

EVALUATION OF HERBICIDE TREATMENTS FOR CONTROL OF WILD GLADIOLUS (*GLADIOLUS SEGETUM*) IN WHEAT

MAJD, R.¹ – CHAMANABAD, H. R. M.¹ – ZAND, E.² – MOHEBODINI, M.³ – KHIAVI, H. K.⁴ –
ALEBRAHIM, M. T.^{1*} – TSENG, T. M.⁵

¹*Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources
University of Mohaghegh Ardabili, Ardabil, Iran (phone: +98-9123501493)*

²*Department of Weed Research, Iranian Institute of Plant Protection, Tehran, Iran*

³*Department of Horticulture, Faculty of Agriculture and Natural Resource
University of Mohaghegh Ardabili, Ardabil, Iran*

⁴*Department of Plant Protection Research, Ardabil Agriculture and Natural Resources
Research and Education center, Ardabil, Iran*

⁵*Department of Plant and Soil Sciences, Mississippi State University
Mississippi State, Mississippi, USA (phone: +1-662-325-4725; fax: +1-662-325-8742)*

*Corresponding author

email: m_brahim@uma.ac.ir; phone: +98-9123501493

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Abstract. Wild gladiolus (*Gladiolus segetum*) is a problematic weed in dry land winter wheat fields of northwest and west provinces in Iran, and accounts for about 23% of yield losses in winter wheat. Greenhouse studies were conducted from 2014 to 2015 to evaluate different herbicide treatments to control of the noxious weed, *Gladiolus segetum*, commonly found in northwest of Iran. The objective was to evaluate potential control possibilities with commercial herbicides containing sulfosulfuron (Apirus®), mesosulfuron+ idosulfuron (Atlantis®), mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican (Othello®), metsulfuron-methyl + sulfosulfuron (Total®), clodinafop-propargyl (Topik®), and glyphosate. Herbicides were applied to fully developed stems (10 cm), at the 2-3 leaf stage of the weed which coincides with the tillering stage in wheat. Similar to sulfosulfuron and mesosulfuron + idosulfuron, higher rates of glyphosate resulted in higher biomass reduction of *G. segetum* in both years. But the application rates of glyphosate were remarkably higher compared to sulfosulfuron and mesosulfuron + idosulfuron. The ED₅₀ values of glyphosate were 1751.99 and 1919.93 g ai ha⁻¹, and the ED₉₀ values were 6349.96 and 7031.84 g ai ha⁻¹, in 2014 and 2015, respectively.

Keywords: dose response, dry weight, effective dose, noxious weed, weed management

Introduction

Gladiolus spp. from the Iridaceae family (subfamily of Crocoideae) is distributed in Africa, the Mediterranean basin, and Western Asia. It is a large genus that includes approximately 300 species (Goldblatt et al., 2001). *Gladiolus segetum* Ker Gawl and *G. italicus* Mill. are important weeds in dry land winter wheat fields of the Ardabil province in Iran (Ebadi et al., 2004-2007). The species can be identified by a set of characteristics such as narrow leaves, parallel venation, lowest leaf reduced to a subterranean sheathing cataphyll, and tubular or funnel-shaped perianth with synsepalous sepals (Davis, 1984; Dahlgren, 1985). Wild gladiolus (*Gladiolus segetum*) is a native weed in Greece, Turkey, Iraq, and Iran. They invade disturbed fields in the northern and western parts of Iran, particularly wheat, legume and other dryland fields (Rashed Mohassel et al., 2001; Majd et al., 2017). It is a major

constraint to wheat production in Iran, accounting for about 23% of yield losses (Khalaghani, 2008); and weed control in winter wheat relies almost exclusively on herbicides (Deihim-Fard and Zand, 2006).

