Effects of shade and watering frequency on growth and development of glyphosate-resistant and susceptible biotypes of goosegrass (*Eleusine indica* (L.) Gaertn.)

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Summary

The interactions between shading levels and watering frequency on growth of glyphosate-resistant (R) and -susceptible (S) *Eleusine indica* (L.) Gaertn. biotypes was investigated under non-competitive conditions in the greenhouse. Under full sunlight with daily watering frequency, the S biotype produced higher total biomass and number of tillers than the R biotype whilst the R biotype produced a greater number of spikes and matured earlier than the S biotype. Under 80% shade, the number of tillers and total dry weights of both biotypes generally decreased significantly irrespective of watering frequency. Under this condition, increased root partitioning as watering intervals decreased was only observed in the S biotype. Under the same shading level, the S biotype partitioned less dry matter to inflorescences but more into vegetative parts as compared to the R biotype at 1- and 7-day watering intervals.

Introduction

Eleusine indica (L.) Gaertn, a tufted annual grass commonly known as goosegrass, is one of most serious weeds in vegetable cultivation, orchards, and oil palm and rubber plantations as well as in wastelands and roadsides of the Malaysian Peninsular (Holm *et al.* 1977). Glyphosate has established itself as the leading post-emergence, systemic, non-selective herbicide for the control of annual and perennial weeds since its introduction in 1974. Its success can be attributed to the fact that it is safe to the environment and few weeds have developed resistance in spite of its widespread use over the last 27 years.

The first appearance of glyphosate resistance was recorded in 1996 in *Lolium rigidum* Gaud. (Powles *et al.* 1998). A further case of glyphosate resistance involves *Eleusine indica* (L) Gaertn which was found in orchards, vegetable fields, and nurseries as well as in young oil palm plantations in several regions of Peninsular Malaysia (Teng and Teo 1999, Lim and Ngim 2000). On-site field trials in Bidor, Perak have demonstrated that the resistant (R) biotype of *E. indica* was able to survive glyphosate at more than four times the commercial recommended rate of 1.08 kg a.e. ha^{-1} (Teng and Teo 1999). Lim and Ngim (2000) reported that glyphosate at 5.76 kg a.e. ha^{-1} gave only about 25% control of the R biotype in Teluk Intan, Perak. The level of resistance in the R biotype in Teluk Intan was found to be 8- to 12-fold higher than the susceptible biotype.

Studies on triazine-resistant weeds have demonstrated that the resistant biotype weeds showed reduced vigour compared to the susceptible counterparts when the herbicide was not used (Holt and Radosevich 1983, Marriage and Warwick 1980, Warwick 1980). Other investigations on weeds resistant to non-triazine herbicides, however, have indicated that the resistant biotype may be as fit as its susceptible counterpart (Warwick and Black 1994, Haigler *et al.* 1994, Harris *et al.* 1995, Purba *et al.* 1996).

To date, limited fitness research has been conducted with glyphosate-resistant weed biotypes. Hence, this study was initiated to characterize fitness differences in growth, development and resource allocation between resistant and susceptible biotypes under different shading levels and watering regimes. Understanding the survival strategies of glyphosate-resistant and the susceptible biotypes could be used to improve the management of resistant *E. indica*.

Materials and methods

Seed source

Eleusine indica seeds were collected from Bidor, Perak in Peninsular Malaysia where glyphosate-resistant *E. indica* has been identified (Teng and Teo 1999). The putative glyphosate-resistant seeds were collected from a soursop (*Anona muricata*) farm at Batu 12, Bidor which had been treated with glyphosate at least 10 times per year for five years while the putative susceptible seeds were collected from a nearby roadside population approximately 2 km from the resistant population. Inflorescences from each plant were placed individually inside an envelope and a total of 30 plants were collected for each population.

Screening for the R and S biotypes

Seeds of 10 plants from each of the putative resistant and susceptible populations were germinated in an incubator at 35/20°C day/night. The photoperiod was set at 12 h with a light intensity of 50 µE m^{-2} sec^{-1}. After two weeks, eight seedlings of each plant were transplanted into 28 × 56 cm trays containing commercial potting mix soil and grown in the greenhouse at 29 ± 4°C, with a 12 h photoperiod and a light intensity of 800 µE m^{-2} sec^{-1}. Plants were watered twice daily. Three weeks after transplanting, four seedlings of each plant were screened for resistance to glyphosate by spraying the seedlings at the recommended rate of 1.08 kg a.e. ha^{-1} in order to confirm resistance or susceptibility.

