Weed Biology and Management

Weed Biology and Management **11**, 64–71 (2011)

RESEARCH PAPER

Response of wild barley (*Hordeum spontaneum*) and winter wheat (*Triticum aestivum*) to sulfosulfuron: The role of degradation

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Wild barley (*Hordeum spontaneum*) is one of the most troublesome weed species in winter wheat (*Triticum aestivum*) in Iran. Two bioassay experiments were conducted in order to study the response of wild barley and wheat to different herbicides and to study the efficacy of pre-emergence (PRE), postemergence (POST), and PRE followed by POST applications of sulfosulfuron on wild barely. Moreover, the degradation of sulfosulfuron was studied by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The results showed that wild barley was highly tolerant to clodinafop-propargyl and its dry weight was reduced by only 15%, compared to the control, at the recommended dose (64 g ai ha^{-1}). Sulfosulfuron reduced the wild barley biomass by ≤50% at the highest dose (90 g ai ha^{-1}) in the first bioassay but by not more than 20% and 12% at the recommended dose (22 g ai ha^{-1}) in the first and second bioassay, respectively. Significant differences were found among the application methods of sulfosulfuron, with the POST application being the least effective method. In contrast to the POST application, wild barley was severely injured by the PRE application of sulfosulfuron, with an ED_{50} dose of 7.3 g ai ha^{-1}. The degradation study showed that wild barley can metabolize sulfosulfuron that is applied POST, but at a lower rate than wheat. By 4 h after application, wild barley had metabolized 26% of the sulfosulfuron, compared to 46% by wheat. In conclusion, wild barley can metabolize the recommended dose of sulfosulfuron that is applied POST; thus, the PRE application of sulfosulfuron or other integrated methods should be considered for the effective control of wild barley in wheat.

Keywords: herbicide degradation, sulfosulfuron, wheat, wild barley.

Wild barley (*Hordeum spontaneum* Koch.), the wild ancestor of domesticated barley (*Hordeum vulgare* L.) (Zohary & Hopf 2000), is among the most common and most troublesome weed species in wheat (*Triticum aestivum* L.) in Iran (Montazeri *et al.* 2005). Wild barley is now present in >16 provinces in Iran (Baghestani *et al.* 2007) and poses a threat to wheat production. The germination, growth, and development of wild barley is similar to wheat; thus, there are few effective control options in wheat. In contrast to other common weeds, wild barley is not considered as a problematic weed globally and only a few studies have been published on wild barley (Zand *et al.* 2007; Baghestani *et al.* 2008). Presently, there is no herbicide that is registered for the selective control of wild barley in wheat. Indeed, wild barley tolerates most of the wheat-selective herbicides at their recommended dose. Currently, sulfosulfuron and sulfosulfuron plus metsulfuron-methyl are considered to be the most effective herbicides for controlling wild barley in wheat (Zand *et al.* 2007; Baghestani *et al.* 2008). They both belong to the class of sulfonylurea herbicides, which inhibit the acetolactate synthase (ALS) enzyme.

Communicated by Y. Yogo.
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Received 10 July 2010; accepted 16 January 2011

doi:10.1111/j.1445-6664.2011.00410.x

However, higher-than-recommended doses are required to achieve appropriate control. Sulfosulfuron can be applied pre-emergence (PRE) and some experiments have shown a higher efficacy level of PRE, compared to postemergence (POST), application in wheat (Blackshaw & Hamman 1998) and tomato (Eizenberg *et al*. 2003). However, crop injury and rotational restrictions must be considered (Kelley & Peeper 2003; Lyon *et al*. 2003) when applying sulfosulfuron PRE.

The rapid metabolism of clodinafop-propargyl (Kreuz *et al*. 1991), various sulfonylurea herbicides (King *et al*. 2003; deBoer *et al*. 2006), and sulfosulfuron (Olson *et al*. 2000) has been cited as the main mechanism of herbicide selectivity in wheat. Also, there are some reports that barley can metabolize clodinafop-propargyl (Kreuz *et al*. 1991), metsulfuron-methyl (Anderson *et al*. 1989), and AE F13006003 (a mixture of two sulfonylurea herbicides, mesosulfuron-methyl plus iodosulfuron-methyl-sodium) (King *et al*. 2003). Due to the close similarities of wild barley to wheat and barley, it can be assumed that the response of wild barley to different studied herbicides is related to the metabolism rate of these herbicides in wild barley to unlethal metabolites.

