



Full Length Article

Control Measures of Sprangletop (*Leptochloa chinensis*) Resistant Biotype using Propanil, Quinclorac and Cyhalofop-butyl

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Abstract

Sprangletop is one of the most common and dominant weed species in rice fields. Repeated use of the same herbicides in the same field over time to combat this menace can lead to weed resistance to the herbicides causing >50% yield loss of direct-seeded rice in Malaysia. Thus, experiments were conducted to quantify the resistance levels and to evaluate potential for weed control using rates of propanil, quinclorac and cyhalofop-butyl individually or in combination. Initial foliar injury (40 to 55%) was observed in the resistant (R) sprangletop biotype (Kedah-SB8) but finally regain and exhilarate by use of propanil, quinclorac and cyhalofop-butyl at eight-folds the recommended field use rates. The effective dose of propanil, cyhalofop-butyl and quinclorac to inhibit plant growth by 50% (ED₅₀) was 0.58 g m⁻², 0.05 g m⁻² and 0.09 g m⁻², for the R biotype, while for that of the susceptible (S) biotype was 0.20 g m⁻², 0.008 g m⁻² and 0.006 g m⁻², respectively. The ED₅₀ values demonstrate that the sprangletop R biotype (Kedah-SB8) was 2.90 times more resistant to propanil, 6.25 times more resistant to cyhalofop-butyl, and 15.00 times more resistant to quinclorac than the S biotype. Sprangletop R biotype (Kedah-SB8) can be fully controlled by the combined use of recommended rates of propanil (0.55 g m⁻²) and cyhalofop-butyl (0.08 g m⁻²) or with double dosage of recommended field use rate of propanil (1.10 g m⁻²) or cyhalofop-butyl (0.16 g m⁻²) and quinclorac (0.06 g m⁻²), respectively. The above results show that rice growers can take the advantage of the combined used of herbicides to control R biotype of sprangletop grass. © 2014 Friends Science Publishers

Keywords: Control; Herbicides; Sprangletop; Resistant biotype; Propanil; Quinclorac; Cyhalofop-butyl

Introduction

Transplanting rice seedlings is a common practice for rice production but direct seeded rice (DSR) is an attractive option for sustainability of rice production systems (Tomita *et al.*, 2003; Savary *et al.*, 2005; Farooq *et al.*, 2011; Jabran *et al.*, 2012). Direct seeded rice is inevitable due to water scarcity for conventional flooded condition of transplanting rice and labor shortage. Direct seeding of rice has been practiced in Malaysia and rice growing countries in South East Asia since the 1980s (Azmi *et al.*, 2007), but its major drawback is the higher infestation of weeds (Jabran *et al.*, 2012), which can influence rice yield, quality and price. Many weeds, including barnyardgrass, sprangletop, junglerice and southern crabgrass are notorious grass species in direct seeding rice (Chauhan and Johnson, 2011; Chauhan *et al.*, 2011). Among these sprangletop is a serious and seasonal weed which is extensively disseminated in the rice growing regions in the world and ranked third in rice weed ecosystems in Malaysia (Begum *et al.*, 2005). Rice yield is directly associated with water availability, fertilization, pest and weed management. Rice yield can be drastically reduced without weed control measures. Previous studies have demonstrated that due to weed

competition with rice, grain yield can decrease to about 10-35% when weed infestation is partially controlled. Without weeds control yield reduction can be as high as 89% (Yu *et al.*, 2007). Thus, proper and timely application of herbicides has been one of the most reliable and cheaper methods of weed control (Abeysekera and Wickrama, 2005). The presence of weeds in rice crop field present one of the great threats on yield and quality of rice and has become a prominent pest problem in temperate rice cultivation (Ioannis and Kico, 2005) because they compete for moisture, nutrients, and light during rice growing season.

Inappropriate use of herbicides does not control weeds and can consequently lead weed species to become more resistant to herbicides. However, the use of herbicide has to be optimized for efficient weed control, so that growers will not face any unusual drawbacks for following other mechanical practices including grazing, burning, producing cover crops followed by fallow in order to keep weed densities at minimal levels. Growers are producing more profitable crops on the same piece of land, with the use of herbicides and pesticides throughout the world (Tomita *et al.*, 2003). However, the comprehensive application of herbicides, although an effective process to control weed in rice fields, its repeated application of the same herbicides in

the same field over time can cause severe obstacles especially as the weeds can gain relatively more resistance to the herbicides used (Christoffers, 1999).