Four resistant (R) plants that survived glyphosate treatment and four untreated susceptible (S) plants were grown in isolation to facilitate self-pollination. Each plant was grown in a 12.5 cm polybag filled with commercial potting mix soil and was placed under an iron-framed box covered with transparent plastic on each of four sides and a nylon mesh material on the top. These R and S biotypes were grown in separate greenhouse bays to prevent cross-pollination. The R and S biotype seeds collected from this first generation were used in the plant growth and development experiments.

Plant growth and development studies

Scarified seeds of the R and S biotypes collected were germinated in the incubator at fluctuating temperature of 35/20°C with a 12 h photoperiod. After one week, seedlings of both biotypes were transplanted into pots containing commercial potting mix soil and grown in the greenhouse for three weeks. Plants were watered twice daily.

Pots containing four-week-old plants from each biotype were divided into three groups. The first group received full sunlight (0% shade), while the other two groups were placed under green netting providing 40 and 80% shading. The netting was placed at the top and on each side of the shade structures.

From the start of the experiment, the soil was watered to 90% field capacity either daily, every four days or every seven days. These watering regimes were continued for four weeks. The numbers of tillers, inflorescences and spikes for both biotypes were counted at one-week intervals for one month. Dry weight of shoot, inflorescences and root were determined four weeks after the watering regime began. Shoots were cut to soil level and left to dry at 55°C for one week. Roots were separated from soil with gently running water and were dried before weighing. Greenhouse experiments were designed as randomized complete block, split-split plot with three levels of shade as main plots, three water levels as subplots and R and S biotypes as sub-subplots, with four replications.

Statistical analysis
All data were subjected to multiple factor ANOVA and means were compared by LSD tests at the 5% level. Data on number of tillers and spikes at one-week intervals for each biotype was fitted to either a polynomial or logistic function.

Results and discussion

The difference in fitness between the R and S plants may be confounded by differences in response to both biotic and abiotic factors among populations (Jasieniuk *et al*. 1996). In order to minimize fitness differences between the R and S biotypes due to environmental factors, the R and S seeds were collected from similar growing conditions (soil type) and geographic areas (only 2 km apart). Furthermore, for these experiments, these R and S biotype seeds were grown for one generation in the greenhouse at the same temperature and light regimes in order to minimize germination differences due to environmental factors on the parent plants (Gutterman 1985, Fenner 1991).

Table 1 shows the effect of shade and watering frequency on the growth of R and S biotypes of *E. indica*. Under 0% shade, the number of tillers of both biotypes decreased significantly as the watering intervals were increased. However, watering frequency did not affect the number of tillers of either biotype under 40 and 80% shade. Generally, the number of tillers of both biotypes decreased with increasing shading level irrespective of watering frequency. Increasing shading level reduced the number of tillers of R and S plants which were watered daily. Significant reduction in tiller production by both biotypes that received 4- and 7-day watering frequency was observed when shading level increased from 40 to 80%.

When the watering frequency was reduced from daily to four day intervals, the number of spikes of R and S biotypes decreased significantly when the plant were put under zero and 40% shade but not under 80% shade. Increasing shading levels caused a decline in the number of spikes in those R and S plants that received daily watering. Shading did not affect the number of spikes of both biotypes when the plants were watered at 4- and 7-day watering intervals.

Less frequent watering significantly reduced the total dry weight of R and S biotypes under zero and 40% shade but not under 80% shade. Increasing shade level also reduced the dry weights of both biotypes when they were watered either every day or every four days. Plants watered every four days showed significant reduction in the total dry weight when shade level was increased from 40 to 80%. These results indicate that 80% shade had a suppressive effect on the growth of both biotypes, suggesting that both biotypes are shade-intolerant. Previous reports have demonstrated that shade significantly reduced the growth of *Asystasia gangetica* (L.) T.Anders (Ismail and Abdul-Shukor 1998), yellow and purple nutsedge (Patterson 1982) and itchgrass (Patterson 1979). The reduction in dry matter production with shading was due to significant reductions in both net assimilation rate or total amount of leaf area present (Patterson 1980).