The objectives of this study were to: (i) evaluate the response of wild barley and wheat to three different herbicides under greenhouse conditions; (ii) determine the effectiveness of PRE, POST, and PRE followed by POST (PRE + POST) applications of sulfosulfuron in the control of wild barley; and (iii) to study the degradation of sulfosulfuron in wheat and wild barley by using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

MATERIALS AND METHODS

Greenhouse studies were conducted during 2008 and 2010. The first bioassay, conducted at Ferdowsi University of Mashhad, Mashhad, Iran, examined the response of wild barley and wheat to different herbicides. A second bioassay was conducted at the Department of Integrated Pest Management, Aarhus University, Slagelse, Denmark, to study the efficacy of the PRE, POST, and PRE + POST applications of sulfosulfuron on wild barely and thus it was an experiment that studied the metabolism of sulfosulfuron in wheat and wild barley.

Bioassays

In the first bioassay, wild barley seeds were collected from a heavily infested wheat field in Zahedshahr, Fars province, in the south-west of Iran. Eight seeds of wild barley and wheat (cv. Chamran; Mashhad, Khorasan razavi, Iran) were sown 1 cm deep in 15 cm diameter plastic pots (1 L) that were filled with a manure–loam–sand mixture (1:2:1 by volume). The pots were kept in a greenhouse with a photoperiod of 16/8 h light/darkness and with a temperature of ~20°C during the day. The pots were watered as required until harvest. Prior to the herbicide application, the plants were thinned to four uniformly sized plants per pot. The pots were arranged in a completely randomized design with three replicates. The herbicides were applied to wild barley and wheat at dosages that were proportional to 0.25–5.0-fold the recommended dose on the labels. Clodinafop-propargyl (Topic, 80 g L^{-1} EC; Syngenta, Basel, Switzerland) was applied at rates of 0, 16, 32, 64, 100, 240, and 400 g ai ha^{-1}. Sulfosulfuron (Apirus, 75% WG; Monsanto, USA) was applied at rates of 7.5, 15, 22.5, 30, 60, and 90 g ai ha^{-1} and sulfosulfuron plus metsulfuron-methyl (Total 75% + 15% WG; United Phosphorus, India) was applied at rates of 9, 27, 45, 67.5, 90, and 180 g ai ha^{-1}. The untreated pots served as the controls. All the herbicide treatments were applied at the two-to-three leaf stage by using a laboratory sprayer (MATABI Elegance plus; Mashhad, Iran), equipped with a flat-fan nozzle (8001; Mashhad, Iran) that was calibrated to deliver 250 L ha^{-1} of spray solution at 200 kPa. The plants were harvested 3 weeks after the herbicide application, dried for 72 h, and weighed. The relative dry weight was expressed as a percentage of the untreated control.

A second bioassay was conducted on wild barley, using the same seed stock as in the first bioassay. Field soil (sandy loam) was used in this experiment. The seeds were sown 1 cm deep in 1 L pots and then the pots were irrigated from the bottom. Sulfosulfuron (Monitor, 80% WG; Monsanto) was applied PRE, POST, and PRE + POST at rates of 10, 20, 40, 80, and 160 g ai ha^{-1} in a mixture with a non-ionic surfactant (Agropol, AgroDan, Fanoe, Denmark) at 0.2% (v/v). As the same formulation of the first bioassay (Apirus, 75% WG) was not available in Denmark, Monitor (80% WG) was used in the second bioassay and also in the degradation study. In the POST treatments, prior to the herbicide application, the plants were thinned to four uniformly sized plants per pot. The untreated plants were included for each method of application. The experimental design was a completely randomized design with three replicates of each treatment. For the PRE and PRE + POST applications, the herbicide was applied to the soil surface 2 days after sowing but before plant emergence. Following the herbicide application, 20 mL water were added to the soil surface to ensure an equal distribution of the herbicide in the upper soil layer. For the PRE + POST applications, 50% of the dose was applied PRE and 50% was applied POST. The POST treatments were applied at the two-to-three leaf

