technology have resulted in a general shift in rice production systems from transplanted rice to direct seeded rice (Tabbal et al., 2002; Tomita et al., 2003; Savary et al., 2005). In addition, the direct-seeding, or no tillage of rice fields can also prevent soil erosion of rice field (Gomez et al., 2004; Lal, 2004). As a consequence, in rice growing countries, such as Malaysia, Vietnam, and South Korea, the extensive adoption of the direct-seeding technique for rice has coincided with the increased occurrence of weedy rice (Pyon et al., 2000; Watanabe et al., 2000) and barnyardgrass in rice field. Direct seeding of rice is the predominant crop establishment technique in the granary areas in Malaysia since 1970 and thereafter expanded rapidly and covered about 90% area by 2000 (Azmi et al., 2007). Direct-seeded rice allows weeds to germinate and establish with the crop thus compete well for resources. The adoption of semi-dwarf cultivars, high N fertilization, wet-seeded rice and shallow flooding has increased weed growth and competition. Weeds have always been recognized as one of the major constraints on yield and quality of rice and a significant pest problem in temperate rice culture (Ioannis and Kico, 2005) which can reduce rice yields by competing for moisture, nutrients, and light during the growing season. Weed seed contamination of rice grain lowers grain quality and may lower the cash value of the rice crop. Effective weed control is one of the major requirements to ensure a successful wet land rice production (Azmi and Mortimer, 2000). Herbicides have been used intensively in Malaysian rice crops for the last few decades. *Fimbristylis miliacea* and *Spehenoclea zeylandica* has evolved resistance to synthetic auxins (quinclorac) since 1989 and 1995, respectively while *Echinochloa (E) crus-galli* have evolved resistance to propanil due to repeated use of amides (propanil) in many countries of the world including Malaysia (Azmi et al., 2007). Reliance upon herbicides as the primary method of weed control in cropping systems is understandable but repeated use of the same herbicide or group of herbicides with the same mode of action in the

Table 1. *E. crus-galli* seeds collected from Kedah, Malaysia

Name of the location with abbreviated form	
Kampung Permetang - KP	Singkir Yan - SY
Kampung Pida-Tiga - KPT	Tg. Dawai - TD
Sungai Baru - SB1	Dulang Besar - DB
Sungai Baru - SB2	Kampung Bahagia - KB1
Kampung Pida-Empat - KPE	Kampung Bahagia - KB2

same fields may lead to consequential occurrences of herbicide resistance rice fields. About 18 herbicide resistance weed species are identified from Malaysian field of Agriculture (Azmi and Baki, 2002) but little information is available on rice fields weed species which is herbicides resistance. Many weed species available in the rice field could not control due to improper use, rates and methods of application of herbicides. *E. crus-galli*, a weed that is found primarily in direct-seeded rice fields can cause almost total loss of field yields and is the most frequently reported weed in many countries including Malaysia (Azmi and Mortimer, 2000). *E. crus-galli* is prolific annual grass weed that is widely distributed in the tropics and in most of the regions of culture of the rice in the world (Marambe and Amarasinghe, 2002) and it is of great concern to control for maximizing rice production in Malaysia. Therefore, the present investigation was undertaken to identify herbicide R biotypes of *E. crus-galli* and to suggest controlling method of R biotypes by using optimum rates of herbicides application.

Materials and Methods

Echinochloa crus-galli seeds were collected from 10 random locations of rice granary area Muda irrigation scheme, Kedah (6°20'N, 100°22'E), Malaysia. Weed population was derived from five patches into rice fields in the farmer's fields where different kinds of herbicides were applied since 1990. *E. crus-galli* seeds collected from different locations are abbreviated and presented in Table 1. Collected seeds were kept in tight bottles in Weed Science Laboratory, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM). Germination test was done in the laboratory to ensure success of germination. About 2-3 days were required to germinate weed seeds under laboratory while 4-5 days needed in plastic pots, respectively. A series of experiments were carried out in the green house of UKM. Soil samples used in the present study was collected from rice field of Malaysian Agricultural Research and Development Institute, Research Station, Bertam, Pulau Penang, Malaysia. The soil was air-dried and sterilized using autoclave. Experiments were conducted using 15- by 20-cm plastic pots filled with 500 g of clay loam soil. Pots were placed in the greenhouse, where natural light was more than 13-h. Day and night temperatures were 27 to 31^{0}C and 20 to 24 ^{0}C, respectively. No fertilizer was applied during study period. Screening experiment was conducted to determine the R and S biotypes of *E. crus-galli* thereafter R biotypes were tested against different concentration of propanil (zepronex ®) 35 % w/w; quinclorac (facet®) 21.9 % w/w; cyhalofop-butyl (clincer®) 10.1% w/w. Further investigation was carried out to determine the levels and methods of herbicide application to control R biotypes. In all experiments, about 200 seeds from each population were sown per pots at a depth of 1-2 mm into the soil to ensure germination. The pots were saturated with normal irrigation water up to field capacity for proper germination and growth of plants. Seven days after emergence (DAE), three weed seedlings were transferred to each experimental pot as per treatment schedule.

