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Weed Response to ALS-Inhibitor Herbicide (Sulfosulfuron + Metsulfuron Methyl) under Increased Temperature and Carbon Dioxide

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Abstract: Information on the impact of climate change on the growth of weed species and their sensitivity to herbicides could help to establish an efficient weed management strategy. Due to the excessive use of acetolactate synthase (ALS)-inhibitor herbicides, resistance to those herbicides is increasing globally. It is, thus, crucial to find out whether the efficacy of these herbicides will change in the future due to the increase in temperatures and carbon dioxide concentration. Therefore, this work aimed to evaluate the impact of temperature and carbon dioxide (CO_2) changes on the growth of *Amaranthus retroflexus*, *Bromus tectorum*, *Chenopodium album*, and *Echinochloa crus-galli*, including the assessment of sulfosulfuron 75% + metsulfuron methyl 5% efficacy in these weeds. A factorial experiment was performed in a completely randomized design with a factorial arrangement ($2 \times 2 \times 6$), including two CO_2 concentrations (400 and 700 ppm), two temperature regimes (30/20 °C and 34/24 °C day/night), and six herbicide rates (0, 25, 37.5, 50, 62.5, and 75 g ha^{-1}). As a result, it was seen that temperature and CO_2 concentration changes influenced the morphological variables of the weeds. The temperature regime affected the herbicide's effectiveness on *B. tectorum* and *E. crus-galli*. The herbicide's efficacy on weed species was affected by the interaction of herbicide rates and the temperature regime, except for on *E. crus-galli*; the highest efficacy was observed at 30/20 °C and at a rate 50% higher (75 g ha^{-1}) than the recommended one (50 g ha^{-1}). Except for *E. crus-galli*, increasing CO_2 concentrations enhanced the herbicide efficacy and ALS enzyme activity inhibition in all the weed species, but had the greatest effect on C_3 weeds. We found that temperature and CO_2 levels can alter the efficacy of weed control with herbicides, with clear differences between C_3 and C_4 plants. As a result, increased temperature and CO_2 concentration will possibly allow better control of weed species such as *B. tectorum*, *C. album* and *A. retroflexus* at lower doses of the ALS herbicide under investigation.

Keywords: carbon dioxide; climate change; herbicide efficiency; temperature

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1. Introduction

Global climate change is one of the main concerns for the future sustainability of our development, because of its impact on numerous socioeconomic sectors of human activity [1]. Changes in temperature, atmospheric carbon dioxide (CO_2), and frequent and extreme weather events could have significant impacts on weed populations and their management [2–4]. From 1980 to 2020, atmospheric CO_2 concentration increased from 340 ppm to 411 ppm, and it was estimated that CO_2 would reach 600–1000 ppm at the end of the 21st century [5]. High concentrations of CO_2 improve plant growth, directly affecting photosynthesis activity, this being decisively influenced by the photosynthetic pathway of plants (C_3 or C_4). In general, C_3 weeds will respond more favorably to increased atmospheric CO_2 than C_4 ones [3]. Different responses of C_3 and C_4 plants to the increase in

CO_2 concentration and temperature could lead to important consequences for weed–crop interaction [3].

Environmental conditions affect plant and herbicide interaction. Despite their importance, there is not much information on the effects of herbicides on weeds in the context of climate change, like reduced efficacy and, eventually, herbicide resistance. High concentrations of CO_2 reduce stomatal conductance, which can alter the efficacy of foliar herbicides [6]. Some studies have indicated that the efficacy of glyphosate in controlling *Paspalum dilatatum*, *Conyza canadensis*, and *Chenopodium album* could be reduced at high CO_2 concentrations [7–9]. When herbicides are sprayed on weed leaves at high temperatures, leaf cuticles become more fluid and more readily penetrated by fat-soluble compounds, thus demonstrating low selectivity [10].

According to reports, herbicides of the sulfonylureas group were first introduced to the market in 1982 and, in most cases, they exert a good control of weeds [11]. This herbicide class inhibits the activity of the acetolactate synthase (ALS) enzyme, which is responsible for the biosynthesis of leucine, isoleucine, and valine amino acids in plants. Inhibition of ALS activity leads to the starvation of the plant for these amino acids, and it is this which is thought to be the primary mechanism responsible for the plant death caused by ALS-inhibiting herbicides. However, other secondary effects of ALS inhibition, such as the buildup of 2-ketobutyrate and the disruption of protein synthesis and of photosynthate transport, have also been implicated in plant death [12]. The mixture of sulfosulfuron 75% + metsulfuron methyl 5% as a post-emergence herbicide is one of the sulfonylurea class herbicides used for controlling narrow and broadleaf weeds of wheat. However, there is a lack of studies on the effect of climate change on sulfonylureas, and, in order to optimize herbicide rates for acceptable weed control in the future, there is a need to gain more understanding of the interactions between climate change and herbicide efficacy. Therefore, this research was aimed at evaluating the effects of CO_2 and rising temperature on the performance of sulfosulfuron 75% + metsulfuron methyl 5% in controlling *Chenopodium album*, *Amaranthus retroflexus*, *Bromus tectorum*, and *Echinochloa crus-galli*.

2. Materials and Methods

2.1. Plant Material, Growth Conditions, and Treatments

A factorial experiment was performed in a completely randomized design with three factors (two CO_2 concentrations, two different temperatures and six herbicide rates) in four replications. Two C_4 (*A. retroflexus* and *E. crus-galli*) and two C_3 (*C. album* and *B. tectorum*) plant species were used in this experiment. Seeds of barnyard grass (*E. crus-galli*) and cheat grass (*B. tectorum*) as grass, and red-root pigweed (*A. retroflexus*) and common lambsquarters (*C. album*) as broadleaf were collected from the Research Farm of the University of Zanjan (35° 35′ N, 47° 15′ E).