stage of wild barley. All the treatments were applied with a cabinet sprayer that was equipped with a boom and two flat-fan nozzles (ISO Hardi; Jens Kristensen, Ringsted, Denmark), delivering a spray volume of 163 L ha^{-1}. The pots were placed in a glasshouse (minimum temperature of 14°C), with supplemental light extending the photoperiod to 16 h per day. All the plants were irrigated from the bottom throughout the experiment. The plants were harvested 4 weeks after the herbicide application in the PRE and POST treatments and 4 weeks after the POST application in the PRE + POST treatment. Then, the foliage fresh and dry weights were recorded.

In both bioassay experiments, the four-parameter log-logistic model (eqn 1) was fitted to the data by using the open-source statistical software, R 2.6.2 (R Development Core Team 2006), utilizing the *drc* statistical addition package (Knezevic *et al.* 2007):

$$Y = c + (d - c / 1 + \exp[b(\log x - \log e)]), \quad (1)$$

where Y is the response that is expressed as a percentage of the untreated control, c and d are asymptotic values of Y at the lower and upper limits, respectively, b is the slope of the curve around the point of inflection, and e is the herbicide rate giving a response halfway between d and c (the ED_{50} for plant dry weight). If $c = 0$, then the four-parameter model reduces to the three-parameter model (eqn 2), with the lower limit being zero:

$$Y = d / 1 + \exp(b[\log x - \log e]). \quad (2)$$

The goodness of fit was evaluated by using lack-of-fit F-tests. The ED_{25}, ED_{50}, and ED_{90} doses were estimated for each treatment. The assumption of parallel dose–response curves was assessed by comparing the residual sum of the squares of the regressions assuming non-parallel and parallel dose–response curves.

Degradation study

Reagents and chemicals

All the reagents and solvents that were used were of gradient grade (Merck, Darmstadt, Germany) and high-purity water for the analysis was obtained in-house with a MilliQ system (Millipore Corporation, Bedford, MA, USA). Sulfosulfuron (95.5% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

Plant material and herbicide application

The study was conducted on wild barley and wheat in a completely randomized design with three replicates. The seeds were sown 1 cm deep in 1 L pots. Then, the pots were irrigated from the bottom. After emergence, eight plants in each pot were kept for both wheat and wild barley. At the two-to-three leaf stage, the plants were sprayed with commercially formulated sulfosulfuron (Monitor, 80% WG) at 24 g ai ha^{-1}. Although the plant density was higher than in the bioassay studies, there was no effect on the herbicide retention or plant growth during the degradation study period. Sulfosulfuron was applied with a cabinet sprayer that was equipped with a boom and two flat-fan nozzles (ISO Hardi), delivering a spray volume of 157 L ha^{-1}. After the herbicide application, all the plants were returned to the greenhouse until harvest time. Whole plants were harvested 1, 4, 8, and 24 h after treatment (HAT) for both wild barley and wheat. At each harvest time, 2 g of plant material were dipped into a jar containing 40 mL of wash solution (0.1% of a non-ionic surfactant [Contact, Jens Kristensen, Ringsted, Denmark] and 10% (v/v) methanol). The jar was gently shaken for 1 min to wash off the unabsorbed herbicide (Devine *et al.* 1984). A 10 mL aliquot of the wash solution was assayed for the unabsorbed herbicide. Then, the plant material was blotted dry on paper towels, frozen in liquid nitrogen, and pulverized with a mortar and pestle. Next, 1.5 g of the homogenized sample was transferred to a 50 mL polypropylene conical tube (Falcon; Becton Dickinson Labware, NJ, USA) and 5 mL of methanol were added to each tube to stop further degradation. Blank samples of each plant were obtained from the untreated control pots. The samples were stored immediately at −20°C until their analysis.