Since wild gladiolus has high vigor and emerges simultaneously with winter wheat, it can become dominant and has the potential to cause heavy yield losses in infested wheat fields. It is thus necessary to identify strategies to effectively manage this problematic weed as like as others which can be nonchemical including fertilizer management had beneficial effect against Velvetleaf (Aghaie et al., 2013), physical (Majd et al., 2017) or chemical by applying new herbicides or using alternative herbicide to delay resistance (Alebrahim et al., 2017; Tahmasebi et al., 2018). Additionally, since wheat fields in different regions of Iran have variable climatic conditions, there is a need to tailor weed control strategies specific to each region. Finding an effective weed control strategy will greatly depend on the use of herbicides at effective doses; therefore, selecting an appropriate herbicide and dose is most important. Ebadi et al. (2015) reported that biomass of wild gladiolus was reduced by incorporating cultural strategies with herbicide mixtures such as clodinafop-propargyl, difenzoquat + 2,4-D, Topik + 2,4-D, clodinafop-propargyl + tribenuron-methyl, and glyphosate + fallow. The above mentioned mixtures had no differences in their effect on wild gladiolus. The highest and lowest percentage of biomass reduction was obtained by hand weeding and spraying with 2,4-D, respectively. In addition, their results revealed that clodinafop-propargyl controlled wild gladiolus and other weeds results in an increase in crop yield.

Herbicide dose response curves help determine the selectivity and efficacy of herbicides and in some situations help unravel the dose-range for effective weed control and minimal crop damage. Relationships of dose response depends on the weed species, range of doses used, herbicide type, and various environmental conditions, such as temperature, humidity, wind velocity, CO₂ and O₂ levels, and geographical altitude (Streibig et al., 1993). In our current study we selected several herbicides that belong to three mode-of-action groups: acetolactate synthase (ALS) inhibitors (including mesosulfuron-methyl + idosulfuron-methyl-sodium + diflufenican, mesosulfuron + idosulfuron, metsulfuron-methyl + sulfosulfuron, and sulfosulfuron), inhibitors of aromatic amino acid biosynthesis (including glyphosate), and acetyl-CoA carboxylase inhibitors (including clodinafop-propargyl). Herbicides such as the pyrimidinyl salicylates (Shimizu et al., 1994), triazolopyrimidine sulfonamide (Subramanian and Gerwick, 1989), imidazolinone (Shaner et al., 1984), and sulfonylurea (LaRossa and Schloss, 1984; Ray, 1984) herbicides has ALS as their primary target site of action. The ALS enzyme catalyzes the biosynthetic pathway for the production of three branched-chain amino acids such as valine, leucine and isoleucine. Exposure of plants to ALS-inhibitors therefore inhibits production of the three branched-chain amino acids.

Glyphosate [N-(phosphonomethyl)glycine], is a nonselective, broad spectrum herbicide discovered in 1971 (Baird et al., 1971), and was introduced in 1974 (Franz et al., 1997). Glyphosate was one of the first commercially important herbicide whose site of action was characterized as a single target enzyme in plants, and is the only herbicide known to inhibit 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS). EPSPS catalyzes the penultimate reaction of the shikimate (Shk) pathway (Gruys and Sikorski, 1999) in certain bacteria and plants.

The cyclohexanediones (CHD) and aryloxyphenoxypropanoates (AOPP) inhibits the plastidic enzyme acetyl-CoA carboxylase (ACCase). CHD and AOPP herbicides are used to control a wide selection of grass weeds in both monocot and dicot crops. The basis of

selectivity differs between dicot and grasses: in dicots, tolerance is based on the inherent insensitivity of the ACCase to these herbicides, whereas in certain cereal crops, selectivity is based on higher rates of herbicide detoxification in the crop species (Devine and Shimabukuro, 1994).

The goal of our study was to compare the efficacy of different herbicides treatments, sulfosulfuron (Apirus®), mesosulfuron + idosulfuron (Atlantis®), mesosulfuron methyl + iodosulfuron-methyl-sodium + diflufenican (Othello®), metsulfuron-methyl + sulfosulfuron (Total®), clodinafop-propargyl (Topik®), and glyphosate (Roundup®), on the dry weight of wild gladiolus populations. These herbicides were selected because they are primarily used for weed control in wheat in Iran.