Screening of R and S biotypes of E. crus-galli

Screening experiments of *E. crus-galli* over 10 populations was tested against propanil, quinclorac and cyhalofop-butyl. A factorial arrangement of treatments (*E. crus-galli* biotypes by herbicide treatment) in a completely randomized design with three replications was used. A non-treated control was included for comparison. Recommended and double rates of each herbicides such as propanil at rates of 5.50 and 11.00 kg a.i. ha^{-1}, quinclorac at rates of 0.30 and 0.60 kg a.i. ha^{-1} and cyhalofop butyl at rates of 0.80 and 1.60 kg a.i. ha^{-1} were tested against 10 populations. Seedlings of all populations were screened at 28 days after transplanting (DAT) to observe resistant to propanil, quinclorac and cyhalofop-butyl at recommended and double rates in order to confirm resistance or susceptibility. Plant growth was monitored by recording fresh weight of above ground plant tops at the time of harvest (42 DAE). After harvesting of weeds, above ground shoots were washed in water and soaked with tissue paper and then recorded of fresh weight. All weed populations were harvested for fresh weight yield. Visual assessments were done as a suitable alternative and are certainly much quicker than weight assessments. Visual assessment was performed on spot evaluation by eye estimation. Assessment of herbicide activity was determined by foliage fresh weight and visual assessment by scoring plant mortality and

Table 2. Visual evaluation indices of *E. crus-galli*

Physical status of weed plants	Visual Score	Remarks
Green shoot and leaves	1	Highly resistant
Green shoot and leaves light green colour	2	Resistant
Green shoot and pale yellow colour leaves	3	Partial resistant
Partial control (dead)	4	Susceptible
Completely control (dead)	5	Strongly susceptible

Table 3. Visual scoring of *E. crus-galli* populations as affected by herbicides

Source of weed population	Herbicides (kg ha^{-1}) Propanil		Quinclorac		Cyhalofop-butyl	
	†5.50	††11.00	†0.30	††0.60	†0.80	††1.60
KP	4	5	4	5	4	5
KPT	1	2	4	5	4	5
SB 1	1	2	4	5	5	5
SB 2	2	3	4	5	1	2
KPE	2	2	4	5	1	5
SY	2	2	4	5	4	4
TD	2	2	5	5	2	3
DB	1	1	5	5	2	5
KB 1	1	1	2	2	2	5
KB 2	1	1	5	5	4	5

†-recommended rate; ††-double rate

Scoring indices:

1 = green shoot and leaves; 2 = green shoot and leaves light green colour; 3 = green shoot and pale yellow colour leaves; 4 = partial control (dead) and 5 = completely control (dead)

greenness of shoot and leaves. The assessment was accredited by numerical score. The highest and lowest score indicated maximum weed plant mortality and greenness of shoot and leaves colour, respectively. Visual score indices are presented in Table 2.

Dose response of herbicide on R biotypes of E. crus-galli

The seeds were selected from the identified resistant (R) and susceptible (S) biotypes as confirmed by screening test and then germinated separately as described in screening experiment. The biotypes KB1 and KP were selected as R and S against propanil, quinclorac and cyhalofop-butyl by both recommended and double rates, respectively. Propanil at rates of 0, 1.375, 2.75, 5.50 (recommended rate), 11.00, 22.00 and 44.00 kg a.i. ha^{-1}; quinclorac at rates of 0, 0.075, 0.15, 0.30 (recommended rate), 0.60, 1.20 and 2.40 kg a.i. ha^{-1} and cyhalofop-butyl at rates of 0, 0.20, 0.40, 0.80 (recommended rate), 1.60, 3.20 and 6.40 kg a.i. ha^{-1} were tested against both KB1 and KP. A factorial arrangement of treatments (KB1 and KP biotypes by herbicide treatment) in a completely randomized design with three replications was used. Both biotypes were sprayed with propanil, quinclorac and cyhalofop-butyl at 35 DAG. The above-ground living tissue remaining on each plant was harvested and weighed at 7-d after spraying (42 DAG). Fresh weight data was used to check the accuracy of the visual assessments and the consistency of results between subsequent assays. For the susceptible standard in single dose assays, aim to achieve an 80-95 % reduction in foliage fresh weight was considered (Moss, 1999). Both a highly resistant (expected 0-5% reduction) and partially resistant (about 50 % reduction in foliage fresh weight) standards were included in this study.