The spray retention experiment was conducted with a dye method (Xie *et al.* 1995; Nelson & Penner 2006) in order to determine the initial amount of herbicide deposition on each plant species, with eight replicates. Prior to the herbicide application, the number of plants in each pot was reduced to one. The spray solutions consisted of sulfosulfuron (Monitor, 80% WG) in a mixture with 0.2% non-ionic surfactant (Contact, AgroDan, Fanoe, Denmark) and brilliant-sulfoflavin, a fluorescent dye, at a concentration of 1 g L^{-1}. After the spray deposits had dried, the plants were cut at soil level, placed in a 60 mL jar containing 40 mL of a solution of 0.1% of the non-ionic surfactant, and shaken for 1 min to wash the dye from the plant. Within 12 h, the dye concentrations were quantified by using a spectrophotometer (Hewlett Packard, Allerød, Denmark [the equipment is a Hewlett Packard HP1100]) with excitation and emission wave lengths of 410 nm and 518 nm, respectively. A single standard curve was used to determine the micrograms of dye that were retained. The equation for the standard curve was linear ($R^2 = 0.99$). The foliage dry weight of each plant was determined following drying at 70°C for 48 h. The level of spray retention was expressed in μg of spray solution retained per g of plant dry weight. The data

Table 1. Estimated ED_{50} and ED_{25} doses for wild barley (*Hordeum spontaneum*) and wheat (*Triticum aestivum*) in response to different herbicides

Herbicide	ED_{50}† (g ai ha^{-1})		ED_{25}† (g ai ha^{-1})	
	Wild barley	Wheat	Wild barley	Wheat
Clodinafop-propargyl	>400.0	>400	88.3 (7.53)	>400.0
Sulfosulfuron	90.5 (9.96)	>90	31.8 (5.12)	51.2 (13.41)
Sulfosulfuron plus metsulfuron-methyl	83.0 (15.03)	>180	20.6 (6.94)	>180.0

† ED_{25} and ED_{50} refer to the herbicide dose that is required for a 25% and 50% biomass reduction, compared to the untreated control, respectively. The values in parentheses represent the standard error.

were subjected to an ANOVA and the means were separated by using the Least Significant Difference test with a 5% level of probability. As the plants could not be dried before analyzing the parent compound with LC-MS/MS in the degradation study, the dry matter content of each plant species was determined by measuring the fresh and dry weights of 20 individual plants from each species.

Extraction of the parent herbicide from the plant tissue

After removing the samples from the refrigerator, 10 mL of methanol were added to each Falcon tube and the tubes were mixed gently and put in an ultrasonic bath (ABC-Sonic; ABC Hansen Engineering, Hørsholm, Denmark) for 15 min. After shaking for 1 h on an automatic shaker, the samples were centrifuged for 20 min at 4000 rpm (3300 g) on a centrifuge (Multifuge 3S-R; Heraeus, Osterode, Germany). Then, 500 µL supernatant from each tube was transferred to another tube that was diluted with 500 µL MilliQ water. The final volume (1 mL) of each sample was filtered through a 0.45 µm filter and analyzed with LC-MS/MS.

Liquid chromatography coupled with tandem mass spectrometry

The chromatographic separation was carried out with a chromatograph (1100; Hewlett-Packard, Allerød, Denmark) with gradient elution. First, 10 µL were injected on a 250 mm × 2.1 mm, 5 µm column (BDS Hypersil C18; Thermo Electron Corporation, Waltham, MA, USA). The A-eluent was 99% 10 mM ammonium acetate and 1% methanol and the B-eluent was 10% 10 mM ammonium acetate and 90% methanol. The MS/MS detection was carried out with an instrument (Applied Biosystems Sciex API 2000, tandem mass spectrometer, Toronto, Canada) in positive multiple reaction monitoring (MRM) ionization mode for sulfosulfuron (m/z 471 → 211). The detection limits were 5 µg kg^{-1} for sulfosulfuron.