Materials and Methods

Study sites

Experiments were carried out from May 20, 2014 to August 22, 2014 and from May 20, 2015 to August 22, 2015 in the research greenhouse located in the College of Agriculture and Natural Resources, University of Mohaghegh Ardabili (longitude 48°17.600' 20.72" E, latitude 38°12.607' 29.51" N, and 1,386 m altitude), Ardabil, Iran.

Experimental design and treatments

The experiment was a completely randomized design (CRD) with 4 replications, and were independently repeated in time. Six herbicides with a range of doses were applied and the dry weight of gladiolus measured (*Table 1*). In April 2014, mature corms of wild gladiolus were collected from several fields of north-western part of Iran, from Germe city; located near Ardabil at longitude 48° 9' 24.46" E, latitude 39° 6' 36.20" N, and 1,350 m altitude. The rate per hectare was calculated based on the surface area of the pot (942 cm²). Herbicides were applied using a CO₂-pressurized backpack sprayer fitted with 8002 VS flat fan nozzles, MATABI model, calibrated to a deliver 250 L ha⁻¹ at 276 KPa, while maintaining the constant speed of 4.8 KPH. Herbicides were applied to fully developed stems (10 cm), at the two-to-three leaf stage of the weed and coincided with the tillering stage of wheat. Irrigation was performed every week from the beginning to end of experiments. Temperature was 19-25°C. Fertigation by using NPK (20:20:20) was done during experiments.

Table 1. List of herbicides used in the study

Herbicide	Rate (g ai ha ⁻¹)	Recommended Rate for Wheat in Iran (g ai ha ⁻¹) (Zand et al., 2017)
Sulfosulfuron (Apirus®)	0, 3.75, 7.5, 15, 22.5, 30	20
Mesosulfuron + Idosulfuron (Atlantis®)	0, 6, 18, 24, 30, 36	24
Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican (Othello®)	0, 33, 66, 82.5, 99, 132	96
Metsulfuron-methyl + sulfosulfuron (Total®)	0, 22.5, 27, 31.5, 36, 40.5, 45	33
Clodinafop-propargyl (Topik®)	0, 0.032, 0.064, 0.080, 0.096, 0.128	60
Glyphosate®	0, 1025, 1230, 1435, 1640, 1845	1600

Sampling procedure

Shoots of weeds were collected from the soil surface in each pot, four weeks after herbicide treatment application. After drying in an oven at 70°C (for 72 h) dry weights were measured.

Statistical analysis

The dry weight per pot, y , was estimated using a log-logistic dose-response model with dose x in ai/haby R software:

$$y = \frac{d}{1 + \exp(b \cdot [\log(x) - \log(ED_{50})])} \quad (\text{Eq.1})$$

where, b denotes the relative slope around ED_{50} (Effective Dose), which is the dose required to produce one-half of the dry-weight relative to the upper limit, d . The dose-response curve takes a snapshot of the dry matter production at the time of harvesting the biomass (Ritz et al., 2006).

Results

The analysis of residuals and test for lack of fit ($p < 0.05$) confirmed the assumption that the data had constant variance, were normally distributed, and the dose-response curves described the variation in data. The efficacy of the herbicides were compared based on the ED_{50} and ED_{90} levels (Tables 2, 3, 4 and 5). As noted in Figs. 1 and 2, the doses were concentrated around the middle of the dose-response curve in order to obtain a precise ED_{50} .

