Environmental factors affecting sethoxydim efficacy on glyphosate-resistant and -susceptible biotypes of *Eleusine indica*

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Summary

Glasshouse experiments were conducted to evaluate the interaction between shading levels and watering frequency before and after sethoxydim treatment on the growth of glyphosate-resistant (R) and -susceptible (S) biotypes of *Eleusine indica*. The efficacy of sethoxydim on the R and S biotypes at 0.30 kg a.i. ha^{-1} was not affected by reduced soil moisture level after herbicidal application. However, water stress experienced by the R and S biotypes both before and after sethoxydim treatment could reduce sethoxydim control by 7–20%. Control of the R and S biotypes with sethoxydim was lower at 0% shade than 60% shade, with both biotypes showing 51–72% and 78–80% shoot dry weight reduction at 0 and 60% shade, respectively. The R biotype was more susceptible to sethoxydim application than the S biotype when both biotypes experienced water stress before the herbicidal treatment, irrespective of the soil moisture level after the herbicidal treatment. Field experiment was conducted to examine the effects of dry and wet seasons on sethoxydim efficacy. The results showed that sethoxydim provided 90% control of the R and S biotypes during the wet season (350 mm) whereas control of both biotypes by sethoxydim was reduced by 30–50% during the dry season (139 mm).

Introduction

Sethoxydim is a systemic post-emergence herbicide that acts selectively on grasses (Poaceae) by inhibiting lipid synthesis. However, the effectiveness of sethoxydim on the control of weeds such as *Eleusine indica* (L.) Gartn. (goosegrass) is affected by environmental conditions before, at and after application of the herbicide. Before spraying, environmental factors such as light and soil moisture influence the size and form of the shoots, thereby affecting the efficacy of post-emergence herbicides (Caseley 1996). At the time of herbicide application, the presence of water on the leaves may increase or decrease performance or have no effect what so ever, depending on the amount of water, the type of herbicide, its formulation and the plant species concerned (Caseley 1996).

Sethoxydim efficacy varies depending on soil moisture content, shading, dosage and weed species. A study by Chernicky *et al.* (1984) demonstrated that soil moisture did not have a significant effect on the control of *Digitaria sanguinalis* (L.) Scop. by sethoxydim but control of *Brachiaria platyphylla* Griseb. by sethoxydim at 0.21 kg a.i. ha^{-1} decreased by 10% when this weed grew under low soil moisture conditions (Scott *et al.* 1998). Retzinger *et al.* (1983) studied the efficacy of sethoxydim on the control of *Sorghum halepense*, which grew together with soybean. Sethoxydim application at 0.28 kg a.i. ha^{-1} provided an estimated 98% of the potential soybean yield increase when rainfall was adequate but a higher concentration of 0.41 kg a.i. ha^{-1} was required to obtain the same yield when plants were grown under moisture stress conditions.

Studies on the effect of shading on the efficacy of herbicides have shown varied responses. For instance, it was reported that shading increased the control of *Paspalum conjugatum* Berg. with glyphosate (Ismail and Ibrahim 1996) and paraquat (Ipor and Price 1991). However, Adkins *et al.* (1998) reported that phytotoxicity of glyphosate on *Avena fatua* L. and *Urochloa panicoides* Beauv. was not affected by shading. A study by Chandrasena and Sagar (1986) demonstrated that control of *Elymus repens* (L.) Gould with fluazifop-butyl decreased with increasing shade levels.

This present study was initiated to examine (1) the effects of shading and watering frequency before and after sethoxydim treatment on the growth of the glyphosate-resistant (R) and -susceptible (S) biotypes of *Eleusine indica* under glasshouse conditions and (2) the effects of dry and wet seasons on the efficacy of sethoxydim under field conditions. This paper is part of a series of studies on various aspects of biology of the R and S biotypes of *E. indica* (Ismail *et al.* 2002, 2003, Ng *et al.* 2004).

Materials and methods

Seed source

Collection of the seeds of the R and S biotypes of *E. indica* was carried out as reported previously (Ismail *et al.* 2003). The putative R seeds were collected from a soursop (*Anona muriacata*) farm at Batu 12, Bidor while the putative S seeds were obtained from a nearby roadside population, approximately 2 km from the R population.