Sprangletop has become resistant to propanil as a result of repeated application of anilides (propanil) in different countries of the world including Malaysia. About 18 weed species have been identified as resistant to herbicides in Malaysia since 1980 (Azmi *et al.*, 2007). The continuous use of the cyhalofop-butyl in intensive rice producing areas in Kedah, Malaysia has become a great concern due to the very poor control of the sprangletop weed. Field records have shown that propanil and quinclorac has been used continuously at this location since 1990. In addition, observations have indicated that no crop rotation practices were implemented throughout this period except rice after rice (Ho *et al.*, 2008). Therefore, the objectives of this study were to quantify herbicide resistance in sprangletop biotypes and to test control measures using three groups of herbicides, namely the aryloxyphenoxy propanoate (APP) herbicide: cyhalofop-butyl, the auxin herbicide: quinclorac and the anilide herbicide: propanil, alone or in combination.

Materials and Methods

Experimental Site and Management

Sprangletop (*Leptochloa chinensis* (L.) Nees) weed seeds were collected from rice fields in Kedah (6°20'N, 100°22'E), Malaysia. From each location, 10 plants were randomly selected to collect mature seeds and each location covered an area about 5-x5 m². Seeds from 10 random locations were mixed a homogeneous combined sample and a total of 10 combined sample seeds were made to test against each herbicide. Plastic pots (20 x 25 cm) were used in greenhouse experiments to identify R and S biotype. About 1500 g air-dried sterilized clay loam soil was used in each plastic pot to grow weed plants to test against three herbicides. During experimental period the sunshine hour was more than 12-h and day and night temperatures at the greenhouse were 29°C±2°C and 22°C±2°C, respectively. Weed seeds were sown in pots and after germination four weed seedlings were kept in each pot for treatment with each herbicide. Normal irrigation water was applied to saturate up to field capacity for proper germination and growth of the plants.

Identification of Resistant and Susceptible Biotype Experiments

Herbicides were used in amounts equivalent to recommended rate (manufacturer's guideline) and double the recommended rate to determine R and S biotypes. Each herbicide was evaluated against 10 composite weed populations. Untreated control plants were included as check against each herbicide. Each herbicide along with weed biotypes was treated as an individual experiment. Herbicides dosages are shown in Table 1(a). All (10)

biotypes were replicated four times and each experiment was conducted under completely randomized design (CRD). The seedlings of all weed biotypes were sprayed with herbicides at 4 weeks after germination (WAG) when weed plant was at four to five leaves stage. Weed control was evaluated visually. Visual control ratings were taken at 1, 2, 3 and 4 weeks after treatment (DAT). The control rating was fixed on a scale of 0 to 10. With 0 means to the weed plants are fully healthy and observed in the untreated plants, and 10 represent to complete suppression of growth. Both treated and untreated above ground shoots were harvested at 4 WAT and were washed properly with running water then soaked with absorbent paper. The shoot fresh weight (SFW) was recorded using a digital sensitive balance. The SFW was converted to percentage reduction compared to untreated healthy plants. The reduction of SFW was used as an indicator to monitor weed plant growth suppression. The SFW data was used as counterpart to check the accuracy of the visual evaluation and the similarity of results for next assays. For subsequent tests, SFW reduction >85% was represented as S biotypes, while <5% and 50% decline considered as R and partially R, respectively (Moss, 1995). Data were subjected to analysis of variance and treatment means were separated with Duncan's New Multiple Range Test (DMRT) at the 5% level.

Herbicides Dose-Response Experiments

Kedah-SB8 sprangletop grass was selected as common R biotype to all the herbicides applied, and this biotype was used exclusively for the following experiments. Each herbicide was used at rates equivalent to 0.25, 0.5, 1, 2, 4 and 8 times the recommended rate. Non-treated plants were included against each herbicide as control. Herbicides dosages are presented in Table 1b. Kedah-SB8 resistant biotype was sprayed with propanil, quinclorac and cyhalofop-butyl at 4 WAG approximately at the four to five-leaf stage (<20 cm in height). Visual assessment ratings were recorded at 1, 2, 3 and 4 WAT. The SFW reduction in percentage was recorded at 4 WAT and it was explained in the previous experiment. Each herbicide with six levels along with untreated control treatment was tested in an individual experiment. Each experiment was arranged in CRD with four replications. Data were subjected to analysis of variance and treatment means were separated with Duncan's New Multiple Range Test (DMRT) at the 5% level. The log-logistic model was used against herbicide R biotypes to estimate of the herbicide rates that declined the SFW by 50% (ED₅₀) when compared to untreated weed plants (Seefeldt *et al.*, 1995).