Table 2. The nonlinear log-logistic regression fit of dry weight in the first year. Standard errors in parenthesis, and with upper 95% confidence interval of ED_{50} (g ai ha⁻¹)

Herbicide	Upper limit	Slope	ED ₅₀
Sulfosulfuron	0.36(0.01)	0.87(0.10)	20.6(2.72)
Mesosulfuron + Idosulfuron	0.33(0.02)	3.80(2.80)	44.03(4.48)
Glyphosate	1.24(0.03)	1.70(0.18)	1751(74.77)
Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican	0.43(0.01)	0.64(0.13)	102.7(18.48)
Clodinafop-propargyl	0.35(0.01)	1.33(0.31)	193.8(27.55)
Metsulfuron-methyl + sulfosulfuron	0.33(0.01)	1.84(0.31)	61.24(4.55)

Table 3. The nonlinear log-logistic regression fit of dry weight in the second year. Standard errors in parenthesis, and with upper 95% confidence interval of ED_{50} (g ai ha⁻¹)

Herbicide	Upper limit	Slope	ED ₅₀
Sulfosulfuron	0.42 (0.01)	0.67(0.08)	20.8(3.43)
Mesosulfuron + Idosulfuron	0.42(0.02)	3.02(0.92)	38.29(2.12)
Glyphosate	1.20(0.02)	1.69(0.13)	1919(59.59)
Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican	0.39(0.01)	0.74(0.13)	161.2(23.29)
Clodinafop-propargyl	0.31(0.01)	1.12(0.16)	92.57(9.58)
Metsulfuron-methyl + sulfosulfuron	0.37(0.01)	0.85(0.29)	71.05(16.31)

Table 4. ED_{90} ($g\ ai\ ha^{-1}$) levels of the first experiment with 95% confidence intervals for the first year

Herbicide	Estimate ED_{90}	Upper limit ED_{90}
Sulfosulfuron	255	392
Mesosulfuron + Idosulfuron	78	160.28
Glyphosate	6349	8217.82
Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican	3077	7477.74
Clodinafop-propargyl	1005	1991.63
Metsulfuron-methyl + sulfosulfuron	201	302.19

Table 5. ED_{90} ($g\ ai\ ha^{-1}$) levels of the second experiment with 95% confidence intervals for the second year

Herbicide	Estimate ED_{90}	Upper limit ED_{90}
Sulfosulfuron	541	985.38
Mesosulfuron + Idosulfuron	79	117.13
Glyphosate	7031	8601.46
Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican	3053	6581.38
Clodinafop-propargyl	651	1045.21
Metsulfuron-methyl + sulfosulfuron	918	2908.76

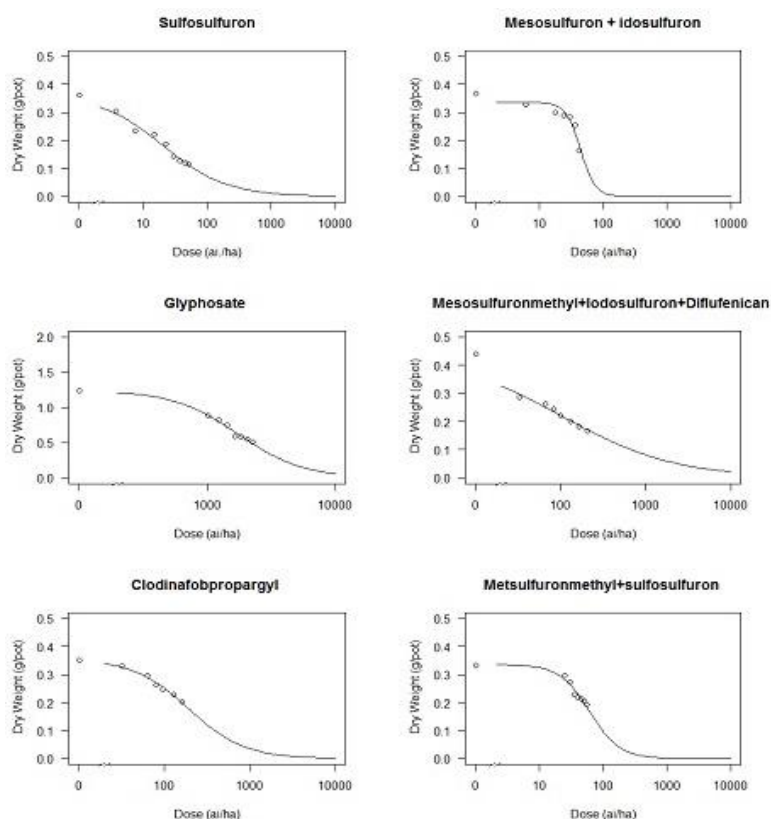


Figure 1. Estimated dose response curves of wild gladiolus to six herbicide treatments. Data shown as dry weight in the first year