Under full sunlight and with daily waterings, the S biotype produced higher total biomass than the R biotype. These observations agree with other studies involving triazine-resistant weeds such as *Solanum nigrum* (Jacobs *et al*. 1988) and *Senecio vulgaris* L. (Holt 1988) as well as graminicide-resistant *E. indica* (Marshall *et al*. 1993).

At 0% shade (full sunlight), watering frequency did not affect on the partitioning into the root of the S and R biotypes. Under 40% shade, however, both biotypes showed an increase in root partitioning

Table 1. The effects of light and watering frequency on number of tillers, spikes, total dry weight, and percentage total dry weight of the vegetative parts, inflorescences and roots of the R and S biotypes of *E. indica*.

Shade	No. tillers per plant							No. spikes per plant						
	R			S				R			S			
(%)	1*	4**	7***	1	4	7	LSD	1	4	7	1	4	7	LSD
0	14.33	11.00	10.00	20.00	12.00	11.00	2.40	81.33	13.00	5.67	34.33	6.33	3.00	9.00
40	11.00	8.67	8.67	11.67	11.33	10.67	2.40	34.67	15.67	11.33	25.00	8.33	5.67	9.00
80	4.00	3.67	4.33	3.37	4.33	3.67	2.40	13.33	5.00	7.67	5.33	5.33	6.00	9.00
LSD	2.40	2.40	2.40	2.40	2.40	2.40		9.00	9.00	9.00	9.00	9.00	9.00	

Shade	Total dry weight (g)							Root (% of total dry weight)						
	R			S				R			S			
(%)	1	4	7	1	4	7	LSD	1	4	7	1	4	7	LSD
0	8.28	4.68	2.77	12.27	5.09	2.98	1.08	11.94	15.66	14.87	12.80	16.51	15.84	3.73
40	7.57	3.84	2.29	8.26	4.08	3.34	1.08	7.83	10.24	12.95	9.25	11.81	15.47	3.73
80	1.71	1.67	1.44	2.27	1.62	1.92	1.08	6.66	8.13	9.08	7.51	11.13	12.54	3.73
LSD	1.08	1.08	1.08	1.08	1.08	1.08		3.73	3.73	3.73	3.73	3.73	3.73	

Shade	Inflorescences (% of total dry weight)							Vegetative parts (% of total dry weight)						
	R			S				R			S			
(%)	1	4	7	1	4	7	LSD	1	4	7	1	4	7	LSD
0	22.78	11.66	13.96	9.50	4.30	1.07	5.49	65.28	72.67	71.18	77.70	78.84	83.10	5.02
40	17.56	13.3	20.35	9.57	7.94	7.55	5.49	74.61	76.46	66.70	81.17	80.25	76.98	5.02
80	21.63	16.25	22.33	13.45	10.23	11.21	5.49	72.30	75.62	68.58	79.04	78.64	76.24	5.02
LSD	5.49	5.49	5.49	5.49	5.49	5.49		5.02	5.02	5.02	5.02	5.02	5.02	

*1-day watering interval, ** 4-day watering interval, *** 7-day watering interval.

as the watering frequency was decreased from daily to weekly intervals. Under 80% shade, less watering frequency increased partitioning into root of the S biotype but not the R biotype, reflecting greater plasticity of the susceptible biotype when confronted with environmental fluctuations such as water availability. Hence, under limited light conditions (80% shade), the S biotype may be more competitive than the R biotype. The increased partitioning into the root as observed in the S biotype as watering interval increased would ensure plant survival under drought conditions. Daily and 4-day watering intervals of both biotypes gave significant decreases in the percentage dry weight of root as shading levels were increased from zero to 80%.

Increasing watering intervals from daily to weekly reduced the percentage dry weight of inflorescences of both R and S biotypes under 0% shade. When watering was done at 7-day intervals, increasing shade levels increased the percentage dry weight of inflorescences in both biotypes. Under full sunlight, the R biotype allocated greater biomass into inflorescences compared to the S biotype when the plants were subjected to daily watering. This finding is in agreement with previous reports on growth and development of triazine-resistant *S. vulgaris* (Holt 1988, Holt and Radosevich 1983) and graminicide-resistant *E. indica* (Marshall *et al*. 1993).