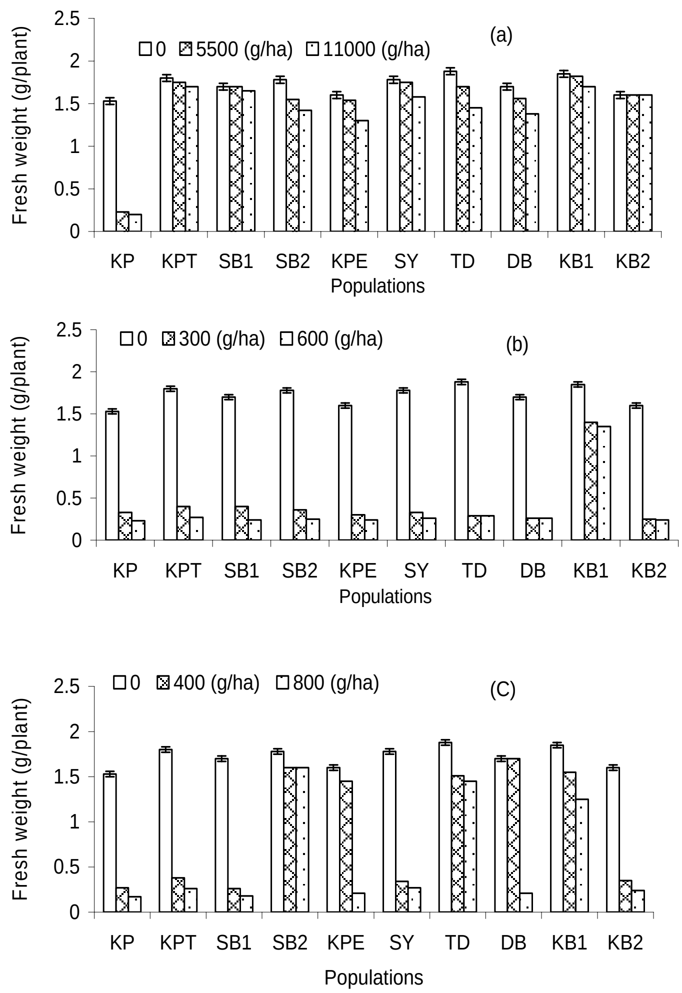

Fig. 1. Shoot fresh weight as affected by (a) propanil, (b) quinclorac and (c) cyhalfop-butyl (Error bar indicates LSD at 0.01)

Table 4. Regression equation and coefficients of determination (R^2) for the relationship between herbicides concentration and shoot fresh weight of resistant KB1 and susceptible KP biotypes.

Herbicides	Regression equation [a]	R^2
Propanil for R biotype	$Y = 2.4106 - 0.0841X + 0.0011X^2$	0.9748
Propanil for S biotype	$Y = 1.9204 - 0.1603X + 0.0028X^2$	0.7615
Quinclorac for R biotype	$Y = 2.0508 - 1.2837X + 0.3139X^2$	0.9342
Quinclorac for S biotype	$Y = 1.5237 - 2.3133X + 0.7495X^2$	0.6359
Cyhalofop-butyl for R biotype	$Y = 1.9761 - 0.8462X + 0.0899X^2$	0.9545
Cyhalofop-butyl for S biotype	$Y = 1.6081- 0.9141X + 0.1101X^2$	0.7772

[a]Y, shoot fresh weight; X, herbicides in kg ha^{-1}

Effects of herbicides on R biotype of E. crus-galli

For control measure of R biotype of KB1, further experiment was carried out with recommended and double rates of propanil (5.50, 11.00 kg ha^{-1}), quinclorac (0.30, 0.60 kg ha^{-1}) and cyhalofop-butyl (0.80, 1.60 kg ha^{-1}) singly or in combination, respectively. Non treated plant was also used as check. The experiment was carried out under completely randomized design with four replications. Plants were sprayed with the appropriate concentration of each herbicide at 35 DAG. Seven days after spraying, the above-ground living tissue remaining on each plant was harvested (42 DAE) and weighed.

Statistical analysis

The shoot fresh weight was plotted against the propanil, quinclorac and cyhalofop-butyl rate in order to determine the propanil, quinclorac and cyhalofop-butyl dose that caused a 50% reduction in shoot fresh weight (ED_{50}). A log logistic model (Seefeldt et al., 1995) was fitted to estimate the ED_{50} and to compare the susceptibility of the biotypes to the herbicides. The dose was expressed in kg ha^{-1}. The data were analyzed using a Statistical Analysis System (SAS, 1999). Following the analysis of variance procedures, differences among treatment means were determined using the LSD comparison method.

Results and Discussion

Screening of R and S biotypes of *E. crus-galli*

Visual assessment showed that weed plants grown from DB, KB1 and KB2 populations scored lowest value (1) by use of propanil at rates of 5.50 (recommended) and 11.00 (double rates) kg ha^{-1}. Plants grown from KPT and SB populations scored 1 and 2 by using propanil at rates of 5.50 and 11.00 kg ha^{-1}, respectively. Plants grown from seeds collected from KPE, SY and TD populations scored 2 at rates of 5.50 and 11.00 kg ha^{-1} of propanil. KP population scored 4 and 5 by using propanil at rates of 5.50 and 11.00 kg ha^{-1}, respectively. Plants grown from seeds collected from all locations except KB1 obtained maximum score (4 to 5) by application of quinclorac at both 0.30 and 0.60 kg ha^{-1} (Table 3). Weed