Sulfosulfuron

The application rates of sulfosulfuron required to reduce wild gladiolus dry matter by 50% in 2014 and 2015, was 20.6 and 20.8 g ai ha⁻¹, respectively (Tables 2 and 3). Moreover, the application rates of sulfosulfuron required to achieve 90% wild gladiolus control in 2014 and 2015, were 255 and 541 g ai ha⁻¹, respectively (Tables 4 and 5). In both instances, the ED₉₀ was significantly different from zero even though there were not many observations at the lower part the curve, which is reflected in the precision of ED₉₀. The recommended rate for this herbicide in wheat is around 20 g ai ha⁻¹ (Table 1). Some studies have shown that the height of weed species and weed biomass at the time of post herbicide applications is an important factor in determining the level of control achieved (Craigmyle et al., 2013; Chahal et al., 2014).

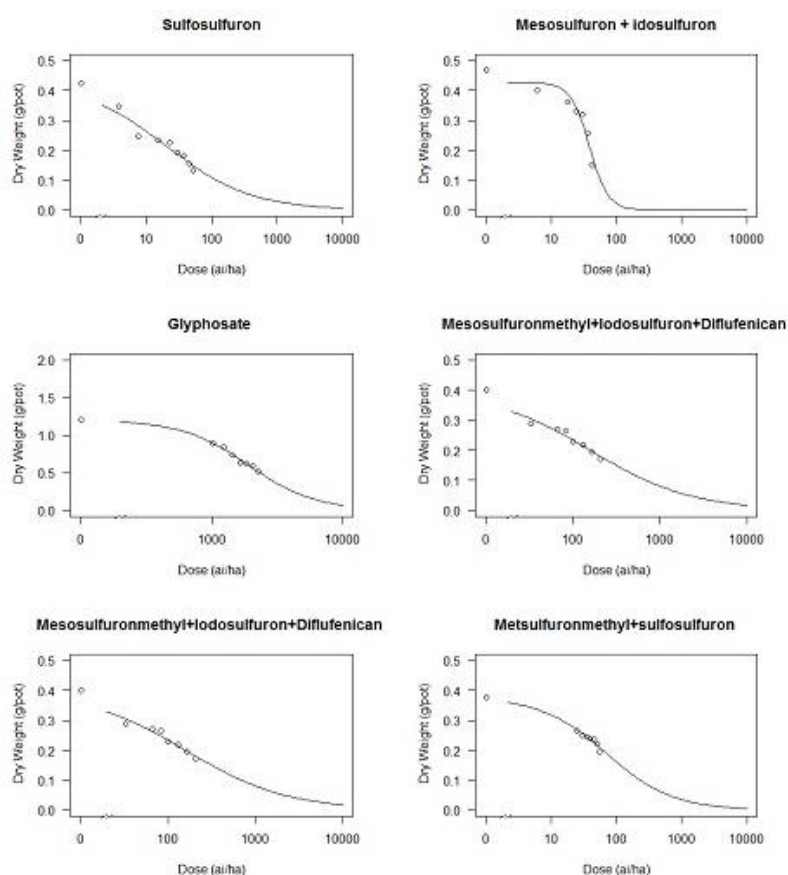


Figure 2. Estimated dose response curves of wild gladiolus to six herbicide treatments. Data shown as dry weight in the second year