Resistant Biotype Control Experiments using Single or Combined Herbicides

Dose-response experiments were implemented to control measures of R biotype of Kedah-SB8. Propanil was used at rates of 0, 0.55 and 1.10 g m⁻², quinclorac at rates of 0, 0.03,

Table 1: Herbicides and their rate used in different experiment

Herbicides	Herbicides application rate (g m ⁻²)						
(a). Identification of resistant and susceptible biotype							
Propanil	0		0.55		1.10		
Quinclorac	0		0.03		0.06		
Cyhalofop-butyl	0		0.08		0.16		
(b). Herbicides application rate in dose response experiment							
Propanil	0	0.1375	0.275	0.55	1.10	2.20	4.40
Quinclorac	0	0.0075	0.015	0.03	0.06	0.12	0.24
Cyhalofop-butyl	0	0.02	0.04	0.08	0.16	0.32	0.64

0.06 g m⁻² and cyhalofop-butyl at rates of 0, 0.08, 0.16 g m⁻² single or in combination. The experiment was conducted under CRD with four replications. Herbicide application schedules and SFW methods followed the dose response experiments as described above. Data were subjected to analysis of variance and treatment means were separated with Duncan's New Multiple Range Test (DMRT) at the 5% level.

Results

Identification of Resistant and Susceptible Biotypes

Propanil, quinclorac and cyhalofop-butyl treated R biotype (Kedah-SB8) showed 40-55% initial injury at 1 WAT whilst at 2 WAT it recovered slightly (35-50%). It was noticed that by 4 WAT the R biotype had almost fully recovered (Table 2). The SFW reduction in both R and S biotypes was affected significantly by herbicides application. The SFW was reduced significantly with higher rates of herbicides ($P < 0.001$) in the S biotype but the R biotype (Kedah-SB8) showed resistance against herbicides even with more than double the recommended field use rate (Table 2). The level of control measures in R biotype (Kedah-SB8) was similar among propanil, quinclorac and cyhalofop-butyl at 4 WAT based on either visual assessment or SFW reduction rate.

Dose Response of Herbicides

Regardless of the type and application rates of herbicides the R biotype (Kedah-SB8) appeared as resistant over time. The SFW of the R biotype (Kedah-SB8) showed a similar

trend of injury level with all herbicides at 1 WAT but the level of initial injury was 65-75% against propanil at rates of 2.20-4.40 g m⁻² which thereafter recovered gradually over time (Table 3). A similar trend was also observed in the case of quinclorac at rates of 0.12-0.24 g m⁻² and cyhalofop-butyl at rates of 0.32-0.64 g m⁻². For both R (Kedah-SB8) and S biotypes, the SFW decreased with higher rates of propanil. However the S biotype showed a more pronounced ($P < 0.001$) decline compared to the R biotype (Kedah-SB8) of sprangletop. The S biotypes SFW was reduced by about 95% with the application of recommended (0.55 g m⁻²) and double rates (1.10 g m⁻²) of propanil (Table 4) suggesting no perceptible difference in phytotoxicity of propanil between recommended field use rate or two-fold to eight-fold rates. The susceptible biotype had 100% mortality at the highest levels of propanil (4.40 g m⁻²), whereas the R biotype (Kedah-SB8) showed 55% mortality (Table 4). The resistant Kedah-SB8 sprangletop biotype had 50% mortality at the highest rates of quinclorac (0.24 g m⁻²), while the S biotype showed 85% mortality in SFW by quinclorac at recommended rate (0.03 g m⁻²), which was identical with higher rates used (Table 4). Similarly the Kedah-SB8 sprangletop biotype showed resistance at an even higher rate of quinclorac (0.24 g m⁻²). The shoot fresh weight of the S biotype declined >85% at recommended field use rate (0.08 g m⁻²), and identical with higher rates of cyhalofop-butyl (Table 4).