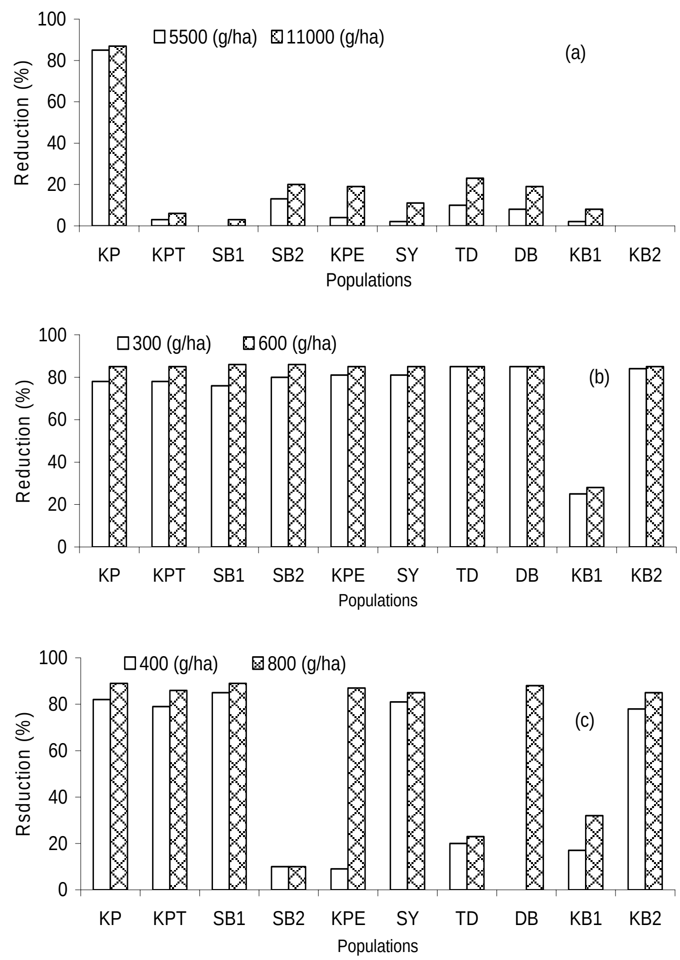

Fig. 2. Shoot fresh weight reduction as affected by (a) propanil, (b) quinclorac and (c) cyhalfop-butyl

plants grown from seeds collected from SB2 scored 1 and 2 by application of cyhalofop-butyl at rates of 0.80 and 1.60 kg ha^{-1}, respectively. On the other hand DB and KB1 populations treated with Cyhalofop-butyl scored 2 and 5 at both rates. Plants grown from seeds collected from KP, KPT, SB1, SY and KB2 populations scored 4 and 5 by rates of 0.80 and 1.60 kg ha^{-1} of Cyhalofop-butyl, respectively (Table 3). Fresh weight was influenced by the application of herbicides. Fresh weight ranged from 1.53 to 1.88 g $plant^{-1}$ in non treated control

Table 5. ED_{50} estimates from dose response curves for *E. crus-galli* shoot fresh weight

Herbicides	ED_{50} (kg a.i. ha^{-1}) R	ED_{50} (kg a.i. ha^{-1}) S	Resistance Index
Propanil	11.27	2.40	4.70
Cyhalofop-butyl	1.22	0.07	17.43
Quinclorac	0.95	0.09	10.56

plants. Maximum fresh weight was obtained by the plants grown from seeds collected from TD and KB1 control populations. The lowest fresh weight was obtained by plants grown from seeds collected from KP population of control treatments (Fig. 1).

Propanil treated plants produced fresh weight ranged from 0.20 to 1.82 g $plant^{-1}$. The highest and lowest fresh weight was obtained by the plants grown from seeds collected from KB1 and KP populations followed by KPT population. Intermediate fresh weight was obtained from the plants grown from seeds collected from other populations (Fig. 1a).

The highest reduction (85-87%) of shoot fresh weight was recorded from plants grown from seeds collected from KP population (Fig. 2a). The application of propanil interrupts the photosynthetic electron transport chain in photosynthesis and thus blocks the ability of the plant to turn light energy into chemical energy. As a result fresh weight was reduced drastically by the application of propanil (Daniell et al., 2006). Similar results were found by (Ioannis and Kico, 2005) and they reported that the shoot fresh weight of barnyard grass biotype was reduced by 78 and 85% by application of propanil at the rate of 10.4 kg ha^{-1}. Susceptible and R biotypes were identified based on combined evaluation of fresh weight, reduction of shoot fresh weight and visual score. Plants populations of KPT, SB1, SB2, KPE, SY, TD, DB, KB1 and KB2 recorded greater fresh weight, minimum reduction of shoot fresh weight and lowest visual score suggested that all are considered highly R biotypes to propanil. Only KP population obtained lower fresh weight, highest visual score and maximum reduction rate of shoot fresh weight indicated S to propanil. Quinclorac treated plant population of KB1 produced the highest fresh weight (1.35-1.40 g $plant^{-1}$) and all other populations obtained poor fresh weight compared to control (Fig. 1b).

Reduction of shoot fresh weight over control plants ranged from 25-86% and reduction of shoot fresh weight was remarkably higher in all populations except KB1 (Fig. 2b). Considering tested parameters such as lower fresh weight, maximum reduction of shoot fresh weight and highest scored by visual assessments of plants grown from seeds collected from all locations except KB1 population indicated S biotypes to quinclorac. Plants population of KB1 identified as R biotype to quinclorac resulting higher fresh weight, minimum reduction of shoot fresh weight over control plants and lower score index (Figs. 1b and 2b).

Weed plants treated by cyhalofop-butyl at rates of 0.80 and 1.60 kg ha^{-1} obtained 0.17 to 1.70 g $plant^{-1}$fresh