Under 0% shade, increasing the watering intervals from daily to 7-day intervals increased the percentage dry weight of vegetative parts of R and S biotypes. Increasing shading from 0 to 80% at 7-day watering frequency reduced the percentage dry weights of the vegetative parts of the S biotype. However, under 80% shading and at 1-, 4- and 7-day watering intervals, the S biotype partitioned less dry matter into inflorescences but more into vegetative parts as compared to the R biotype. These findings suggest that the S biotype may be superior than the R biotype as increasing reinvestment of dry matter in the production of new photosynthetic tissue (vegetative part) enhances their survival chances under limited light conditions.

Figure 1 shows the effects of 0% shade at 1-, 4- and 7-day watering intervals on the development of spikes and tillers in both biotypes. Under 0% shade and with daily watering, the S biotype had greater number of tillers than the R biotype at 51, 58 and 65 days after planting (DAP). There was no significant difference in the number of spikes of the two biotypes through 58 DAP. However, at 65 DAP the R biotype exhibited greater number of spikes compared to the S biotype. With a 4-day watering interval, the number of tillers were similar in both biotypes up to 65 DAP. There were no difference in the number of spikes in the two biotypes except at 65 DAP, where the R biotype produced greater number of spikes than the S biotype. The S biotype exhibited a greater number of tillers than the R biotype after 51 DAP with weekly watering. There was no differences between the biotypes in the number of spikes throughout 65 DAP. Inhibition of tiller and spike production was observed in both biotypes as watering intervals decreased from daily to weekly under 0% shade.

