

## Application Timing Affects S-metolachlor Bioavailability in Soil

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### ABSTRACT

A bioassay, based on the response of wheat roots, was used to quantify the concentrations of bioavailable S-metolachlor when applied at different timings. The application timings of S-metolachlor (0.48 kg/ha) were 20 days before crop sowing (DBS), at crop sowing (AS) applied either post-sowing pre-emergence (PSPE) or incorporated by sowing (IBS). The upper 0 to 5 cm soil layer was sampled from all treatments at 0, 8, 14, 23 and 33 days after crop sowing (DAS). The concentration of bioavailable S-metolachlor was similar between the application timings AS (IBS) and AS (PSPE) at both 0 DAS (94 to 96%) and 8 DAS (86 to 89%). After this period, herbicide bioavailability was significantly greater in the AS (IBS) than AS (PSPE). The bioavailability of S-metolachlor was always greater for the herbicide applied AS (IBS) than applied at 20 DBS. The bioavailability of the herbicide applied at 20 DBS was 55% of the original applied herbicide at seeding. On the last sampling time (33 DAS) the bioavailability of S-metolachlor was 45, 27 and 28% of the original amount applied for application timings of AS (IBS), AS (PSPE) and 20 DBS, respectively. The implications of this information for weed management strategies are discussed.

**Key words :** Herbicide dissipation, application time, crop injury, weed control efficiency

### INTRODUCTION

Annual ryegrass (*Lolium rigidum* Gaud.) is the most important weed of the cropping systems in southern Australia. In this region, herbicides are the main weed control techniques used. Trifluralin (a soil-applied herbicide) is being widely used for the control of this weed; however, cases of resistance have been reported against this herbicide (McAlister *et al.*, 1995). With several more cases of trifluralin resistance being discovered, there is a need to find an effective herbicide with a different mode of action.

S-metolachlor, a chloroacetamide herbicide, is widely used in many crops to control annual grasses and some annual broadleaf weeds (Mueller *et al.*, 1999; Rouchaud *et al.*, 1999). In Australia, this herbicide is not recommended for annual ryegrass control in wheat (*Triticum aestivum* L.) due to phytotoxic effects on the crop when used at rates needed to control annual ryegrass. However, changing the application timing from at or immediately after planting to pre-plant could reduce the damage to wheat. For example, S-metolachlor could be applied well before crop sowing, so that it dissipates to a level that is safe for the crop as well as effective on annual ryegrass. The present study was undertaken to quantify the impact of herbicide application timings on the bioavailability of S-metolachlor in the soil.

### MATERIALS AND METHODS

#### Experimental Details

An experiment was conducted during the growing season of 2005 at the Roseworthy Campus Farm of the University of Adelaide, South Australia. The soil texture at the site was clay loam with 2.6% organic matter and a pH of 7.5. Wheat (Krichauff variety) was sown at the rate of 90 kg/ha under no-till conditions with 16-mm wide knife-point openers. S-metolachlor was applied 20 days before crop sowing (DBS) or at sowing (AS) either incorporated by sowing (IBS) or post sowing pre-emergence (PSPE) at the rate of 0 (control), 0.48 and 0.96 kg/ha. The herbicide was applied using a 5-m wide boom sprayer that delivered 100 l/ha spray solution through flat fan nozzles at a spray pressure of 200 kPa, mounted on a four-wheel motorbike (speed–5 km/h). The experiment was arranged in the field in a split plot design with application timing as the main plots and dose of S-metolachlor as the sub-plots. There were three replicates of each treatment.

#### Soil Sampling

The soil samples were taken from the field experiment treated with S-metolachlor at 0.48 and 0.96

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kg/ha and from application timings of 20 DAS, AS (IBS) and AS (PSPE). The upper 0 to 5 cm soil layer was sampled from all replications at 0, 8, 14, 23 and 33 days after crop sowing (DAS), taking 10 4.5 cm-diameter cores randomly from each plot. During sampling in the field, the soil samples were placed in sealed plastic bags and stored temporarily in Styrofoam boxes containing ice. The soil samples were frozen at -18°C until analysis.

