Comparative growth studies of glyphosate-resistant and susceptible *Eleusine indica* (L.) Gaertn. (goosegrass) biotypes from the Malaysian Peninsular

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Summary

A comparative study of four putative glyphosate-resistant (R) and four glyphosate-susceptible (S) biotypes of *Eleusine indica* (L.) Gaertn from four locations in the Malaysian Peninsular namely Bidor, Chaah, Lenggeng, and Temerloh was conducted under non-competitive conditions over a 10 week period to determine the relative competitive fitness of the resistant and susceptible biotypes. The test characteristics compared included growth habit, plant height, number of tillers, above ground dry weight, number of inflorescences and dry weight of the inflorescences (reproductive weight).

The R and S biotypes exhibited no distinct differences among populations or within biotypes except the S biotype from Temerloh which had a prostrate growth habit compared to the others which had an erect growth habit. There were significant differences (P = 0.05) in plant height at week 2, 6 and 8 between the biotypes. The R biotypes showed an increase in height but there were no significant differences in plant height within the R biotype populations. However, there were significant differences in height within the S biotype populations at nearly every harvest date. As for the number of inflorescences and reproductive weight, the R biotype produced a higher number of inflorescences at week 10 and could have shown a high reproductive weight if the shedding of seeds did not occur around that time.

Introduction

In 'The World's worst weeds', Holm *et al.* (1977) list *Eleusine indica* (L.) Gaertn. as the fifth most troublesome weed in the world. In Malaysia, glyphosate-resistant *E. indica* populations have been reported in several oil palm plantations, vegetable farms and fruit orchards (Teng and Teo 1999) after years of repeated usage of the herbicide glyphosate. The mechanism of *E. indica* resistance to glyphosate has been extensively studied (Baerson *et al.* 2002, Ng *et al.* 2003), where the resistance in *E. indica* is attributable to a mutation in the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; EC 2.5.1.19) gene.

Theoretical models developed to predict the rate of resistance evolution in weed populations include the relative fitness of resistant and susceptible biotypes as important parameters (Gressel and Segel 1978). Fitness describes the potential evolutionary success of a phenotype, based on its survival and reproductive success (Radosevich *et al.* 1997). In the absence of selection pressure such as herbicide use, Gressel and Segel (1978) reported that resistant weeds would not be able to compete with their susceptible counterparts. This is because a consequence of herbicide resistance in weed biotypes may be manifested as reduced fitness when compared to the susceptible biotypes (Gressel and Segel 1982).

Relative measures of fitness describe the potential evolutionary success of a genotype based on survival, competitive ability and ultimately, reproductive success with the most fit individual leaving the greatest number of offspring, thereby contributing a greater proportion of its genes to the gene pool of the population. Usually, differences in fitness between susceptible and resistant biotypes are characterized only by plant productivity or competitiveness (Warwick and Black 1994).

The existence of negative correlation between fitness and resistance would have important long term implications, and unless the susceptible biotype in a resistant weed population is more fit than the resistant biotype, the relative frequency of the resistant individuals in the population is likely to decline only slowly (if at all) when herbicide selection pressure is removed (Maxwell *et al.* 1990). Additionally, whether resistant biotypes can spread into and become established in weed populations previously not exposed to the herbicide, will depend not only on agricultural practice, but also on the relative fitness of the two biotypes in the absence of the herbicide (Gressel and Segel 1990, Maxwell *et al.* 1990). In some instances, biotypes of a weed species with tolerance to phenoxy herbicides in cropping situations may be morphologically different from non-tolerant populations (Hume 1988), but this does not necessarily mean diminished fitness of the tolerant biotype.

Resistance to the Group C triazine herbicides is usually associated with reduced fitness of the resistant plants (Gressel and Ben-Sinai 1985, Holt *et al.* 1981). However, investigations of resistance in Group A (ACCase inhibitors) or B (ALS inhibitors) showed that resistance is not associated with fitness penalties (Holt 1996). Furthermore, Pedersen *et al.* (2007) reported that glyphosate resistance is not associated with a major fitness penalty in glyphosate resistant *Lolium rigidum* from Australia.