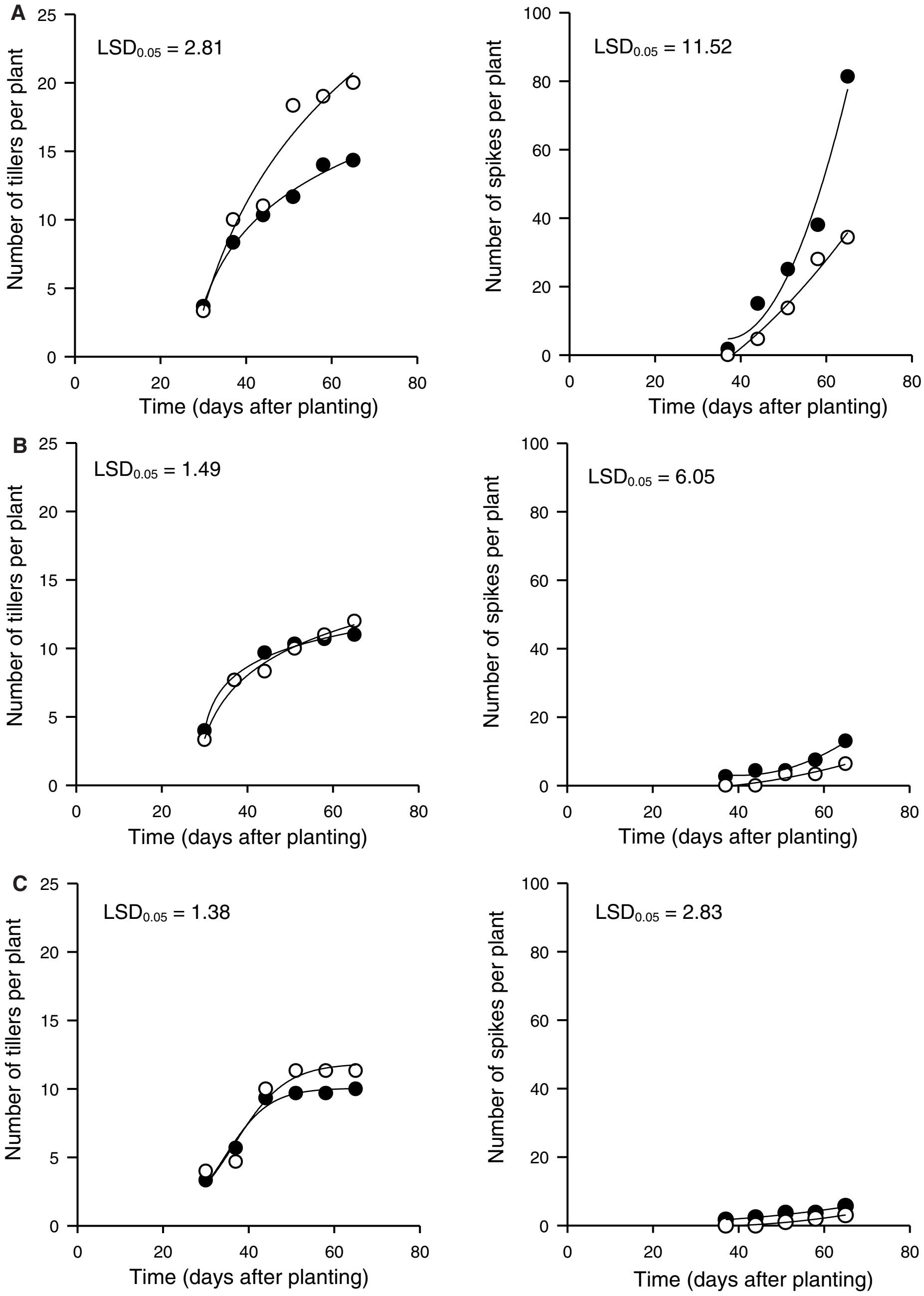

Figure 1. Effects of 0% shade at 1 (A), 4 (B) and 7-day (C) watering intervals on development of spikes and tillers in resistant (●) and susceptible (○) biotypes of *E. indica*.

Figure 2 shows the effects of varying watering intervals on spike and tiller productions in the R and S biotypes when placed under 40% shade. There were no differences in the number of tillers and spikes produced by either biotype when the plants were watered daily. There were also no differences in the number of tillers and spikes at the 4-day watering intervals during the first 51 DAP. S biotype exhibited greater number of tillers than the R biotype at 58 and 65 DAP but differences in the number of spikes remained insignificant between R and S biotypes throughout 65 DAP. With 7-day watering intervals, there was no significant different in the number of tillers and spikes of the R and S biotypes throughout 58 DAP. The S biotype, however, had a greater number of tillers and a lower number of spikes compared to the R biotype at 65 DAP. The number of spikes produced in both biotypes decreased with increasing watering intervals under 40% shade.