RESULTS AND DISCUSSION

Bioassays

In the first bioassay, wild barley was highly tolerant to clodinafop-propargyl and its dry weight was reduced by only 15%, compared to the control, at the recommended dose (64 g ai ha^{-1}). At the highest dose (400 g ai ha^{-1}), the biomass reduction of wild barley and wheat was 33% and 9.5%, respectively (data not shown). Therefore, only the ED_{25} doses could be estimated (Table 1). Zand *et al.* (2007) also reported that the application of clodinafop-propargyl at the recommended dose resulted in only a minor biomass reduction of wild barley in the wheat field. The safener, cloquintocet-mexyl (CGA 185072), selectively protects cereals from the herbicide, clodinafop-propargyl. Kreuz *et al.* (1991) reported that the safener accelerated the metabolism of clodinafop-propargyl in wheat and, to a lesser degree, in barley, but not in maize.

Sulfosulfuron reduced the wild barley biomass by ≤50% at the highest dose (90 g ai ha^{-1}), but by not more than 20% at the recommended dose (22 g ai ha^{-1}) (data not shown). Wild barley was highly tolerant to sulfosulfuron, but was more susceptible than wheat at higher doses. The ED_{25} and ED_{50} doses for wild barley were 31.8 and 90.5 g ai ha^{-1}, respectively (Table 1). Baghestani *et al.* (2008) reported that different populations of wild barley responded differently to the POST application of sulfosulfuron but that, in most cases, doses higher than the recommended dose were required to effectively control wild barley in wheat. The wheat biomass was reduced by ≤30% by the highest dose (90 g ai ha^{-1}, which is fourfold higher than the recommended dose) (data not shown).

Sulfosulfuron plus metsulfuron-methyl reduced the wild barley biomass by 32% at the recommended dose (45 g ai ha^{-1}) and by ≤61% at the highest dose (180 g ai ha^{-1}), while the wheat biomass was reduced by 21% at the highest dose (data not shown). The ED_{25} and

Table 2. Regression parameters and estimated ED_{25}, ED_{50}, and ED_{90} doses for wild barley (*Hordeum spontaneum*) in response to different application methods of sulfosulfuron

Application method	b†	c‡	d‡	ED_{25}§ (g ai ha^{-1})	ED_{50}§ (g ai ha^{-1})	ED_{90}§ (g ai ha^{-1})
PRE	1.5 (0.13)	10.8 (1.66)	99.8 (1.62)	3.5 (0.39)	7.3 (0.56)	31.7 (4.27)
PRE + POST	1.5 (0.13)	10.8 (1.66)	99.8 (1.62)	8.5 (0.7)	17.8 (1.07)	77.3 (11.40)
POST	–	–	–	–	>160.0	>160.0

† b is the slope around the ED_{50}; ‡ c and d are the upper and lower asymptotes at zero and very high doses; §the ED_{25}, ED_{50}, and ED_{90} refer to the herbicide dose that is required for a 25%, 50%, and 90% biomass reduction, compared to the untreated control, respectively. The values in parentheses represent the standard error. POST, postemergence; PRE, pre-emergence; PRE + POST, pre-emergence followed by postemergence.

ED_{50} doses for wild barley were 20.6 and 83.0 g ai ha^{-1}, respectively (Table 1). Zand *et al.* (2007) reported that, among several herbicides, sulfosulfuron plus metsulfuron-methyl was the only effective one on wild barley, reducing its biomass by ≤67% in wheat. Although it was more effective on wild barley and caused less injury to wheat, wild barley was relatively tolerant to the recommended dose.

In the second bioassay, significant differences were found between the application methods, with the POST application being the least effective method. In comparison to the previous experiment, wild barley was more tolerant to the POST application of sulfosulfuron, with a biomass reduction of only 31% at the highest dose. Therefore, the ED_{50} dose for the POST application could not be estimated (Table 2). Similar results have been reported by Baghestani *et al.* (2008), who found no more than a 30% biomass reduction of wild barley in some regions of Iran.

The PRE application of sulfosulfuron resulted in a biomass reduction of wild barley of 70% at the recommended dose (Fig. 1). In contrast to the POST application, wild barley was severely injured by the PRE application of sulfosulfuron, with an ED_{50} dose of 7.3 g ai ha^{-1} (threefold less than the recommended dose). The superiority of the preplant-incorporated or PRE application of sulfosulfuron over the POST application has been reported for wild barley control (Baghestani *et al.* 2008), other weed species in wheat (Blackshaw & Hamman 1998), and tomato (Eizenberg *et al.* 2003). In the present experiment, winter wheat showed a high tolerance to sulfosulfuron when it was applied PRE, even at higher doses (data not shown).