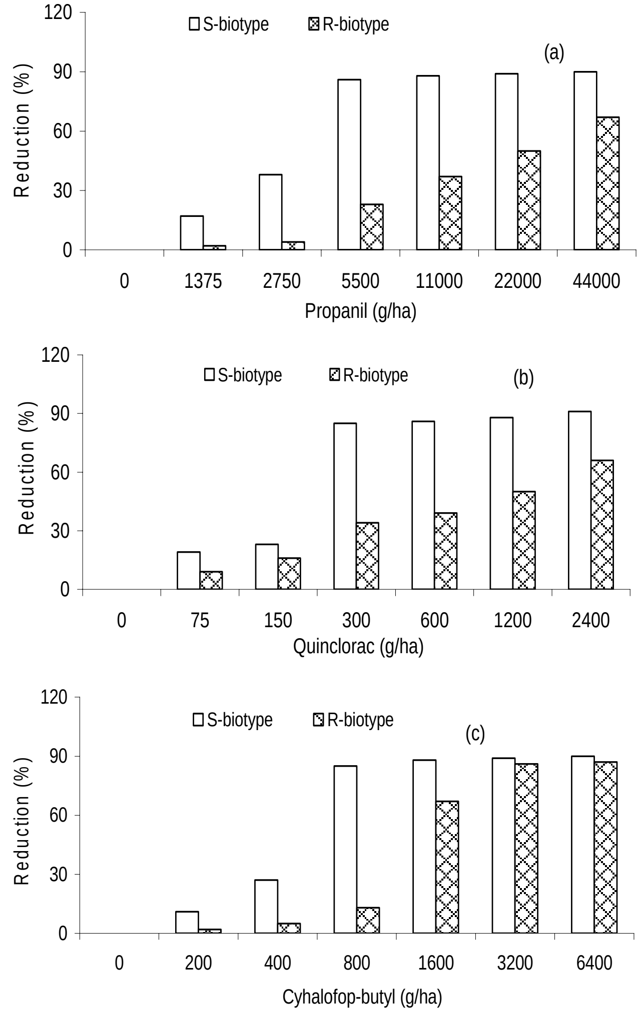

Fig. 3. Shoot fresh weight reduction of KB1 R biotype as affected by propanil, quinclorac and cyhalofop-butyl

weight. Plant population of DB population with cyhalofop-butyl at the rate of 0.80 kg ha^{-1} and SB2 population by both rates (0.80 and 1.60 kg ha^{-1}) produced higher fresh weight followed by TD and KPE, KB1 plant population at the rate of 0.80 kg ha^{-1}, respectively. Plant populations of DB and KB1 produced higher and lower fresh weight at rates of 0.80 and 1.60 kg ha^{-1} of cyhalofop-butyl, respectively. Plant populations of KP, KPT, SB1, SY and KB2 recorded poor fresh weight by both rates of cyhalofop-butyl (Fig. 1c). Reduction in the shoot fresh weight over control plants was also higher in SB1 population followed by KP, KPT, SY and KB2 population. Considering lower fresh weight with higher visual scoring and maximum reduction of shoot fresh weight, KP, KPT, SB1, SY and KB2 populations indicated S biotypes while higher fresh weight, lower scoring indices and minimum reduction of shoot fresh weight of SB2, KPE, TD, and KB1 populations indicated R biotypes to cyhalofop-butyl, respectively (Fig. 2c). DB considered R only at the rate of 0.80 kg ha^{-1}. Screening experiments showed that above ground fresh weight was

Table 6. Effect of herbicides on the fresh weight and shoot reduction of R biotype

Herbicides rate (kg ha^{-1}) (R – recommended rate; D – double rate)	Shoot fresh weight (g)	Shoot reduction (%)
Control	1.87	0
Propanil - 5.50 kg ha^{-1} R	1.82	2
Propanil - 11.00 kg ha^{-1} D	1.70	8
Quinclorac - 0.30 kg ha^{-1} R	1.40	25
Quinclorac - 0.60 kg ha^{-1} D	1.35	28
Cyhalofop-butyl 0.80 kg ha^{-1} R	1.55	17
Cyhalofop-butyl 1.60 kg ha^{-1} D	1.25	32
Propanil - R + Quinclorac - R	0.26	86
Propanil - R + Cyhalofop-butyl - R	1.31	30
Quinclorac - R + Cyhalofop-butyl - R	0.24	87
Propanil - R + Quinclorac - R + Cyhalofop-butyl - R	0.19	90
Propanil - D + Quinclorac - D	0.22	88
Propanil - D + Cyhalofop-butyl – D	0.26	86
Quinclorac - D + Cyhalofop-butyl - D	0.15	92
Propanil - D + Quinclorac - D + Cyhalofop-butyl - D	0.11	94
LSD 0.01	0.15	-

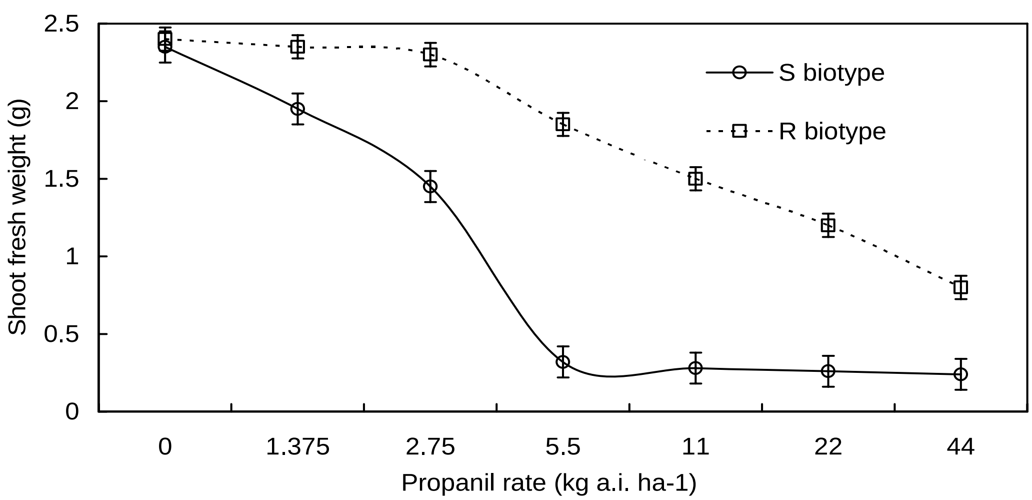

Fig. 4. Effects of propanil concentrations on the shoot fresh weight of E. crusgalli

directly associated with visual assessment scoring. Resistant and susceptible biotypes were identified based on possessing superior shoot fresh weight and visual scoring.