The ED₅₀ values of propanil for the R biotype (Kedah-SB8) and S biotypes were 0.58 and 0.20 g m⁻², respectively, showing that the R biotype was 2.90 times more tolerant against propanil than the S biotype. However, the ED₅₀ value of propanil for the R biotype was slightly stronger than the recommended rates (0.55 g m⁻²) of propanil, which can control the S biotype (Table 5). The R biotype (Kedah-SB8) was 15 times more resistance to quinclorac compared to the S biotype based on the values of ED₅₀. Moreover, the ED₅₀ value of quinclorac for this biotype was significantly superior to the recommended (0.03 g m⁻²) rate of quinclorac. However in contrast to propanil or cyhalofop-butyl, the R biotype (Kedah-SB8) showed a greater level of resistance to quinclorac. For example, the ED₅₀ values of cyhalofop-butyl for the R biotype (Kedah-SB8) and S biotypes were 0.05 and 0.008 g m⁻²,

Table 2: Visual evaluation at 1, 2, 3 and 4 weeks after treatment (WAT) and shoot fresh weight reduction at 4 WAT from destructive sampling of the Kedah-SB8 sprangletop biotype with propanil, quinclorac and cyhalofop-butyl

Herbicides	Rate (g m ⁻²)	Percent visual control (WAT) Kedah-SB8				Shoot fresh weight reduction (%) at 4 WAT	
		1	2	3	4	Kedah-SB8	Susceptible biotype
Propanil	0.55	40 b	35 b	30 b	25 b	25 b	95 a
Propanil	1.10	52 a	45 a	35 a	30 a	30 a	98 a
Untreated	-	0 c	0 c	0 c	0 c	0 c	0 b
Quinclorac	0.03	45b	40 b	30 b	25 b	25 b	90 a
Quinclorac	0.06	50 a	45 a	40 a	30 a	30 a	95 a
Untreated	-	0 c	0 c	0 c	0 c	0 c	0 b
Cyhalofop-butyl	0.08	45 b	40 b	35 b	30 b	30 b	95 a
Cyhalofop-butyl	0.16	55 a	50 a	45 a	40 a	35 a	98 a
Untreated	-	0 c	0 c	0 c	0 c	0 c	0 b

Means followed by the same letters are not significantly different for each treatment means ($P < 0.05$) by DMRT

Table 3: Percent visual control at 1, 2, 3 and 4 weeks after treatment (WAT) of the Kedah-SB8 sprangletop biotype against different rates of propanil, quinclorac and cyhalofop-butyl

Herbicides	Rate (g m ⁻²)	Visual control (%) over time (WAT)			
		1	2	3	4
Propanil	0.1375	15 d	10 d	5 d	3 d
Propanil	0.275	25 c	15 d	10 d	5 d
Propanil	0.55	45 b	35 c	25 c	20 c
Propanil	1.10	55 b	50 b	40 b	35 b
Propanil	2.20	65 ab	60 ab	55 a	45 b
Propanil	4.40	75 a	70 a	65 a	60 a
Untreated	-	0 e	0 e	0 e	0 e
Quinclorac	0.0075	25 d	20 d	15 d	10 d
Quinclorac	0.015	30 d	25 d	20 d	15 d
Quinclorac	0.03	50 c	45 c	40 c	35 c
Quinclorac	0.06	60 b	55 b	50 b	45 b
Quinclorac	0.12	70 a	65 a	60 a	55 a
Quinclorac	0.24	75 a	72 a	66 a	63 a
Untreated	-	0 e	0 e	0 e	0 e
Cyhalofop-butyl	0.02	15 c	12 c	10 c	5 d
Cyhalofop-butyl	0.04	25 c	20 c	15 c	10 d
Cyhalofop-butyl	0.08	50 b	45 b	40 b	30 c
Cyhalofop-butyl	0.16	65 b	60 b	55 b	47 b
Cyhalofop-butyl	0.32	75 a	70 a	65 a	63 a
Cyhalofop-butyl	0.64	78 a	75 a	72 a	70 a
Untreated	-	0 d	0 d	0 d	0 e

Means followed by the same letters are not significantly different for each treatment means ($P < 0.05$) by DMRT

respectively designating that the R biotype (Kedah-SB8) was greater than 6 times more tolerant of cyhalofop-butyl than the S biotype (Table 5).