Given the importance of glyphosate as the best selling herbicide, it is important to establish whether or not glyphosate resistance is associated with any fitness penalties in *Eleusine indica*. Thus, this study was conducted to compare the growth and development of the R and S biotypes of *Eleusine indica* from several populations under non-competitive conditions to determine the relative fitness of the glyphosate-resistant and glyphosate-susceptible biotypes of *E. indica* from Peninsular Malaysia.

Materials and methods

Plant materials

Mature *Eleusine indica* seeds from four areas in Peninsular Malaysia and reported to be glyphosate-resistant and glyphosate-susceptible were collected from Bidor (State of Perak), Chaah (State of Johore), Lenggeng (State of Negeri Sembilan) and Temerloh (State of Pahang). Inflorescences of 20 individual plants consisting of 10 putative glyphosate-resistant and 10 putative glyphosate-susceptible plants from each area were collected and placed in different envelopes.

The seeds collected were germinated in polybags in the greenhouse at the Plant Biotechnology Laboratory, UKM. The polybags were placed in blocks where each block represented the S and R biotypes from one area. To avoid cross pollination contamination, each block was isolated by a boundary of about 1 meter from the next block. Screening of the R and S biotypes was done by applying glyphosate at the recommended dosage of 1.08 kg a.e. ha^{-1} on two week old seedlings. The herbicide applications were conducted using a knapsack sprayer at a water volume of 450 L ha^{-1} and pressure of 100 kPa. Plant evaluation was conducted three weeks after treatment and plants that showed visual development of necrosis and chlorosis were considered as S plants while those that survived the treatment were designated as R plants. Other germinated seeds from the same plants were then allowed to reach maturity and the inflorescences of each mature plant were collected and kept separately.

Germination of the seeds from the R and S plants was then conducted and after one week, the seedlings were transferred to black polybags (measuring 8 cm across) containing 'Right Grow' commercial potting mix (distributed by Kosas Profil Sdn. Bhd.) and grown in the greenhouse at 29 ± 4°C, with light intensity of 800 µE $m^{-2}s^{-1}$ and a 12 hour photoperiod. Two seedlings were planted per bag with a total of 640 seedlings in 320 polybags. The plants were watered twice daily.

Experimental design and parameters

The experimental design was a randomized complete block with four replications. Harvesting was done at 2, 4, 6, 8 and 10 weeks. Five plants per biotype per population were harvested at each harvest interval. Parameters recorded at each harvest included plant height, number of tillers and aboveground dry weight. The recordings for the number of inflorescences and their dry weight (reproductive weight) were done at week 6, 8 and 10. The data for growth habit was recorded at week 8.

Statistical analyses included 1 and 2 way analysis of variance (ANOVA). The total variation among populations was partitioned into 'between biotypes' and 'among populations within biotypes'. As for the latter, it was further partitioned to examine variations among populations within the S and R biotypes.

Results and discussion

Growth form

The most striking difference among all the biotypes was the observation that the S biotype from Temerloh produced a much more prostrate growth form compared to the other biotypes in the greenhouse. This was also observed in the field (Chuah Tse Seng personal communication). Besides, Maxwell *et al.* (1990) reported that the wild graminicide-susceptible biotype produced a much more upright growth form than the graminicide-resistant biotype.

The rest of the S biotypes did not differ in form from the R biotypes in this study. Thus, it is suggested that the growth form variation exhibited by the S Temerloh biotype could be attributed to phenotypic plasticity; the capacity for marked variation in the phenotype as a result of environmental influence on the genotype during development. According to Anderson (1996) and Zimdahl (1993), *E. indica* has an upright growth form under field conditions, but it develops a prostate habit when it is mowed and both forms can produce viable seed. Sunohara and Ikeda (2004) also reported that trampling activities can decrease the leaf blade length to width ratio in *E. indica* which in turn influences the growth habit of the plant.

Plant height

Between the biotypes, there were significant differences in plant height at week 2 (P = 0.0001), 6 (P = 0.0023) and 8 (P = 0.0016), where the R biotype was relatively taller (Table 1). Variation among populations was significant at week 2 (P = 0.0001), 4 (P = 0.0293) and 10 (P = 0.0023). As for the S biotypes, there were significant differences among the populations at all the harvest dates (i.e. week 2: P = 0.0001, week 4: P = 0.0006, week 8: P = 0.0010, and week 10: P = 0.0001) except at week 6. At this harvest date, the S biotype plants from Lenggeng were relatively taller than the plants of the S biotype from the other locations. No significant difference in plant height was recorded for the different populations of the R biotype throughout the experiment.