### Bioassay Procedure

A bioassay experiment was conducted in a naturally-lit glasshouse to determine the bioavailability of S-metolachlor in the field. The temperature in the glasshouse fluctuated between  $25 \pm 5^\circ\text{C}$  during the day and  $15 \pm 5^\circ\text{C}$  during the night. Preliminary experiments were conducted to develop a suitable bioassay technique that was simple to use (after Chauhan *et al.*, 2006). Wheat, oat [*Avena sativa* (L.) cv. Swan], annual ryegrass and canary grass (*Phalaris aquatica* L.) were tested by germinating seeds in pots containing the herbicide ranging from 0 to 0.64  $\mu\text{g/g}$  soil. Assuming a bulk soil density of 1.4  $\text{g/cm}^3$  (Chauhan *et al.*, 2006), the dose of the applied S-metolachlor at 0.48 and 0.96 kg /ha was calculated to be equivalent to 0.32 and 0.64  $\mu\text{g/g}$  soil, respectively. Wheat was the only species found to be suitable for the bioassay; therefore, inhibition of wheat root growth was selected as the bioassay criterion to study the bioavailability of S-metolachlor.

The frozen soil samples were thawed for 6 h at room temperature before placing in the pots. The soil sample from each plot was thoroughly mixed and 550 g samples were placed in 600-ml clear-plastic pots. The pots had holes at the bottom and aluminium foil was wrapped around the pots to eliminate light effects on the roots. Concurrently, a standard curve of wheat root response to increasing S-metolachlor dose was developed. Non-treated soil that had been air-dried was used to prepare standards between 0 and 0.64 mg S-metolachlor/g soil.

Six wheat seeds were seeded in each pot at a depth of 0.5 cm and a thin layer of non-treated soil was spread on the surface. The pots were then placed in the glasshouse and arranged in a randomised complete block design with three replications. Wheat roots were washed from the soil 21 DAS when seedlings in the non-treated soil had reached the 2.5 to 3-leaf stage. The four most uniform seedlings from each pot were used to measure their root length. Wheat roots were scanned with a flatbed

scanner and total root length per plant determined with the WinRHIZO program (after Chauhan *et al.*, 2006).

### Statistical Analysis

The bioavailability experiment was conducted twice, and the data were combined for analyses as there was no interaction between experiment and treatment. An exponential decay curve,  $RL = a \times e^{-bx}$ , was fitted to the mean root length of the plants grown at known herbicide concentrations (standards) by using SigmaPlot, where  $RL$  represents the root length at bioavailable concentration of herbicide  $x$ , and  $a$  and  $b$  are the fitted constants. Bioavailable concentrations of herbicide present in the field soil were estimated by fitting the data for root length (of herbicide-treated soil from the field) to the equation. The estimated bioavailable concentrations of herbicide were then converted to the percentage of the original applied amount. The data were analysed by using two-way ANOVA in a randomised block design with application timing and sampling time (DAS) as the two factors. GenStat was used for statistical analysis of all data (GenStat 8.0, 2005).

## RESULTS AND DISCUSSION

Herbicides in soils are measured with several methods, including high-performance liquid chromatography, gas chromatography and antibodies (Cabras and Melis, 1991; Krause and Niemczyk, 1992; Garimella *et al.*, 2000). Herbicide recovery from soil required for analytical techniques can vary with herbicide concentration, extraction method, and soil type (Garimella *et al.*, 2000). In any case, weed control is not based on the total amount of herbicide present but on its bioavailability. Therefore, a bioassay is an effective procedure to determine the bioavailability of an herbicide in the soil ( Zimdahl and Clark, 1982; Bunting *et al.*, 2003; Parker *et al.*, 2005).