Resistant Biotype Control using Single or Combined Herbicides

The results observed from visual assessment showed an identical trend to the two previous research experiments. The use of single herbicides showed 40-70% foliar injury while its combined application at recommended or double rate recorded the highest foliar injury (>95%) at 1 WAT (Table 6). No significant effect was observed on the reduction of SFW of the R biotype with the single use of either recommended rate or double rate of propanil, quinclorac and cyhalofop-butyl (Table 6). The SFW of R biotype (Kedah-SB8) was significantly ($P < 0.001$) reduced (90%) by the combined effect of recommended field use rates propanil + quinclorac and quinclorac + cyhalofop-butyl. Recommended rates of propanil combined with cyhalofop-butyl also showed great potential to reduce SFW in the R biotype (Table 6).

Discussion

The results of the present findings were similar to the reports with the R biotype of Johnsongrass against graminicides, where the initial injury was 30 to 60% (Kevin and Edward, 2001). Propanil inhibit the photosynthetic electron transport chain in photosynthesis and thus block the ability of the plant to turn light energy into chemical energy. As a consequence

Table 4: Shoot fresh weight reduction (%) at 4 WAT of the Kedah-SB8 and susceptible sprangletop biotype against propanil, quinclorac and cyhalofop-butyl

Sprangletop Biotype	Propanil (g m ⁻²)							
	0	0.1375	0.275	0.55	1.10	2.20	4.40	
Kedah-SB8	0 f	5 e	10 e	20 d	37 c	45 b	55 a	
Susceptible	0 d	25 c	40 b	90 a	95 a	100 a	100 a	
		Quinclorac (g m ⁻²)						
		0	0.0075	0.015	0.03	0.06	0.12	0.24
Kedah-SB8	0 d	5 c	12 c	30 b	35 b	45 a	50 a	
Susceptible	0 d	20 c	50 b	85 a	90 a	90 a	90 a	
		Cyhalofop-butyl (g m ⁻²)						
		0	0.02	0.04	0.08	0.16	0.32	0.64
Kedah-SB8	0	5 d	10 d	20 c	40 b	45 ab	55 a	
Susceptible	0	30 c	50 b	88 a	92 a	95 a	98 a	

Means followed by the same letters are not significantly different for each treatment means ($P < 0.05$) by DMRT

Table 5: Response of the Kedah-SB8 and susceptible sprangletop biotypes to propanil, quinclorac and chalofof-butyl as determined by ED₅₀^a values and resistant/susceptible (R/S) ratio

Herbicides	ED ₅₀ (g m ⁻²)		
	Susceptible	Kedah-SB8	R/S ratio
Propanil	0.20 ± 0.02 ^b	0.58 ± 0.02	2.90
Cyhalofop-butyl	0.008 ± 0.01	0.05 ± 0.01	6.25
Quinclorac	0.006 ± 0.001	0.09 ± 0.01	15.00

^aHerbicide dosages that reduced the shoot biomass by 50% when compared with untreated plants

^bStandard error of the mean

the SFW of the S biotypes was reduced drastically by the use of propanil (Daniell *et al.*, 2006). From other studies it was found that the SFW of barnyard grass S biotype was reduced by 78 to 85% with the use of propanil at the rate of 10.4 kg ha⁻¹ (Ioannis and Kico, 2005). The induction process of the aminocyclopropane carboxylic acid (ACC) synthase activity plays a primary role in the selective herbicide action of quinclorac. This is a common effect of auxin herbicides and auxins, which lead to the accumulation of cyanide and/or ABA depending on the plant species and tissues, the compound concentration in the tissue, and their biological activity (Grossmann, 1998). It has been reported that quinclorac controls annual grasses and some broad leaf weeds but is ineffective on sprangletop. In this study the R biotype (Kedah-SB8) sprangletop lost 25% of its growth by both visual estimation and reduction of SFW at 4 WAT, while the S biotype lost >90% SFW. The results of the biotype identification experiments revealed that the reduction of SFW was directly correlated with visual percent control. Irrespective of herbicides used against the 10 tested biotypes, the R (Kedah-SB8) and S biotypes can be identified based on visual evaluation and SFW reduction rate.