Dose response of R biotype to propanil, quinclorac and cyhalofop-butyl

There was a general decrease in shoot fresh weight of both R and S biotypes as the propanil concentration increased, but the S biotype appeared to have more rapid decline in shoot fresh weight compared to the R biotype. However, at recommended rates of propanil (5.50 kg ha^{-1}), only S biotype had > 85% reduction in shoot fresh weight (Fig. 3a). Furthermore, the decreased shoot fresh weight of S population with the application of propanil at the rate of 5.50 kg ha^{-1} did not differ significantly (P>0.05) from the rate of 11.00 kg ha^{-1}, suggesting no conspicuous difference in phytotoxicity of propanil at the rate of 5.50 and 11.00 kg ha^{-1}. At the highest dose of propanil (44.00 kg ha^{-1}), S population had 90% mortality, whereas R population had 67% mortality (Fig. 3a).

Only S biotype had 85% reduction in shoot fresh weight by quinclorac at the rate of 0.30 kg ha^{-1}. Furthermore, the decreased shoot fresh weight of S biotype with the application of quinclorac at the rate of 0.30 kg ha^{-1} did not differ significantly (P>0.05) at the rate of 1.20 kg ha^{-1}, suggesting no visible difference in phytotoxicity of quinclorac at the rate of 0.30 and 1.20 kg ha^{-1}. At the highest dose of quinclorac (2.40 kg ha^{-1}), S biotype had 91% mortality, whereas R biotype had 66% mortality (Fig. 3b). The shoot fresh weight of S biotype was reduced significantly at the recommended rate of quinclorac but R biotype showed resistant even higher rate of quinclorac (>2.40 kg ha^{-1}). The shoot fresh weight of S biotype with the application of cyhalofop-butyl at the rate

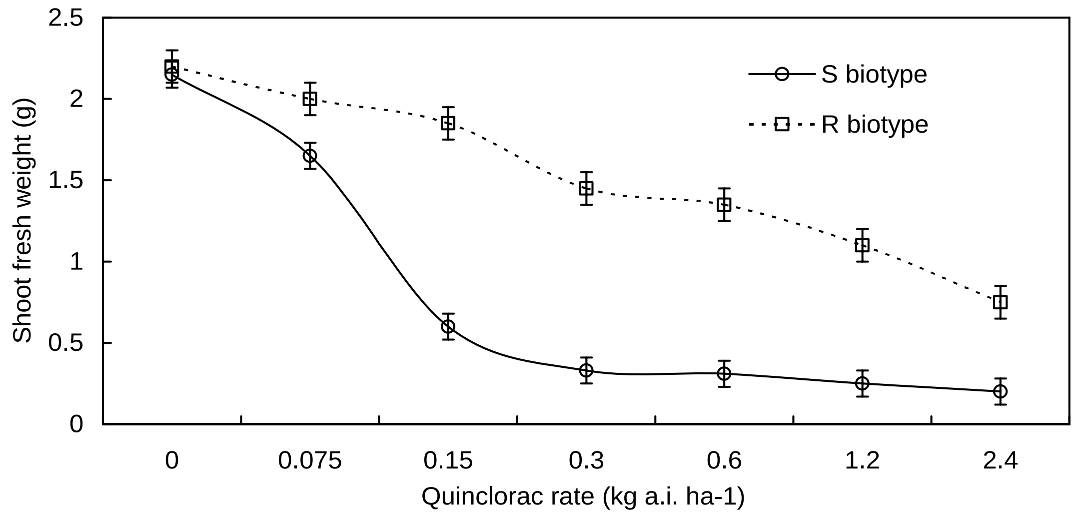

Fig. 5. Effects of Quinclorac concentrations on the shoot fresh weight of E. crus-galli

of 0.80 kg ha^{-1} did not differ significantly (P>0.05) at the rate of 1.60 kg ha^{-1}, indicating invisible difference in phytotoxicity of cyhalofop-butyl at rates of 0.80 and 1.60 kg ha^{-1}. Only S biotype had 85% reduction in shoot fresh weight at recommended rates (0.80 kg ha^{-1}) of cyhalofop-butyl (Fig. 3c). Susceptible biotype was susceptible at the recommended rate of cyhalofop-butyl while R population was also susceptible at the rate of 3.20 kg ha^{-1} and onwards.