In a preliminary experiment, different species were grown at concentrations of 0 to 0.64  $\mu\text{g}$  S-metolachlor/g soil. Oats were found to be tolerant, and both annual ryegrass and canary grass sensitive to the tested concentrations of S-metolachlor (data not shown). Only wheat was found to be suitable for the bioassay as it gave a quantitative response to herbicide dose. Wheat roots were found to be more sensitive to S-metolachlor inhibition and with less variability between individuals compared to shoots. Therefore, inhibition of root growth

was selected as the bioassay criterion to study the bioavailability of S-metolachlor.

A standard curve was constructed by incorporating known amounts of S-metolachlor (0 to 0.64  $\mu\text{g/g}$  soil) into the non-treated soil from the field site (Fig. 1). The concentrations of 0.32 and 0.64  $\mu\text{g}$  S-metolachlor/g soil were equivalent to the field application of 0.48 and 0.96 kg/ha. In the standard curve, 50% inhibition of wheat root growth occurred between 0.08 and 0.16  $\mu\text{g}$  S-metolachlor/g soil. The bioassay was conducted on soil sampled from plots treated with both rates (0.48 and 0.96 kg/ha) of S-metolachlor applied in the field; however, wheat roots were very sensitive in the soils treated with 0.96 kg /ha. Therefore, data are shown only for soil sampled from plots treated with 0.48 kg/ha S-metolachlor.

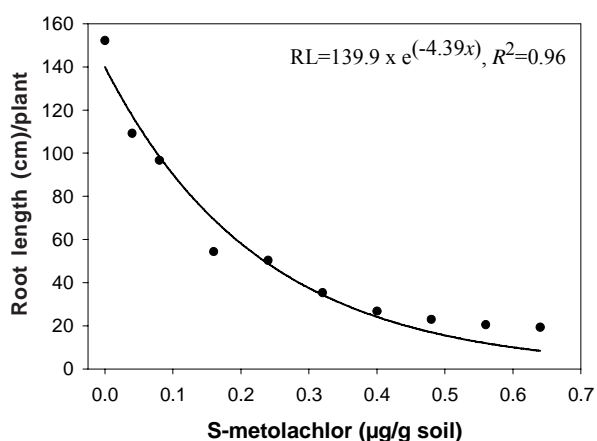


Fig. 1. Exponential relationship between the dose of S-metolachlor (0 to 0.64  $\mu\text{g/g}$  soil) and root length of wheat in the bioassay.

The bioavailable S-metolachlor in the field soil was estimated by fitting the root length data to the equation (Table 1). This bioavailable S-metolachlor was then converted to per cent bioavailability of the applied herbicide (Fig. 2). The concentration of bioavailable S-metolachlor was similar between the application timings AS (IBS) and AS (PSPE) at both 0 DAS (94 to 96%) and 8 DAS (86 to 89%). After this period, herbicide bioavailability was significantly greater in the AS (IBS) than AS (PSPE). As the herbicide applied AS (PSPE) was not incorporated, a greater portion of the herbicide would have been present on the soil surface and vulnerable to photodegradation losses. The bioavailability of S-metolachlor was always greater for the herbicide applied AS (IBS) than applied at 20 DBS. The bioavailability of the herbicide applied at 20 DBS was

Table 1. Wheat root length and bioavailable S-metolachlor determined by bioassay. The applied S-metolachlor [DBS—days before crop sowing; AS (PSPE)—at sowing applied post-sowing pre-emergence; AS (IBS)—at sowing incorporated by sowing] concentration was equivalent to 0.32  $\mu\text{g/g}$  soil. The soil samples were taken at different times after crop sowing (DAS)

Time (DAS)	Wheat root length			Bioavailable S-metolachlor		
	AS (PSPE)	AS (IBS)	20 DBS	AS (PSPE)	AS (IBS)	20 DBS
	-----cm/plant-----			----- $\mu\text{g/g}$ of soil-----		
0	36.6	37.7	64.7	0.31	0.30	0.18
8	40.6	41.7	68.8	0.28	0.28	0.16
14	62.8	53.4	73.0	0.18	0.22	0.15
23	87.0	58.0	77.8	0.11	0.20	0.14
33	96.3	75.1	94.4	0.09	0.14	0.09