Tests for the R and S biotypes

Screening procedures for the R and S biotypes were carried out as described previously (Ismail *et al.* 2003). Seedlings of the putative R and S biotypes were sprayed at the recommended rate of 1.08 kg a.e. ha^{-1}. Seedlings that survived the recommended rate were confirmed as R biotypes, while seedlings which were killed after treatment were considered S biotype. The R and S biotype seeds collected from the first generation were used in the subsequent studies.

Herbicides

The herbicide used in this study was sethoxydim (Expand®, ACM, Malaysia) containing 125 g a.i. L^{-1} solution.

Plant culture and treatments

Seeds of the R and S biotypes were germinated in 36 × 26 × 5 cm plastic trays containing commercial soil potting mix in the glasshouse. After one week, seedlings of both biotypes were transplanted into 12.5 cm pots containing the potting mix and allowed to grow in the glasshouse. The pots containing the one-week-old plants of each biotype were divided into two groups. The first group received full sunlight (0% shade), while the second group was placed under green shade cloth providing 60% shade (320 µE m^{-2} s^{-1}). Temperature at 0 and 60% shade was 35 and 33°C, respectively while relative humidity at 0 and 60% shade was 50 and 90%, respectively. The shade cloth was placed at the top and on the each side of the shade structures.

The soil was watered to 90% field capacity either daily or weekly. These watering regimes were continued for one month. Field capacity was determined after water drained out freely in the columns of soil for 48 hour (Peters 1965). Plants from each biotype and each shade treatment were used in the subsequent experiment 1 and 2.

Experiment 1: Effects of shading and watering frequency on the shoot/root ratio under glasshouse conditions

Plants from each biotype grown under 0 and 60% shade were harvested four weeks after the watering regimes were established as described above. Shoots were cut to soil level and left to dry at 55°C for one week and weighted. Roots were collected and washed with water to remove soil and dry weight recorded. The shoots to roots ratios were determined.

The experimental design was a randomized complete block with four replications. Analysis of variance was performed on the shoot/root ratio of the dry weights and means were compared by Tukey's Honestly Significant Difference (HSD) test at the 5% level of significance.

Experiment 2: Effects of shading and watering frequency on sethoxydim activity under glasshouse conditions

Four weeks after the watering regimes commenced, plants from each biotype and each shade treatment were sprayed with sethoxydim at the rates of 0 or 0.30 kg a.i. ha^{-1} with the addition of the crop oil concentrate (COC) Agri-Dex® (Bayer, Germany) at the rate of 0.05% (v/v), using a Turbo CDA sprayer, delivering 60 L ha^{-1} at spray pressure of 300 kPa.

After the application of sethoxydim, the soil surface was watered either daily or every seven days for each shaded group of the R and S biotypes. The watering regimes were continued for four weeks with enough water supplied at each time to return the soil to 90% field capacity, verified by weighing each pot. Shoots were harvested following the same procedures above, at four weeks after sethoxydim application.

The experimental design was a split-split plot with the two shade levels as the main plots, the two watering regimes as subplots and the two biotypes as sub-subplots. The experiment was replicated four times. Dry weight reduction was calculated as shown below:

100-[(plant dry weight/untreated plant dry weight) × 100]

Arsine transformation was performed on dry weight reduction data before they were subjected to analysis of variance and means were compared by the HSD test at the 5% level of significance.

Experiment 3: Effects of dry and wet seasons on sethoxydim efficacy under field conditions

Seeds from the R and S biotypes were germinated in plastic trays containing commercial soil potting mix in the glasshouse. Three weeks after germination, seedlings of both biotypes were transplanted to 1 × 1 m^2 plots in the field at a density of 120 plants m^{-2}. Two weeks after transplanting, both the R and S biotypes were treated with sethoxydim at the rate of 0 or 0.30 kg a.i. ha^{-1} with the addition of COC at the rate of 0.05% (v/v) as described before for the dry (139 mm) and wet (350 mm) seasons. Daily rainfall records were obtained from a meteorological station located near the experimental field. Plant injury was rated at one and three weeks after the sethoxydim application based on a scale from 0 (no visual effects on plant growth) to 100% (plant death).

The experiment was laid out in completely randomized block design with four replications. Arsine transformation was performed on data of percent control by visual assessment before being subjected to analysis of variance and means were compared by the Tukey test at the 5% level of significance.