In the PRE + POST treatment, the wild barley biomass was reduced by ~47% at the recommended dose, which is lower than that in the PRE treatment (Fig. 1). Splitting the herbicide dose in this method resulted in less wild barley growth suppression (ED_{50} = 17.8 g ai ha^{-1}), in comparison to the whole dose applied PRE (ED_{50} = 7.3 g ai ha^{-1}), but it still showed significant

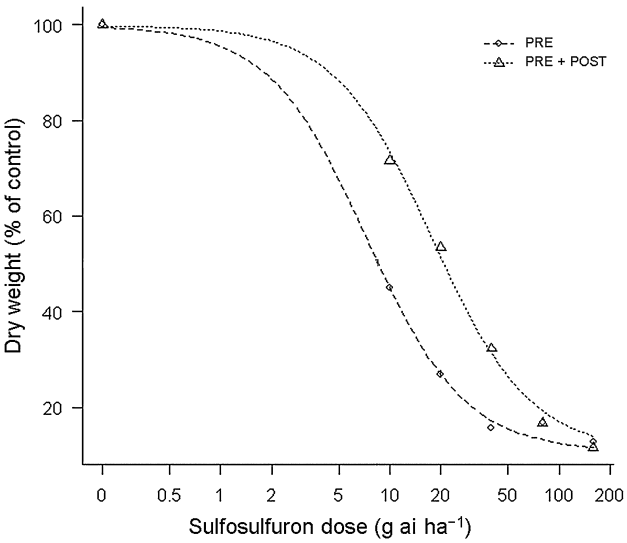

Fig. 1. Biomass response of wild barley (*Hordeum spontaneum*) to pre-emergence (PRE) and pre-emergence followed by postemergence (PRE + POST) sulfosulfuron.

superiority over the POST application (Table 2). The dose–response curves for the PRE and PRE + POST treatments (Fig. 1) were found to be parallel (only differing in their ED parameter); hence, the ratio of doses producing the same effect was independent of the response level.

Degradation study

The amount of spray retention per plant was not significantly different in wheat and wild barley (Table 3). As a result of the lower fresh and dry weights (mg per plant) of wild barley, compared to wheat, the amount of spray retention (expressed as μg g^{-1} fresh or dry weight) was significantly lower in wheat than in wild barley.

In this method, the recovery of sulfosulfuron from the spiked samples was >70%. The results showed that, mostly because of the continuous absorption of the herbicide,

Table 3. Retention of the sulfosulfuron spray solution on wheat (*Triticum aestivum*) and wild barley (*Hordeum spontaneum*)

Plant	Foliage dry weight (mg plant^{-1})	Retention of spray solution			
		μg plant^{-1}	Dry weight (μg g^{-1})	Fresh weight (μg kg^{-1})	Sulfosulfuron† (μg ai kg^{-1} fresh weight)
Wheat	66b‡	14b	208c	32,557c	5009c
Wild barley	28c	11b	393b	44,892a	6906a

† Initial amount of sulfosulfuron retention was estimated for the degradation study; ‡ the means within a column followed by the same letter are not significantly different. The values are the average of eight replications.

Table 4. Amount of sulfosulfuron that was recovered in the wash solution, within the plant tissues, and in the total with LC-MS/MS for wheat (*Triticum aestivum*) and wild barley (*Hordeum spontaneum*)

Plant	Time after treatment (h)	Sulfosulfuron (μg kg^{-1} fresh weight)		
		Wash solution	Plant tissue	Total
Wheat	0†	5008 (101)	–	–
	1	4547 (323)	360 (77)	4907 (398)
	4	4033 (121)	535 (71)	4568 (104)
	8	3827 (418)	463 (67)	4290 (367)
	24	2763 (158)	522 (69)	3285 (204)
Wild barley	0†	6906 (68)	–	–
	1	6193 (143)	555 (2)	6748 (145)
	4	5810 (435)	815 (31)	6625 (427)
	8	4993 (411)	604 (82)	5597 (487)
	24	5360 (390)	796 (80)	6156 (324)

† Values at 0 time were determined by a dye method with eight replications. All the other values are the average of three replications. The values in parentheses represent the standard error.

the amount of sulfosulfuron that was recovered in the wash solution decreased constantly in the two plant species. In contrast, the amount of sulfosulfuron that was recovered from the plant tissues increased within 24 HAT in wheat and wild barley (Table 4).