Mesosulfuron+idosulfuron

In the case of mesosulfuron + idosulfuron application, the ED₅₀ increased almost 50% compared to the Sulfosulfuron (Figs. 1 and 2; and Tables 2 and 3). However, the mixtures had a much steeper dose-response curve than the sulfosulfuron, and thus, the ED₉₀ was much smaller for sulfosulfuron in 2014 and 2015, and were 78 and 79 g ai ha⁻¹, respectively (Tables 4 and 5). The recommended rate for this herbicide in wheat is around 24 g ai ha⁻¹ (Table 1). Kaiser and Gerhards (2015) reported different levels of ED₅₀ for several

multiple-resistant blackgrass (*Alopecurus myosuroides*). There was sufficient control of population ALOMY-S with an ED₅₀ of 2.42 g ai ha⁻¹. ED₅₀ values of 21.36 and 9.19 g ai ha⁻¹, were obtained for ALOMY-R1 and ALOMY-R2, respectively. Why you provide explanation on why you think so?

Glyphosate

Although the ED₅₀ was rather similar for both sulfosulfuron and mesosulfuron + idosulfuron, it is obvious that glyphosate will require much larger rates to attain ED₅₀ in both years (*Figs. 1 and 2; Tables 2 and 3*). The relative slopes of the glyphosate response curves were around 1 and were therefore comparatively flat in nature (*Figs. 1 and 2*). The ED₅₀ of glyphosate was 1751 and 1919 g ai ha⁻¹, in 2014 and 2015, respectively (*Tables 2 and 3*). In 2014 and 2015, the ED₉₀ values of glyphosate were 6349 and 7031 g ai ha⁻¹, respectively (*Tables 4 and 5*). It shows that although effective control of wild gladiolus can be achieved using glyphosate, a rather high dose is required; the recommended rate for this herbicide in wheat is around 1600 g ai ha⁻¹ (*Table 1*). Chahal et al. (2015) showed that the shoot biomass curve indicated a 50 and 90% reduction at 227 and 3704 g ai ha⁻¹, respectively, in 10-cm tall kochia; whereas, the ED₅₀ and ED₉₀ were 612 and 5885 g ai ha⁻¹, respectively for a 20-cm tall kochia.

Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican

Results show that the ED₅₀ for this mixture of compounds had higher efficacy of weed control than glyphosate alone (*Figs. 1 and 2; Tables 2 and 3*). An up to 50% biomass reduction of wild gladiolus was observed at 102.75 and 161.20 g ai ha⁻¹, in the first and second year of experiment, respectively. The application rates of Mesosulfuron-methyl + Iodosulfuron-methyl-sodium + Diflufenican required for 90% reduction in wild gladiolus biomass in 2014 and 2015 were 3077 and 3053 g ai ha⁻¹, respectively. In this case, ED₉₀ values in two experimental years were similar, but was not significantly different from zero (*Tables 4 and 5*); the recommended rate for this herbicide in wheat is around 96 g ai ha⁻¹ (*Table 1*).

Clodinafop-propargyl

The ED₅₀ values of clodinafop-propargyl were 193.84 and 92.57 g ai ha⁻¹ in 2014 and 2015, respectively, with the ED₅₀ in 2014 being two times higher than in 2015. However, the ED₅₀ variation between the two years cancelled out, because the rates were not significantly different from zero (*Tables 2 and 3*). The ED₉₀ values in 2014 and 2015, were 1005 and 651 g ai ha⁻¹, respectively, and the difference is not dramatic considering the precision of the 95% confidence intervals. The relative slope of the dose-response curves in *Figs. 1 and 2* suggest that the herbicide had a similar slope as glyphosate; the recommended rate for this herbicide in wheat is around 60 g ai ha⁻¹ (*Table 1*). Stagnari et al. (2006) conducted a two years experiment to determine the ED₉₀ of clodinafop-propargyl for several weed species. The amount of ED₉₀ for *Lolium multiflorum*, *Avena ludoviciana*, and *Phalaris minor* were 60, 32 and 33.6 g ai ha⁻¹ in the first year, respectively. In the second year of experiment, the amount of ED₉₀ for *Avena ludoviciana* and *Phalaris minor* were 64.5 and 99.8 g ai ha⁻¹, respectively. It indicated that wild gladiolus can tolerate clodinafop-propargyl better than the grasses.