Results and discussion

Experiment 1: Effects of shading and watering frequency on the shoot/root ratios of the R and S biotypes of E. indica before sethoxydim application

Shoot/root ratios of both biotypes increased when plants were shaded under daily watering. The R biotype that experienced water stress (weekly watering), however, exhibited greater plasticity compared to the S biotype because the R biotype showed an increase in shoot partitioning whereas the S biotype did not show any significant difference in the shoot/root ratios, when the shading level increased from 0% to 60% (Table 1).

At 60% shade, a significant reduction on the shoot/root ratio was exhibited as the watering frequency decreased from daily to weekly in both the R and S biotypes. In contrast, under 0% shade, no significant reduction on the shoot/root ratio was observed in both biotypes as the watering frequency was reduced from daily to weekly intervals.

Experiment 2: Effects of interaction between shade levels and watering frequency before and after sethoxydim treatment on the growth of the R and S biotypes of E. indica

Under 60% shade, sethoxydim application reduced the percentage shoot dry weight for both biotypes by 78-80% and its efficacy was not affected by the watering frequency either before or after treatment (Table 2).

At 0% shade, shoot dry weight reduction for both biotypes that received daily watering before sethoxydim treatment was not affected by subsequent watering regimes. Similarly, when the plants were watered weekly before herbicidal treatment, watering regimes after sethoxydim treatment did not affect shoot dry weight reduction for either biotype under 0% shade. However, shoot dry weight reduction of the drought-stressed R and S biotypes (receiving weekly watering both before and after herbicidal treatment) was significantly lower compared to the non-stressed plants (receiving daily watering both before and after herbicidal treatment) under 0% shade. Water stress experienced by both biotypes before and after

Table 1. Effects shading and watering frequency on shoot/root ratios of the glyphosate-resistant (R) and-susceptible (S) biotypes of *E. indica* before sethoxydim application under glasshouse condition.[A]

Watering frequency	Shoot/Root ratio			
	R		S	
	Shading level (%)		Shading level (%)	
	0	60	0	60
Daily	19 ± 2 a	104 ± 10 c	19 ± 2 a	80 ± 10 b
Weekly	19 ± 2 a	72 ± 10 b	13 ± 2 a	20 ± 2 a

[A] Mean ± SE. Means followed by the same alphabet are significantly different at the 5% level of significance.

Table 2. Effects shading level and watering frequency before and after sethoxydim treatment on control of the glyphosate-resistant (R) and-susceptible (S) biotypes of *E. Indica*, expressed as percentage.[A]

Watering frequency before and after sethoxydim treatment		Shoot dry weight reduction (%)			
		R		S	
Before	After	0%	60%	0%	60%
Daily	Daily	68 ± 1 cd	80 ± 2 f	72 ± 1de	79 ± 2 f
Daily	Weekly	68 ± 2 cd	79 ± 2 f	71 ± 2 d	78 ± 1 ef
Weekly	Daily	63 ± 1 bc	80 ± 1 f	53 ± 1 a	80 ± 3 f
Weekly	Weekly	61 ± 1 b	79 ± 1 f	51 ± 1 a	78 ± 1 ef

[A] Mean ± SE. Means followed by the same alphabet are significantly different at the 5% level of significance.

sethoxydim treatment could reduce sethoxydim control by 7–20%; implying sethoxydim is less effective for either biotype of *Eleusine indica* under water stress conditions under 0% shade.

Boydston (1990) reported that drought-stressed *Setaria viridis* (L.) Beauv. showed lower percentage control compared to non-stressed plants when treated with sethoxydim at 0.14 kg a.i. ha^{-1}. However, when the sethoxydim rate was increased from 0.14 to 0.28 kg a.i. ha^{-1}, watering frequency did not have a significant influence on the efficacy of sethoxydim. It appears that the effects of watering regimes on the control of the R and S biotypes of *E. indica* by sethoxydim could probably be overcome if the application rate is increased. However, a study by Chernicky *et al.* (1984) showed that soil moisture did not affect the control of *E. indica* by sethoxydim.

Control of the 0% shaded S biotype that received daily watering before sethoxydim treatment compared to those that received weekly watering before the herbicidal treatment, decreased from 72 to 53% although the plants were watered every day after herbicidal treatment. In contrast, sethoxydim control of the R biotype that did not experience water stress before treatment compared to those that experienced water stress before the herbicidal treatment showed no significant difference when the plants were subjected to daily watering after the herbicidal treatment. This suggests that application of ample water after sethoxydim treatment to the R biotype could regulate the physiological processes interrupted by water stress, and allow the sethoxydim to act as under normal soil moisture conditions. Similar results have been observed with diclofop on barnyardgrass (Dortenzio and Norris 1980).