In contrast to the results from common methods of herbicide absorption, translocation, and metabolism (applying the same initial amount of ^{14}C-herbicide to each plant), in this method, the initial amount of herbicide was significantly different between the plant species. Averaged over harvest intervals, a significantly lower amount of sulfosulfuron was recovered from the wash solution and plant tissues in wheat than in wild barley. As the initial amount of herbicide retention (μg kg^{-1} fresh weight) was different between the plant species, the data of the absorption of sulfosulfuron were expressed as a percentage of the initial herbicide retention for each plant.

The percentage absorption was determined as the difference between the amount of initial spray retention and the amount that was recovered in the wash solution at each time. In both plant species, the level of herbicide absorption was <50% of the applied herbicide over the harvest intervals (Fig. 2). The initial absorption of sulfosulfuron in both plant species was relatively slow, with <20% of the applied herbicide being absorbed at 4 HAT and no significant difference between the plant species being observed. At the later harvest times, however, the level of absorption in wheat was twofold higher than that in wild barley.

Considering the amount of sulfosulfuron that was recovered within the plant tissues (Table 4) and the amount of sulfosulfuron that was absorbed by the plants, the amount of sulfosulfuron that remained within the plant tissues (% of absorbed) was calculated for each harvest time. The amount of sulfosulfuron in wheat

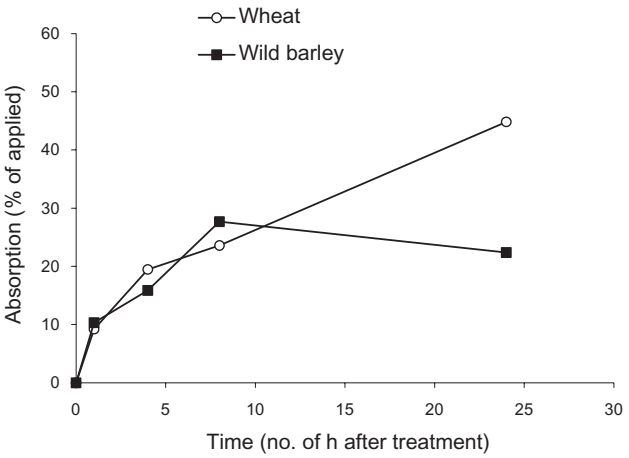

Fig. 2. Absorption of sulfosulfuron in wheat (*Triticum aestivum* L.) and wild barley (*Hordeum spontaneum* L.).

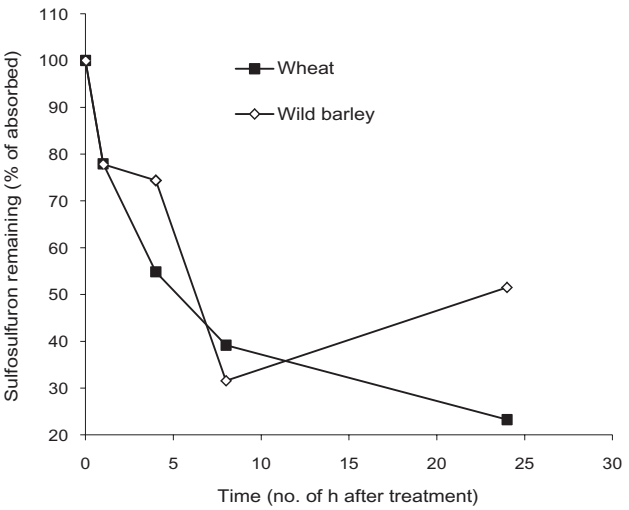

Fig. 3. Degradation of sulfosulfuron in wheat (*Triticum aestivum*) and wild barley (*Hordeum spontaneum*).

decreased more rapidly than in wild barley (Fig. 3). The rapid degradation of sulfonylurea herbicides into non-phototoxic metabolites in wheat have been reported as the main mechanism of herbicide selectivity in wheat (Sweester *et al*. 1982; Olson *et al*. 2000; King *et al*. 2003; Richardson *et al*. 2003; deBoer *et al*. 2006).