Metsulfuron-methyl + sulfosulfuron

Based on the results in *Figs. 1 and 2*, and *Tables 2 and 3*, the differences in ED₅₀ was not that dramatic between the first and second experimental years; ED₅₀ was 61.24 and 71.05 g ai ha⁻¹, respectively. ED₉₀ was 201.35 and 918.36 g ai ha⁻¹ in 2014 and 2015, respectively. There was vast difference in ED₉₀ between the two years, however, the ED₉₀ values were not different from zero (*Tables 4 and 5*). This poor precision of the ED₉₀ could be due to lack of observations to support the lower part of the curve and the much lower relative slope of 0.85 (*Table 3*). The recommended rate for this herbicide in wheat is 33 g ai ha⁻¹ (*Table 1*). Izadi-Darbandi and Aliverdi (2015) reported that the amount of ED₅₀ and ED₉₀ of sulfosulfuron+metsulfuron-methyl to control wild barley in wheat field were 26.64 and 41.95 g ai ha⁻¹, respectively.

Discussion

In our study, results revealed that the highest weed dry weight was associated with glyphosate treatment (*Tables 2 and 3*). This suggests that even after exposure to glyphosate, the weed is able to grow before the herbicide is distributed throughout whole plant and begin exerting its action. Atlantis, a mixture of mesosulfuron+idosulfuron, resulted in the next highest weed dry weight, while sulfosulfuron produced the lowest weed dry weight, thus indicating high efficacy of sulfosulfuron on the weed compared to other herbicides (*Tables 2 and 3*). The relative steepness of the dose-response curves for ED₅₀ were different; for example, a sharp decrease in wild gladiolus dry weight was observed after application of sulfosulfuron. Mesosulfuron+idosulfuron curve declined after a steady phase while other herbicide curves dropped slowly (*Tables 2 and 3; Figs. 1 and 2*). We observed that better control of wild gladiolus was obtained in the second year at the highest dose for all herbicides (*Fig. 2*). It is important to note that optimum efficacy is influenced by weed size, so that when the size of the weed is bigger, the control will be lower (Everitt and Keeling, 2007; Robinson et al., 2012).

Generally, among all herbicides tested, sulfosulfuron had the lowest ED₅₀ in both years, 2014 (20.6 g ai ha⁻¹) and 2015 (20.8 g ai ha⁻¹) (*Tables 2 and 3*); while glyphosate had the highest ED₅₀ in 2014 (1751.99 g ai ha⁻¹) and 2015 (1919.93 g ai ha⁻¹) (*Tables 2 and 3*). This suggests that sulfosulfuron and glyphosate were the highest and lowest performing herbicides with respect to wild gladiolus control. In the case of ED₉₀, mesosulfuron+idosulfuron and glyphosate had the highest and lowest influence on the control of wild gladiolus. The ED₉₀ values of mesosulfuron+idosulfuron were 78 and 79 g ai ha⁻¹ in 2014 and 2015, respectively (*Tables 4 and 5*). Also, the ED₉₀ values of glyphosate were 6349 and 7031g ai ha⁻¹ in 2014 and 2015, respectively (*Tables 4 and 5*).

Conclusion

The greenhouse experiment with six different herbicide treatments identified sulfosulfuron to be the most effective in controlling wild gladiolus. Although a few herbicides were identified to be most effective, it is crucial that we use incorporate the use of different herbicides with various modes of action to prevent or manage herbicide resistance in wild gladiolus. This will encourage herbicide rotation and tank mixing herbicides with different modes of action for effective and sustainable weed control. Further research on studying the effects of natural environmental conditions on the

efficacy of herbicides used in this study with regards to wild gladiolus control is under evaluation.

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