After sethoxydim treatment, the percentage shoot dry weight reduction of the R biotype that experienced water stress before the herbicidal treatment was higher as compared to that of the S biotype that experienced the same water stress, regardless of the watering regimes after the herbicidal treatment. This result implies that the R biotype is more susceptible to sethoxydim application compared to the S biotype when both biotypes are water stressed before the treatment at 0% shade level.

The dry weight of the R and S biotypes was lower at 0% shade than at 60% shade, with both biotypes showing 51–72% and 78–80% reduction at 0 and 60% shade, respectively. The relative inefficiency of sethoxydim against both biotypes at 0% shade may be due to lower shoot/root ratio compared to that at 60% shade. Lower shoot/root ratio at 0% shade could reduce contact surface between plant tissues and sethoxydim, consequently decreasing the amount of sethoxydim absorbed into the plant at 0% shade.

Table 3. Effects of dry and wet seasons on control of the glyphosate-resistant (R) and -susceptible (S) biotypes of *E. indica* one week and three week after sethoxydim application under field conditions.[A]

Season	Visual assessment (%)			
	R		S	
	One week	Three weeks	One week	Three weeks
Wet	50 ± 7 bc	90 ± 4 d	40 ± 7 ab	90 ± 7 d
Dry	50 ± 8 bc	60 ± 9 c	30 ± 4 a	40 ± 4 ab

[A] Mean ± SE. Means followed by the same alphabet are significantly different at the 5% level of significance.

Alternatively, the greater effectiveness of sethoxydim at 60% shade could possibly be attributed to morphological changes of the leaf surface. In general, the leaves of plants when grown under shade produce less epiculticular wax than those exposed to sunlight (Jenks and Ashworth 1999) and this may result in higher uptake of the herbicide. Higher relative humidity (RH) at 60% shade (90% RH) compared to 0% shade (50% RH) in this study might also have contributed the accountable for increased absorption of sethoxydim. Will (1984) demonstrated that sethoxydim control of *Cynodon dactylon* (L.) Pers. increased from 68% to 90% when relative humidity increased from 40% to 100% at 35°C. This was due to greater uptake and translocation of sethoxydim in *C. dactylon* at 100% RH compared to that at 40% RH (Will 1984).

Experiment 3: Dry and wet season effects on the control of the R and S biotypes of E. indica with sethoxydim

Based on visual assessment, during the wet season, sethoxydim provided 40 and 50% control of the R and S biotypes respectively, one week after application. The percentage control of both biotypes increased to 90% three weeks after application during the wet season (Table 3). The R and S biotypes did not experience water stress because total rainfall received by both biotypes one week before and after sethoxydim application was 96.4 mm and 51.6 mm, respectively. Therefore, high soil moisture content both before and after herbicidal treatment could contribute to good control of both biotypes in the field.

During the dry season, the control of the R and S biotypes was observed to be 30% and 50%, respectively, one week after sethoxydim application. Three weeks after herbicidal treatment, weed control was 40% in the S biotype and 60% control in the R biotype. The R and S biotypes experienced water stress because the total rainfall was 2.1 mm and 16.6 mm one week before and after sethoxydim application, respectively (data not shown). Water stress experienced by both biotypes may reduce the efficacy of sethoxydim in the field. The result of this field study clearly showed that both biotypes could be controlled by sethoxydim at 0.30 kg a.i. ha^{-1} during the wet season. However, sethoxydim at the same rate provided poor control of the R and S biotypes during the dry season. This is in agreement with the results observed in the glasshouse study. The R biotype that experienced water stress was however, more susceptible to sethoxydim treatment compared to the S biotype during the dry season.