Results of the plant height trait between biotypes correlates with those reported by Marshall *et al.* (1994) whereby the graminicide-susceptible *E. indica* plants were taller than the R plants at the end of the flowering stage. However, in the case of dinitroaniline-resistant *E. indica*, the height of the plants was similar for both the dinitroaniline resistant and susceptible biotypes (Harris *et al.* 1995).

Differences in fitness between resistant and susceptible biotypes may result from differences in survival, fecundity (Putwain and Mortimer 1989), and competitive ability (Gressel and Segel 1982). As plant height is an indicator of the ability of larger plants to extract resources more efficiently from the soil (Annapurna and Singh 2003), it is believed that the S plants could take advantage of resource abundance and grow more rapidly than the R biotypes, as this is crucial in the survival of the S biotype in a resistant field population.

Aboveground dry weight

There were no significant differences among the biotypes recorded at all harvest intervals except for week 6 (P = 0.0107) where the R biotype had a higher aboveground dry weight (Table 2). There was also a significant difference among populations at week 6 (P = 0.0429) where it was recorded that the Chaah population gave higher aboveground dry weight.

As for the S populations, significant differences were recorded at week 6 (P = 0.0336) and week 8 (P = 0.0017). At week 6, the S population from Lenggeng exhibited the highest aboveground dry weight while at week 8, the S population from Temerloh

Table 1. Plant height of the different biotypes at different harvest intervals.

		Plant height (cm) at harvest				
Biotype	Population	Week 2	Week 4	Week 6	Week 8	Week 10
S	Bidor	7.53	22.47	28.70	45.30	85.70
	Chaah	7.04	17.45	34.41	42.32	82.21
	Lenggeng	8.15	24.95	30.81	46.75	89.08
	Temerloh	4.04	15.90	30.07	32.13	61.82
	Mean	6.69	20.19	31.25	41.62	79.95
R	Bidor	8.70	20.80	33.95	49.54	83.14
	Chaah	8.51	20.63	34.42	46.94	76.33
	Lenggeng	8.87	20.19	37.75	48.77	79.15
	Temerloh	8.81	19.67	35.12	49.82	85.01
	Mean	8.72	20.32	35.31	48.77	80.90

		ANOVA mean squares				
Source of variation	df	Week 2	Week 4	Week 6	Week 8	Week 10
Between biotype	1	0.0001*	0.9218	0.0023*	0.0016*	0.6888
Among populations	3	0.0001*	0.0293*	0.2215	0.1275	0.0023*
Among S populations	3	0.0001*	0.0006*	0.0682	0.0010*	0.0001*
Among R populations	3	0.8636	0.9746	0.4532	0.9315	0.0812

* An asterisk denotes significance at the 5 % level.

had the highest aboveground dry weight compared to the rest.

Among the R populations, significant differences were recorded for week 8 (P = 0.0490) and week 10 (P = 0.0243). At these two harvest intervals, the Chaah R population exhibited the highest aboveground dry weight as compared to the others. Besides genetic variation among populations, significant differences of the aboveground dry weight at different harvest intervals exhibited by the different populations could be partially attributed to experimental conditions, where it must be mentioned that a hot and dry season prevailed from week 5 to week 9.

Generally, there was no significant difference in the aboveground dry weight in the R and S biotypes of *E. indica* under noncompetitive conditions. As a result of plant competition, the proportion of biomass allocated to roots or shoots is an important determinant (Holt and Radosevich 1983). The observed almost equal biomass suggests that the resistant and susceptible biotypes of *E. indica* would probably have similar competitive ability in the absence of any glycine-herbicide, resulting in equal dry matter production and reproductive ability.

Number of tillers

The number of tillers was not significantly different among the biotypes at the different harvest intervals except at week 6 (P = 0.0001) when more tillers were produced by the R biotype than by the S biotype (Table 3). Significant differences, however, were recorded among the populations at weeks 6 (P = 0.0465), 8 (P = 0.0381) and 10 (P= 0.0012) where the highest number of tillers was recorded from the Temerloh

Table 2. Aboveground dry weight recorded at the different harvest intervals.