Figure 3 shows the effects of watering intervals on the development of spikes

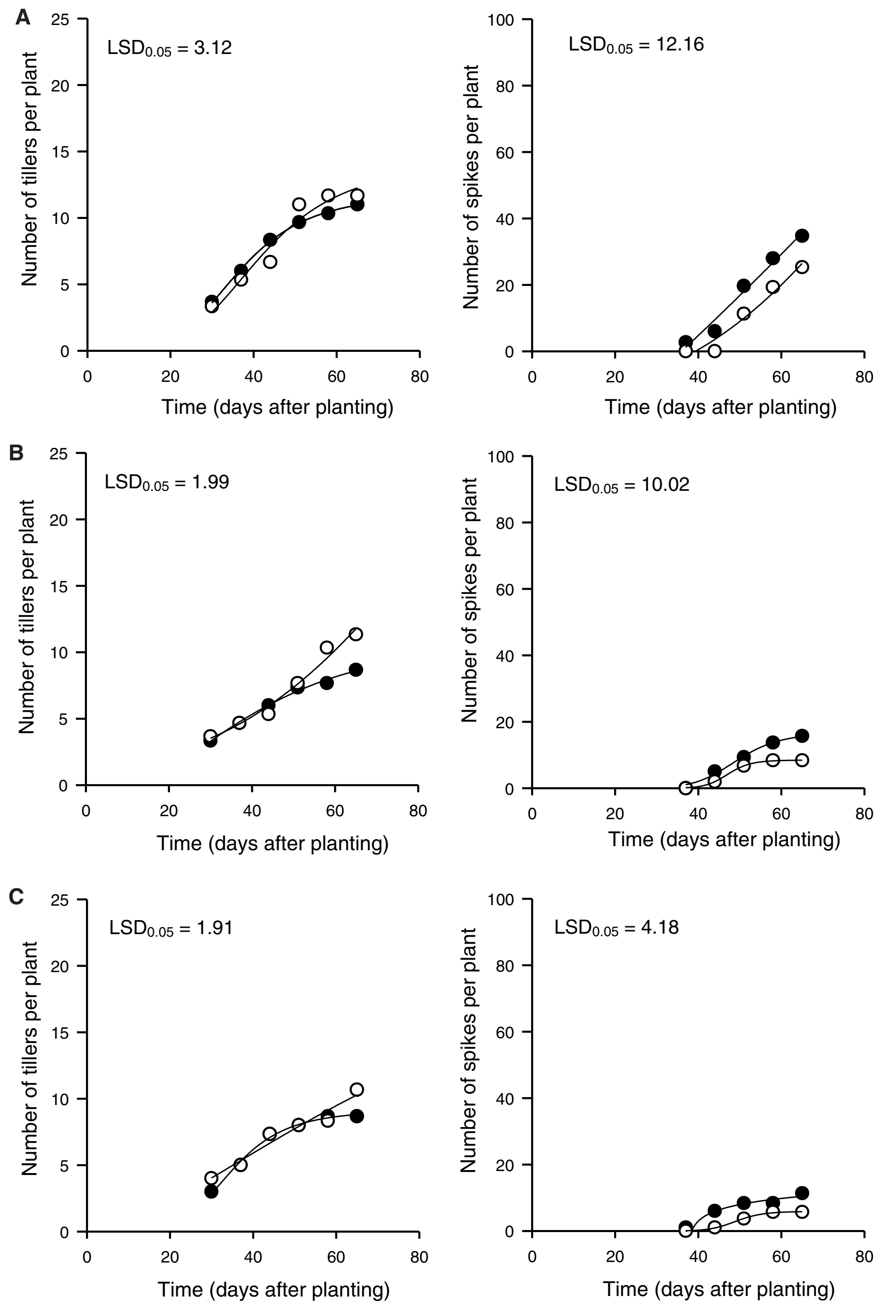

Figure 2. Effects of 40% shade at 1 (A), 4 (B) and 7-day (C) watering intervals on development of spikes and tillers in resistant (●) and susceptible (○) biotypes of *E. indica*.

Table 2. Effects of shading levels and watering regimes on time (days after planting) taken by the resistant (R) and susceptible (S) biotypes to reach 50% of the final spike emergence (T_{50}) of *E. indica*.

Shade	T_{50} (days)					
	R			S		
	Watering frequency			Watering frequency		
(%)	Daily	Every 4 days	Weekly	Daily	Every 4 days	Weekly
0	56	55	48	53	55	56
40	50	49	44	54	47	49
80	44	43	44	42	44	49

and tillers in the R and S biotypes under 80% shade. The biotypes did not differ in terms of the number of tillers produced during the first 65 DAP. However, the R biotype had more spikes than the S biotype from 51 DAP onwards. The R and S biotypes produced similar number of tillers and spikes at 4- and 7-day watering intervals. Tiller and spike productions by both biotypes were suppressed under 80% shade irrespective of watering frequency. Our results demonstrated that spikes appeared (Figures 1, 2 and 3) and matured earlier on the R biotype than did those of the S biotype. Similar observations were reported in graminicide-resistant *E. indica* (Marshall *et al*. 1993).

Table 2 shows the effects of shade levels and watering regimes on time taken (in DAP) to reach 50% of the final spike emergence (T_{50}). The R biotype took less time to reach 50% final spike emergence compared to the S biotype when plants were subjected to 7-day watering interval irrespective of shade levels. Under 0% and 40% shades, as the watering intervals were increased, the time taken to reach T_{50} of the R biotype decreased. However, under 80% shade, watering frequency did not affect the time taken to reach T_{50} of the R biotype. The S biotype took longer to reach T_{50} with increasing watering interval under 80% shade. Under all watering regimes, increase in shade levels reduced the time to reach T_{50} for R and S biotype, suggesting that limited light conditions (80% shade) induced both biotypes to reach T_{50} much earlier as compared to full sunlight conditions.