Regression analysis of *E. crus-galli* fresh weight response to propanil, quinclorac and cyhalofop-butyl indicated that the quadratic equation (Y, % of control; X, kg ha^{-1}) provided the best fit (Table 4). Comparison of the slopes of the regression equations for fresh weight shows that the KB1- R biotype had the less shoot fresh weight reduction rate, whereas the KP- S biotype had the rapid shoot reduction rate in all herbicides. These results indicate clearly that the resistant biotype had the highest growth rate. Similar results were reported by Ioannis et al., (2000), who found that the propanil-resistant biotypes of barnyardgrass had greater growth rate than a susceptible biotype. Fischer et al., (1993) reported that the propanil-resistant biotypes of junglerice had greater leaf area and dry matter accumulation and were taller than a susceptible biotype. In contrast, Radosevich and Holt (1984) reported that barnyardgrass biotypes resistant to photosynthesis-inhibiting herbicides had reduced ecological fitness compared with susceptible biotypes. The dose response curves (Fig. 4) show that the resistance index (RI), that is, the ratio between ED_{50}-R (11.27) and ED_{50}-S (2.40) is 4.7 (Table 5), indicating that the R biotypes is more than 4-fold resistant to propanil than the S biotype. In case of quinclorac application, the dose response curves (Fig. 5) show that the RI, that is, the ratio between ED_{50}-R (0.95) and ED_{50}-S (0.09) is 10.56 (Table 5), indicating that the R biotypes is more than 10-fold resistant to quinclorac than the S biotype. Similarly in the dose response curves of cyhalofop-butyl (Fig. 6) show that the RI, that is, the ratio between ED_{50}-R (1.22) and ED_{50}-S (0.07) is 17.43 (Table 5), indicating that the R biotypes is more than 17-fold resistant to cyhalofop-butyl than the S biotype. ED_{50} values (herbicides concentrations that reduced shoot length by 50% relative to untreated controls), derived from nonlinear regression analysis, indicated six levels of response to propanil, quinclorac and cyhalofop-butyl among R and S populations, respectively. The ED_{50} values varied from 2.40 to 11.27 kg ha^{-1} propanil, 0.07 to 1.22 kg ha^{-1} cyhalofop-butyl and 0.09 to 0.95 kg ha^{-1} for the most susceptible to the most resistant populations, respectively.

Effects of herbicides on R biotype of E. crus-galli

Shoot fresh weight was affected significantly by the application of different herbicides. Propanil, quinclorac and cyhalofop-butyl by both recommended and double doses were not effective to reducing shoot fresh weight (Table 6). Combined application of propanil +quinclorac at recommended rates and quinclorac+cyhalofop-butyl at ecommended rates were effective to reduce shoot fresh weight while recommended rate of propanil+cyhalofop-butyl did not show full potential to reduce shoot fresh weight of R biotype (Table 6). Baldwin et al., (1995) reported that propanil formulations tank-mixed with quinclorac, thiobencarb or pendimethalin were very effective for controlling resistant and susceptible biotypes when applied post-emergence while quinclorac and mixtures of quinclorac with pendimethalin and thiobencarb were very effective when applied pre-emergence. Resistant and susceptible biotypes were controlled in rotational crops by trifluralin, pendimethalin, metolachlor, alachlor, dimethenamid, clomazone and the post emergence graminicides such as clethodim. Resistant barnyard grass biotypes were not controlled with 6.0 to 8.0 kg ha^{-1} of propanil when applied at the

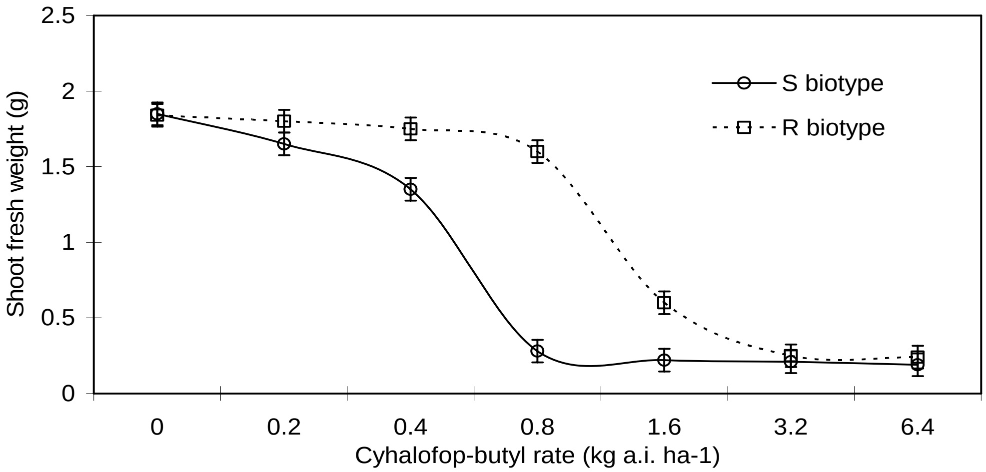

Fig. 6. Effects of Cyhalofop-butyl concentrations on the shoot fresh weight of E. crus-galli

two to three leaf stage in Arkansas (Baltzar and Smith, 1994). Ioannis et al., (2000) also found that propanil at rates of 2.6 or 5.2 kg ha^{-1} was not effective to control resistant biotypes of barnyard grass. Our findings confirms with the findings of Jordan (1997), Baltzar and Smith (1994), Crawford and Jordan (1995). They observed that propanil applied in combination with thiobencard, pendimethalin, molinate or quinclorac controlled more propanil-resistant barnyard grass than the same rate of propanil applied alone. Therefore, the results revealed that recommended rates of propanil+quinclorac or quinclorac+cyhalofop-butyl is suggested to control R biotype of *E. crus-galli*.

Conclusion

The reduction of shoot fresh weight accompanied with visual assessment was a good indicator to identify resistant and susceptible biotype. Out of 10 populations of *E. crus-galli*, only KB1 was found R biotype to propanil, quinclorac and cyhalofop-butyl. The ED_{50} values from the dose-response experiments suggested that the R biotypes was more than four times resistant to propanil, 10 times resistant to quinclorac and 17 times resistant to cyhalofop-butyl, respectively than susceptible biotypes. The study revealed that combined application of quinclorac and propanil at rates of 0.30 and 5.50 kg ha^{-1} or quinclorac and cyhalofop-butyl at rates of 0.30 and 0.80 kg a.i. ha^{-1} was found effective to control KB1 resistant biotype of *Echinochloa crus-galli*, respectively.