		Aboveground dry weight (g) at				
Biotype	Population	Week 2	Week 4	Week 6	Week 8	Week 10
S	Bidor	0.0053	0.0574	0.1729	2.8593	5.7478
	Chaah	0.0047	0.0349	0.2992	2.3080	5.3178
	Lenggeng	0.0047	0.0751	0.3193	2.4814	5.9640
	Temerloh	0.0041	0.0559	0.2353	3.4205	5.2432
	Mean	0.0047	0.0558	0.2567	2.7673	5.5682
R	Bidor	0.0056	0.0748	0.3114	2.5134	6.1498
	Chaah	0.0045	0.0535	0.5201	3.8890	7.9552
	Lenggeng	0.0046	0.0624	0.2895	3.1188	6.0145
	Temerloh	0.0046	0.0537	0.3434	2.4916	5.1098
	Mean	0.0048	0.0611	0.3661	3.0032	6.3073

		ANOVA mean squares				
Source of variation	df	Week 2	Week 4	Week 6	Week 8	Week 10
Between biotype means	1	0.7501	0.6463	0.0107*	0.3157	0.0707
Among populations	3	0.0719	0.4179	0.0429*	0.6250	0.0927
Among S populations	3	0.3944	0.1910	0.0336*	0.0017*	0.6141
Among R populations	3	0.1670	0.8488	0.1150	0.0490*	0.0243*

* An asterisk denotes significance at the 5% level.

Table 3. Number of tillers recorded at harvest intervals of 2, 4, 6, 8 and 10 weeks.

		Number of tillers at harvest week				
Biotype	Population	Week 2	Week 4	Week 6	Week 8	Week 10
S	Bidor	1.0	1.0	1.3	4.9	4.7
	Chaah	1.0	1.0	1.5	4.0	4.4
	Lenggeng	1.0	1.0	2.0	4.0	4.2
	Temerloh	1.0	1.2	2.3	7.0	5.3
	Mean	1.0	1.1	1.8	5.0	4.7
R	Bidor	1.0	1.2	2.4	4.1	5.4
	Chaah	1.0	1.0	2.9	5.3	6.9
	Lenggeng	1.0	1.1	2.1	5.1	3.8
	Temerloh	1.0	1.2	2.8	4.3	4.0
	Mean	1.0	1.1	2.5	4.7	5.0

		ANOVA mean squares				
Source of variation	df	Week 2	Week 4	Week 6	Week 8	Week 10
Between biotype means	1	–	0.6901	0.0001*	0.3607	0.2305
Among populations	3	–	0.1511	0.0465*	0.0381*	0.0012*
Among S populations	3	–	0.2090	0.0018*	0.0001*	0.0554
Among R populations	3	–	0.4648	0.1679	0.1088	0.0001*

* An asterisk denotes significance at the 5 % level.

population at week 6 and 8 and the Chaah population had the highest number of tillers at week 10.

As for the R biotypes, a significant difference was only recorded at week 10 (P = 0.0001). Among the S populations, significant differences were recorded at week 6 (P = 0.0018) and 8 (P = 0.0001) where the Temerloh population had the highest number of tillers.

According to Marshall *et al.* (1994), the graminicide-resistant *E. indica* biotype tillered early in plant development and produced a higher number of tillers than the wild biotype (susceptible). In this study, the R biotype was found to produce more tillers when harvested at week 6, namely when the inflorescences were first recorded in this study. The higher number of tillers early in the reproductive phase could be an adaptation of the R biotype with regard to the proportioning of allocation of biomass resources for maximum potential of the resources.

Among populations, the Temerloh population was found to produce a higher number of tillers at week 6 and 8. This significantly higher number of tillers was mainly contributed by the Temerloh S population. In the growth habit trait, the S biotype from Temerloh recorded a more prostrate habit which could be due to a grazing avoidance mechanism.

The number of tillers is important in comparing the competitive ability of the R and S biotypes. Increased tillering associated with resistance would result in rapid plant establishment and increased leaf area, leading to a significant competitive advantage and therefore the spread of resistance by the resistant biotype (Sharples *et al.* 1997). However in this study, it was found that both the R and S biotypes were not significantly different with regard to the number of tillers. This in turn, could be an indication that the R biotype might not have a competitive advantage over the S biotype.