The current experiments have shown that watering intervals and shade levels affect growth and development of the R and S biotypes. Under non-competitive conditions, the S biotype appears to be more vigorous and productive than the R biotype especially with regards to amassing total biomass. Furthermore, under limited light and water stress conditions, the S biotype has an advantage over the R biotype in the vegetative and root growth. Nevertheless, further study is needed to compare the growth and development of R and S biotypes under competitive conditions using a set of replacement series experiments to assess the relative fitness of both biotypes.

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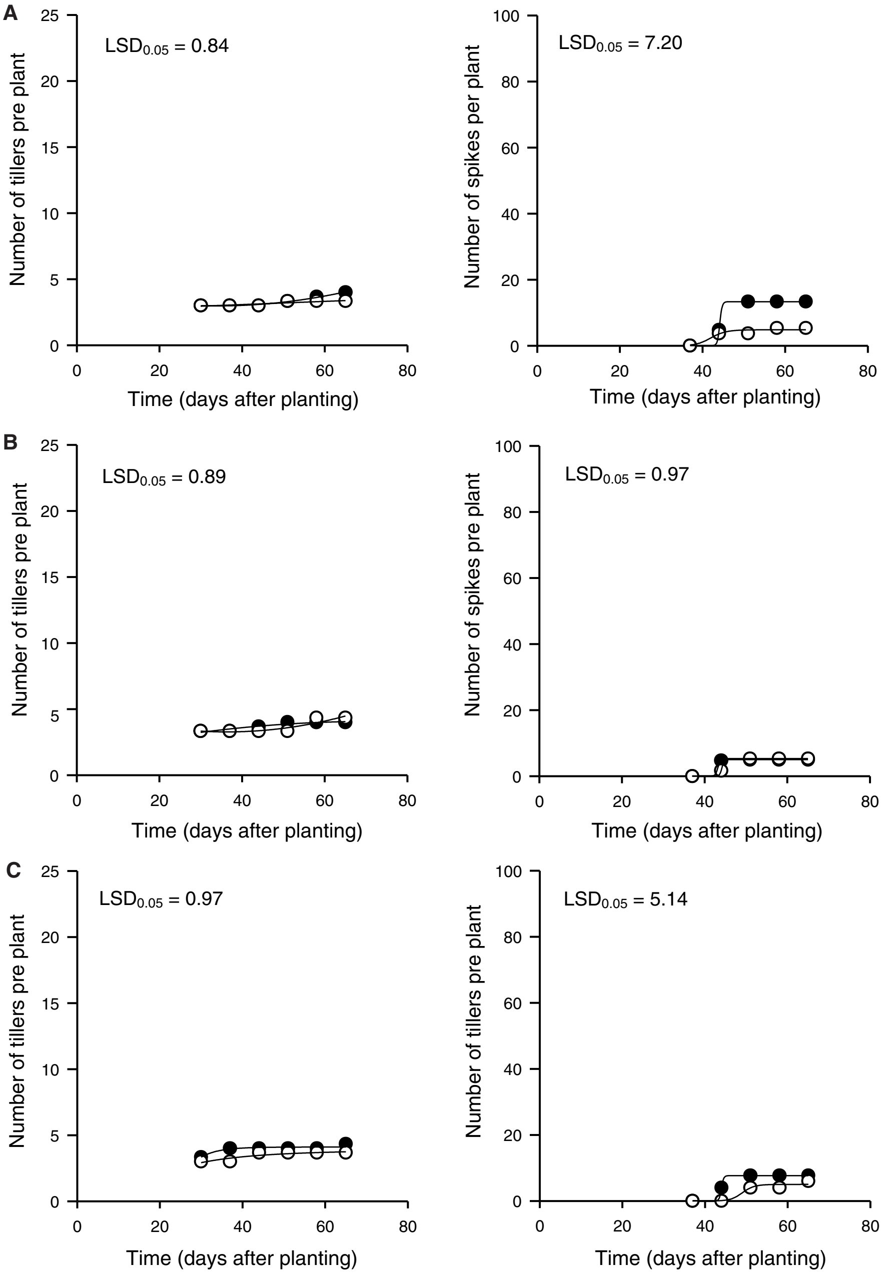

Figure 3. Effects of 80% shade at 1 (A), 4 (B) and 7-day (C) watering intervals on development of spikes and tillers in resistant (●) and susceptible (○) biotypes of *E. indica*.

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