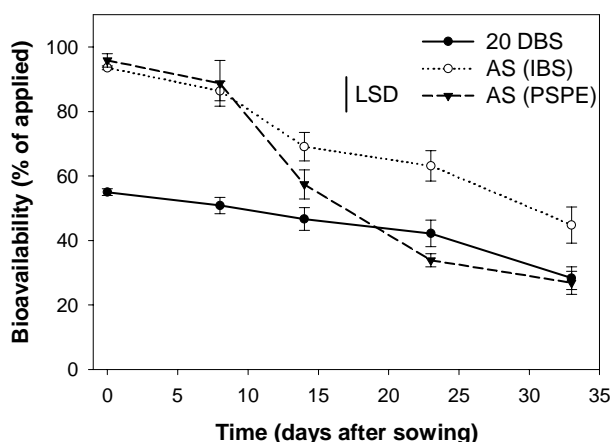


Fig. 2. Bioavailability of S-metolachlor (% of applied) when applied at different times [DBS—days before crop sowing; AS (PSPE)—at sowing applied post-sowing pre-emergence; AS (IBS)—at sowing incorporated by sowing]. A bioassay was used to determine the bioavailability. The applied S-metolachlor concentration was equivalent to 0.32  $\mu\text{g/g}$  soil. Vertical bars with caps represent standard error.

55% of the original applied herbicide at seeding. On the last sampling time (33 DAS) the bioavailability of S-metolachlor was 45, 27 and 28% of the original amount applied for application timings of AS (IBS), AS (PSPE) and 20 DBS, respectively.

Lower amount of bioavailable S-metolachlor in the 20 DBS treatment at the time of sowing (0 DAS) could be due to photodegradation losses occurring since its application (Mathew and Khan, 1996). Photodegradation could be major contributor to dissipation in the field, particularly under prolonged lack of rainfall

when the herbicide remains on the soil surface rather than being incorporated by rainfall.

Despite the bioavailable S-metolachlor declining by 45% between application at 20 DBS and seeding (Fig. 2), effective control (79%) of annual ryegrass was found in the field (Table 2; Chauhan *et al.*, 2007). This suggests the possibility that S-metolachlor may be absorbed into seeds of annual ryegrass during the pre-seeding period. Seedling emergence of wheat in the field was not affected when the herbicide was applied 20 DBS, whereas it was significantly reduced when the herbicide applied at sowing [AS (IBS) or AS (PSPE)] compared to the non-treated control (Table 2; Chauhan, 2006; Chauhan *et al.*, 2007). Although S-metolachlor applied at crop sowing provided an effective control of annual ryegrass, it was phytotoxic to wheat.

Table 2. Effect of S-metolachlor (0.48 kg/ha) application timing [DBS-days before crop sowing; AS (PSPE)–at sowing applied post-sowing pre-emergence; AS (IBS)–at sowing incorporated by sowing] on the relative emergence of annual ryegrass (ARG) and wheat (cited by Chauhan, 2006; Chauhan *et al.*, 2007). The same letters within the column represent non-significant difference with the control on the basis of LSD at P≤0.05

Application timing	ARG emergence ------(relative to the control plot)-----	Wheat emergence
AS (IBS)	0.18 <sup>a</sup>	0.56 <sup>b</sup>
AS (PSPE)	0.17 <sup>a</sup>	0.42 <sup>b</sup>
20 DBS	0.21 <sup>a</sup>	0.93 <sup>a</sup>

In conclusion, S-metolachlor at 0.48 kg /ha could be safely applied around 20 DBS so that its bioavailability dissipates to a level that is safe for the crop as well as effective on annual ryegrass. Pre-seeding application of S-metolachlor has the potential to increase the speed of crop seeding operations, as there may not be a requirement to apply an herbicide at seeding. Above all, it may provide a means of effective control of herbicide-resistant annual ryegrass.

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