The results of this study showed that wild barley can metabolize sulfosulfuron, but at a lower rate than wheat. By 4 HAT, wild barley had metabolized 26% of sulfosulfuron, compared to 46% by wheat (Fig. 3). The metabolism of sulfonylurea herbicides previously has been reported in barley, showing the ability of barley species to metabolize this group of herbicides. King *et al*. (2003) reported that Italian ryegrass (*Lolium multiflorum*) metabolized 34% of AE F13006003 (mesosulfuron-methyl plus iodosulfuron-methyl-sodium) 96 HAT, but that the rate of metabolism was faster in wheat and barley. Anderson *et al*. (1989) reported that metsulfuron-methyl was metabolized very quickly in wheat and barley plant tissues. The faster degradation of sulfosulfuron in wheat than in wild barley was reflected in the bioassay experiments as a lower phototoxicity in wheat at the recommended dose.

The results of the metabolism experiment revealed a higher rate of sulfosulfuron metabolism in wheat than in wild barley. Generally, the primary mechanism of naturally occurring tolerance to ALS inhibitors is the metabolism of the active ingredient that prevents lethal herbicide levels from reaching the target site (Saari *et al*. 1994). Sulfonylurea metabolism also has been reported in susceptible weed species, like the metabolism of chloransulam-methyl in ALS-sensitive smooth pigweed (Amaranthus hybridus) (Poston *et al*. 2001) or florasulam metabolism in hempnettle (*Galeopsis tetrahit* L.), smartweed (*Polygonum lapathifolium*), and cleavers (*Galium aparine* L.) (deBoer *et al*. 2006). However, a more rapid rate of metabolism was reported as the main reason for the higher tolerance of crop species. Wild barley is not susceptible to sulfosulfuron, with no mortality seen at even the highest doses of sulfosulfuron. To date, there is no report regarding herbicide degradation in wild barley. However, considering the literature regarding sulfonylurea metabolism in wheat and the genetic and physiologic similarities of wild barley and wheat, metabolism in wild barley cannot be ruled out. Monaco *et al*. (2004) categorized the 15 grasses into three main groups in response to sulfosulfuron and stated that the generally unresponsive group of grasses (e.g. wheat) rapidly metabolized the herbicide and were highly tolerant to it. But, in the second group, the level of responsiveness was partially dependent on the plants' age and/or stage of development: the plants were less tolerant in the early growth stage, as compared to the late growth stage. The third group included those that were completely susceptible to sulfosulfuron. It seems that wild barley can be categorized into the second group. The results of this study also showed that wild barley was more tolerant to sulfosulfuron at the four-to-six leaf stage than at the two-to-three leaf stage (data not shown).

CONCLUSION

Wild barley showed a tolerance to the POST application of sulfosulfuron at the recommended dose, but was more susceptible to higher doses than wheat. It seems that wild barley, like wheat, can metabolize sulfosulfuron that is

applied POST. Furthermore, this study illustrated the importance of the PRE application of sulfosulfuron for the effective control of wild barley in wheat, but additional research is required to determine the appropriate dose of herbicide for effective weed control with the lowest level of injury to wheat or other rotational crops. Although the use of sulfosulfuron in higher-than-recommended doses could be a solution in heavily infested fields, soil contamination, rotational crop restrictions, and crop injury should be considered. There are several reports that showed no injury of wheat at higher doses of sulfosulfuron (Blackshaw & Hamman 1998), but there are others that reported wheat injury when using higher doses, depending on the environmental conditions (Geier & Stahlman 1996; Kelley & Peeper 2003). Wild barley is naturally tolerant to the majority of wheat-selective herbicides and it seems that integrated approaches are required in order to effectively suppress wild barley in wheat and to reduce seed production.

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