It may be concluded that the efficacy of sethoxydim in controlling both biotypes of *E. indica* at 0.30 kg a.i. ha^{-1} was not affected by the soil moisture level after the herbicidal application. However, water stress experienced by both biotypes before and after sethoxydim treatment could reduce sethoxydim phytotoxicity. Hence, it is suggested that during the dry season, a higher rate of sethoxydim could be applied to control both drought-stressed biotypes. Alternatively, a lower rate of sethoxydim is suggested to control both biotypes grown under 60% shade (compared to those exposed to full sunlight) because shading could increase sethoxydim efficacy. Further study is yet to be conducted under field conditions to verify these recommendations. The R biotype was, however, found to be more susceptible to sethoxydim application than the S biotype when both biotypes experienced water stress before herbicidal treatment irrespective of the soil moisture level after the herbicidal treatment.

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A short review of the impact and management of weedy rice

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Summary

Weedy rice consists of the early shattering biotypes of cultivated rice (*Oryza sativa* L.) and the natural hybrids of *Oryza* spp. and the cultivated rice varieties. Some important characteristics of weedy rice include comparatively early maturation, easy shattering, short shattering periods, red pericarp (in some strains), short grain, long awns and tall plant habit. Continuous direct seeding over the seasons and poor water management are the main factors enhancing infestation with weedy rice biotypes. Under moderate infestation (15–20 panicles m^{-2}) there occurs about 50–60% yield loss and with high infestations (20–30 panicles m^{-2}) up to 70–80% yield loss. Weedy rice seeds are an important contaminant of rice seeds, as they diminish farmers' income qualitatively and quantitatively. On an average there is 17–28% indirect cost through land preparation and 3–5% direct cost through manual weeding or roguing, incurred for controlling the weed. The farmers may get a return of US$105–374 ha^{-1} if the weed is controlled properly. An integrated approach including the use of clean certified seeds, sequential tillage for land preparation, burning of rice straw on the field, spraying of herbicides as a pre-planting procedure, manual weeding at the mid stage of rice growth and the use of transgenic herbicide-resistant rice cultivars, are suggested options to control weedy rice populations. The practice of water seeding and transplanting of rice seedlings instead of dry seeding and the rotation of rice crop with other upland crops like soybean and maize are recommended practices to effectively manage the weedy rice problem.

Introduction

Weedy rice is characterized by undesirable early shattering biotypes that are morphologically very similar to cultivated rice varieties and can cause severe yield reduction in rice crops (Azmi *et al*. 1994). Weedy rice is known by different names such as, 'padi angin' in Malaysia, 'lua lon' in Vietnam, 'lutao' in China, 'akamai' in Japan, 'sharei' in Korea, 'khao pa' in Laos, 'khao nok' in Thailand, 'jhora dhan' in Bangladesh and 'red rice' in USA and Canada (Watanabe *et al*. 1996, Watanabe *et al*. 2000). Weedy rice consists of a range of biotypes belonging to numerous species. The wild relatives of rice with the AA genome can hybridize with cultivated rice (*O. sativa* var. *sylvatica*) and can evolve into weedy types (Catling 1992).

The wild species *O. barthii* and *O. longistaminata* or weedy biotypes from the cultivated *O. glaberima* are among the most noxious weeds in West Africa and the Sahel region of Africa (Ferrero 2003a). In Vietnam and the South-East Asian countries, the species *O. granulata, O. officinalis, O. rufipogon* and *O. nivara* are sometimes classified as weedy rice (Ferrero 2003a). Red rice (*O. sativa* f. *spontanea*) is the most common weedy biotype of cultivated rice in all rice growing areas in temperate regions (Ferrero 2003a). This weedy rice has red pericarp and dark coloured grains. In temperate areas, red rice is observed as late maturing, tall plants with pubescent leaves and hulls, a longer seed dormancy period and easy seed shattering. *O. latifolia* (locally called 'Arroz pato') is a taller (2 m height) weedy rice species that is widespread in Central America (Castro-Espitia 1999). Both the weedy rice and cultivated rice have the same number of chromosomes (2n = 24, genome = AA). However, some *Oryza* species e.g. *O. minuta* and *O. ridleyi* are tetraploid, having 48 chromosomes, they are less weedy in nature, and some have a different genome e.g. *O. officinalis* (2n = 24, genome = CC). (Table 1) (Oka 1990, Gressel 1999). Ten different genome types of *Oryza* species are AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH, and JJKK. The wild relatives of rice with different genome types have significant reproductive isolation and usually do not hybridize under natural conditions. The wild species with AA genome are of most concern because they are responsible for gene flow to cultivated rice (Lu 2004). The species *O. sativa* hybridizes and produces viable offspring close to the wild relatives,