Inflorescence branches and reproductive weight

Generally for inflorescence and reproductive weight, there were no significant differences observed for the R and S biotypes except at week 10 when the R biotype produced more inflorescences (P = 0.0369) (Table 4). However no significant difference was observed. This could be due to earlier seed shedding of the R biotypes as compared to the S biotypes.

Among populations, no significant difference was observed either for the number of inflorescence branches or the reproductive weight. Instead, significant differences were found in the number of inflorescence branches among the S biotype populations at week 6 (P = 0.0001) and 8 (P = 0.0008) and also for the reproductive weight at week 6 (P = 0.0005) and 8 (P = 0.0343) where the Bidor S population exhibited a higher number of inflorescence branches and reproductive weight than the other S populations. Among the R biotypes, a significant difference was recorded at week 8 for the number of inflorescence branches (P = 0.0016) and reproductive weight (P = 0.0274) where the R biotype from Chaah had a higher number of inflorescence branches and reproductive weight.

The findings of this study are consistent with that of Murphy *et al.* (1986) whereby it was reported that the dinitroaniline-susceptible and -resistant biotypes of *E. indica* generally exhibited a similar range of variability, with the exception of total inflorescence dry weight (reproductive weight in this study). The difference of weight seen in the observed reproductive weight however, could be attributed to the earlier maturation of seeds in the resistant biotypes. Marshall *et al.* (1994) also reported that a graminicide-resistant biotype of *E. indica* had a higher proportion of seed bearing structures compared to the wild (susceptible) biotype.

In herbicide resistance, the survival and spread of a resistant weed biotype in the field is determined by both the competitive ability and resistance mechanism. Thus, the long-term outcome of competition will depend on the ultimate reproductive output of the biotypes involved (Conard and Radosevich 1979).

The results of this study demonstrate that under non-competitive greenhouse conditions the growth of the R biotype was similar to that of the S biotype for plant height, aboveground dry weight, number of tillers, number of inflorescence branches and reproductive weight. The fact that the biomass was almost equal suggested that the glyphosate-resistant and -susceptible biotypes of *E. indica* have similar competitive ability in the absence of glycine-herbicides, resulting in equal dry matter weight and reproductive

Table 4. Number of inflorescence branches (IB) and reproductive weight, (WI) (g) recorded when harvested at week 2, 4, 6, 8 and 10.

		Week 6		Week 8		Week 10	
Biotype	Population	IB	WI (g)	IB	WI (g)	IB	WI (g)
S	Bidor	1.1	0.0188	17.1	0.4608	36.0	1.5963
	Chaah	0	0	9.1	0.3080	28.2	1.1933
	Lenggeng	0.7	0.0109	9.4	0.2511	27.2	1.4908
	Temerloh	0.1	0.0010	12.6	0.3826	31.0	1.5900
	Mean	0.5	0.0077	12.0	0.3506	30.6	1.5418
R	Bidor	0.2	0.0023	5.1	0.2087	42.8	1.4727
	Chaah	0.8	0.0173	21.2	0.5840	44.0	1.8977
	Lenggeng	0.7	0.0160	13.2	0.3681	30.7	1.5215
	Temerloh	1.0	0.0126	15.9	0.4331	30.2	1.2753
	Mean	0.6	0.0120	13.8	0.3985	36.9	1.4676

		ANOVA mean squares					
		Week 6		Week 8		Week 10	
Source of variation	df	IB	WI	IB	WI	IB	WI
Between biotype	1	0.2766	0.1901	0.2993	0.3646	0.0369*	0.5996
Among populations	3	0.6846	0.5436	0.2408	0.2280	0.0540	0.9431
Among S populations	3	0.0001*	0.0005*	0.0008*	0.0343*	0.2653	0.5025
Among R populations	3	0.1197	0.1964	0.0016*	0.0274*	0.0740	0.1183

* An asterisk denotes significance at the 5 % level.

ability. The difference in the reproductive weight however, could be attributed to the earlier maturation of seeds from the resistant biotype. As no significant difference in competitive ability was observed between the R and S biotypes, it appears that the glyphosate resistance trait is not associated with growth penalty under noncompetitive conditions. Therefore, further investigations to compare the growth and development of the R and S populations of *Eleusine indica* under competitive conditions are required.

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