Visual evaluations along with SFW reduction from the dose-response experiments were similar with regard to the response of the R biotype (Kedah-SB8). In these experiments, higher levels of herbicides were able to control both the R (Kedah-SB8) and S biotypes. However, the R biotype (Kedah-SB8) demonstrated poor

Table 6: Visual evaluation at 1, 2, 3 and 4 weeks after treatment (WAT) and shoot fresh weight reduction at 28 DAT of the Kedah-SB8 sprangletop biotype with propanil, quinclorac and cyhalofop-butyl alone and combination

Herbicides	Rate (g m ⁻²)	Percent visual control (DAT)				Shoot fresh weight reduction (%) at 4 WAT
		1	2	3	4	
Propanil	0.55	40 e	35 e	30 d	25 d	25 d
Propanil	1.10	55 d	45 d	40 cd	35 bc	30 cd
Quinclorac (Quin)	0.03	50 d	45 d	40 cd	30 cd	32 cd
Quinclorac	0.06	65 c	55 c	45 c	40 bc	36 bc
Cyhalofop-butyl (Cyhal)	0.08	55 d	50 d	40 cd	35 bc	30 cd
Cyhalofop-butyl	0.16	70 bc	70 b	60 b	45 b	40 b
Propanil + Quin	0.55 + 0.03	95 a	93 a	92 a	90 a	90 a
Propanil + Quin	1.10 + 0.06	99 a	95 a	92 a	90 a	92a
Propanil + Cyhal	0.55 + 0.08	95 a	93 a	91 a	90 a	95 a
Propanil + Cyhal	1.10 + 0.16	98 a	95 a	92 a	90 a	90 a
Quin + Cyhal	0.03 + 0.08	99 a	97 a	95 a	90 a	90 a
Quin + Cyhal	0.06 + 0.16	100 a	100 a	97 a	95 a	92 a
Propanil + Quin + Cyhal	0.55+0.03+0.08	100 a	100 a	95 a	90 a	90 a
Propanil + Quin + Cyhal	1.10+0.06+0.16	100 a	100 a	98 a	95 a	94 a
Untreated	-	0 f	0 f	0 e	0 e	0 e

Means followed by the same letters are not significantly different for each treatment means ($P < 0.05$) by DMRT

levels of resistance to cyhalofop-butyl and quinclorac. Foliar applications of cyhalofop-butyl responses are likely to be linked to the mechanism of translocation and metabolism. The light chlorosis and dehydration symptoms in herbicide treated leaves, causing severe arrest of initial growth, are probably linked to its faster translocation to the meristem area from the treated leaf, followed by its rapid metabolism. In addition, the ED₅₀ values of propanil, quinclorac and cyhalofop-butyl for the R biotype were well above the recommended rates.

The results of the present study confirms the observation of previous research findings, that propanil used in combination with thiobencard, pendimethalin, molinate or quinclorac controlled effectively propanil-R barnyard grass than the same rate of propanil applied individually (Baltazar and Smith, 1994; Crawford and Jordan, 1995; Jordan, 1997). Chauhan and Abugho (2012) found that the combined application of penoxsulam and cyhalofop at the four leaf stage can control 89 to 100% of Chinese sprangletop weed. Combined application of herbicides is a useful and effective practice in intensive crop production to reduce weed with herbicide resistance (Khaliq *et al.*, 2012). Therefore, the results of this study suggest that recommended rates of propanil+quinclorac or quinclorac+cyhalofop-butyl are applied to control Kedah-SB8 sprangletop R biotype.

In conclusion, the depreciation of shoot fresh weight associated with visual assessment is an excellent index to determine the R and S biotypes. The ED₅₀ values from the dose-response experiments suggest that the Kedah-SB8 sprangletop R biotype is 2.90 times more resistant to propanil, 15.00 times more resistant to quinclorac and 6.25 times more resistant to cyhalofop-butyl, respectively than the S biotypes. The combined use of quinclorac and propanil at recommended rates of 0.03 and 0.55 g m⁻², quinclorac and cyhalofop-butyl at rates of 0.03 and 0.08 g m⁻² or propanil and cyhalofop-butyl at rates of 0.55 and

0.08 g m⁻², respectively were able to successfully control Kedah-SB8 sprangletop R biotype.

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