Acknowledgement

We thank IRPA 01-02-04-0778 PR 0068/05-05 and MOSTI, Malaysia for their financial help to conducting this research task.

References

Azmi M, Baki BB (2002) Impact of continuous direct seeding rice culture on weed species diversity in the Malaysian rice ecosystem. In: Omar R, Ali RZ, Latif MT, Lihan T, Adam JH (eds) Proceedings of the regional symposium on environment and natural resources, Kuala Lumpur, 10-11 April 2002. 1:61-67

Azmi M, Chin DV, Vongsaroj P, Johnson DE (2007) Emerging issues in weed management of direct-seeded rice in Malaysia, Vietnam, and Thailand. Adv Agron 93:196-198

Azmi M, Mortimer M (2000) Weed species shift in response to serial herbicide application in wet-seeded rice in Malaysia. Pandey S et al., (eds) Direct Seeding Research Strategies and Opportunities. IRRI, Philippines, pp. 357-367

Baldwin FL, Talbert RE, Carey VF, Kitt MJ, Helms RS, Black HL, Smith RJJr (1995) A review of propanil resistant *Echinochloa crus-galli* in Arkansas and field advice for its management in dry seeded rice. Brighton crop protection conference: weeds. Proceedings of an international conference, Brighton, UK, 20-23 November 1995. 2: 577-585

Baltazar AM, Smith RJJr (1994) Propanil-resistant barnyardgrass (*Echinochloa crus-galli*) control in rice (*Oryza sativa*). Weed Tech 8:576-581

Crawford SH, Jordan DL (1995) Comparison of single and multiple applications of

propanil and residual herbicides in dry-seeded rice (*Oryza sativa*). Weed Technol 9:153-157

Daniell H, Sarojini G, Kumarachinnayan P, Kulandaivelu G (2006) Action of propanil on in vivo

chlorophyll a fluorescence in Echinochloa crus-galli and rice. Weed Res 21(3-4):171 - 177

Fageria NK, Baligar VC (2003) Upland rice and allelopathy. Commun Soil Sci Plant Anal 34:1311–1329

Fischer AJ, Granados E, Trujillo D (1993) Propanil resistance in populations of junglerice
(*Echinochloa colona*) in Colombian rice fields. Weed Sci 41:201–206

Gomez JA, Romero P, Giraldez JV, Fereres E (2004) Experimental assessment of runoff and soil erosion in an olive grove on a Vertic soil in southern Spain as affected by soil management. Soil Use Manag 20:426–431

Ioannis BV, Ilias GE, Kico VD (2000) Propanil-Resistant Barnyardgrass (*Echinochloa crus-galli*) Biotypes Found in Greece. Weed Technol 14(3):524-529

Ioannis V, Kico D (2005) Red rice (*Oryza sativa* L.) and barnyardgrass (*Echinochloa*
spp.) biotype susceptibility to postemergence-applied imazamox. Weed Biol Manag 5:46-52

Jordan DL (1997) Efficacy of reduced rates of quinclorac applied with propanilor propanil plus molinate in dry-seeded rice (*Oryza sativa*). Weed Sci 45:824-828

Lal R (2004) Carbon sequestration in soils of central Asia. Land Degrad Dev 15:563–572

Marambe B, Amarasinghe L (2002) Propanil-resistant barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] in Sri Lanka: Seedling growth under different temperatures and control. Weed Biol Manag 2(4):194-199

Moss S (1999) Detecting herbicide resistance. Guidelines for conducting diagnostic tests and interpreting results. http://www.plantprotection.org/HRAC/

Pyon JY, Kwon WY, Guh JO (2000) Distribution, emergence, and control of Korean weedy rice. In: Proceedings of Wild and Weedy Rice in Rice Ecosystems in Asia – A Review (Ho Chi Minh City, Vietnam, 10–11 August 1998). International Rice Research Institute, Los Banos, the Philippines, pp. 37–40

Radosevich SR, Holt J (1984) Weed Ecology, Implications for Vegetation Management. Chapter
6. Wiley J (ed), New York pp. 195–241

SAS Statistical Analysis Systems (1999) SAS/STAT user's guide. Version 8.1, Cary, NC. Statistical Analysis System Institute

Savary S, Castilla NP, Elazegui FA, Teng PS (2005) Multiple effects of two drivers of agricultural change, labor shortage and water scarcity, on rice pest profiles in tropical Asia. Field Crop Res 91: 263–271

Seefeldt SS, Jensen JE, Fuerst EP (1995) Log-logistic analysis of herbicide dose response relationships. Weed Technol 9:218-227

Tabbal DF, Bouman BAM, Bhuiyan SI, Sibayan EB, Sattar MA (2002) On-farm strategies for reducing water input in irrigated rice; case studies in the Philippines. Agric Water Manag 56: 93–112

Tomita S, Miyagama S, Kono Y, Noichana C, Inamura T, Nagata Y (2003) Rice yield losses by competition with weeds in rainfed paddy fields in north-east Thailand. Weed Biol Manag 3:162–171

Watanabe H, Vaughan DA, Tomooka N (2000) Weedy rice complexes: case studies from Malaysia, Vietnam, and Surinam. In: Proceedings of Wild and Weedy Rice in Rice Ecosystems in Asia – A Review (Ho Chi Minh City, Vietnam, 10–11 August 1998). International Rice Research Institute, Los Banos, the Philippines, pp. 25–34