Seeds of *C. album* and *A. retroflexus* were exposed to light and temperature fluctuation treatments to break the dormancy, and scarification was used to break the dormancy of *E. crus-galli* seeds. Seeds of *B. tectorum* did not showed dormancy. Ten seeds of each weed were sown in plastic pots (25 cm × 35 cm), filled with the 75% perlite + 25% coco-peat mixture. Pots were placed in a standard growth chamber (STC 1300, Noor Sanat Azma Ferdous, Iran) under two concentrations of CO_2 (400 and 700 ppm) and two temperatures (day/night temperature 30/20 °C and 34/24 °C). The light required for the growth of the plants was provided by LED bulbs installed inside the growth chamber. Also, the CO_2 concentration was measured by CO_2-sensitive sensors, and, if needed, it was automatically injected from the CO_2 gas capsule connected to the device. Pots were irrigated twice a week with Hoagland solution (until the end of the growth stage). After seed germination, they were thinned, and only four plants were kept in each pot. Finally, six rates of Total ® herbicide (sulfosulfuron 75% + metsulfuron methyl 5%), including (1) the herbicide rate recommended by the manufacturer (50 g ha^{-1}), (2) 25% below it (37.5 g ha^{-1}), (3) 25% above it (62.5 g ha^{-1}), (4) 50% below the recommended rate (25 g ha^{-1}), (5) 50% above it (75 g ha^{-1}), and (6) distilled water as a control (no herbicide), were sprayed on plants at

the six-leaf stage. Herbicide was applied with a backpack sprayer equipped with flood-jet nozzle, calibrated to deliver 200 L ha^{-1} at 250 kPa. The distance between the nozzle and the target was 50 cm. Ten days after spraying, plant parameters were measured.

Weeds were monitored daily after herbicide application. They started withering 3–5 days after herbicide application and showed chlorosis symptoms nearly 10–15 days after spraying.

2.2. Herbicide Efficiency

Ten days after spraying, herbicide damage on weeds was assessed by the European Weed Research Council (EWRC) scoring system [13] (Table 1). Scoring was carried out by two people separately.

Table 1. Modified European Weed Research Council Ratings Scale used to score herbicide effectiveness.

Category Number	Herbicide Effectiveness on Weeds	Weed Control (%)
1	None	0–29.9
2	Very bad	30–54.9
3	Bad	55–69.9
4	Weak	70–81.9
5	Moderate	82–89.9
6	Good to acceptable	90–94.9
7	Very good	95–97.9
8	Excellent	98–99.9
9	Total plant death	100

2.3. Morphological Variable Measurement

At the end of the experiment, roots were removed from the soil and washed with water, and their lengths and root volume were measured later by immersing the roots in a graduated cylinder (500 $\pm$ 1 cc). Plant biomass was dried in an oven at 70 °C for 48 h, and dry weights were determined. Analyses were conducted on total dry weights of root and shoot, separately. Plant height was measured by a ruler.

2.4. Enzyme Assay

The activity of the acetolactate synthase (ALS) enzyme was determined by the Milfin et al. [14] method with three replications. In brief, 100 mg of upper leaves samples from all treatments were used for the extraction and precipitation of protein. Next, about 0.5 mg of the precipitated protein was incubated for 1 h at 30 °C in a buffer containing 40 mM Na-pyruvate, 0.32 mM thiamin pyrophosphate, 0.5 mM $MnSO_4$, and 20 mM Na-phosphate, pH 7.5. After that, the reaction was terminated by the addition of $ZnSO_4$, (5 mM). After centrifugation, the supernatant was acidified with HC1 (37%). Then, 1.7% (w/v) a-naphthol and 0.17% (w/v) creatin were added and incubated at room temperature for 1 h. Finally, the absorbance was recorded at 530 nm with a spectrophotometer (PerkinElmer-lambda 25, Waltham, MA, USA).

2.5. Statistical Analysis

The data were analyzed by ANOVA, and the means were contrasted by the Tukey HSD test ($p \leq 0.05$) using SAS ver. 9 (SAS Institute Inc., Cary, NC, USA).

3. Results

Temperature regimes affected the plant height, root/shoot ratio, root volume, and enzyme activity of *A. retroflexus* (Table 2). The highest root volume and inhibition of ALS enzyme activity were observed at 34/24 °C (Table 3). In contrast, the maximum plant height and root/shoot ratio were obtained at 30/20 °C (day/night) (Table 3). Increasing the CO_2 concentration also affected plant height, total biomass, enzyme activity, and herbicide

efficacy (Table 2). Herbicide rates also influenced plant height, shoot dry weight, total biomass, root volume, enzyme activity, and herbicide efficacy in these species (Table 2).

Temperature and CO_2 greatly affected the *B. tectorum* variables. The temperature regimes affected the root dry weight, root volume, root/shoot ratio, enzyme activity, and herbicide efficacy, and the CO_2 concentration also affected the root dry weight, total biomass, root volume, and herbicide efficacy. On the other hand, herbicide rates had a notable impact on all variables, except for the root/shoot ratio (Table 2).

Table 2. Analysis of variance of the effects of temperature changes, CO_2 concentration, and different rates of sulfosulfuron 75% + metsulfuron methyl 5%on morphological characteristics, enzyme activity, and herbicide efficacy in C_3 and C_4 weeds.

	Mean Square									
	Species	**df**	**Height (cm)**	**SdW (g pot^{-1})**	**RdW (g pot^{-1})**	**Biomass (g pot^{-1})**	**R/S**	**RV (cm^3)**	**ASL Activity**	**HE (%)**
A. retroflexus	Temperature (T)	1	394.07 **	0.49 ns	83.40 ns	0.02 ns	13.70 **	1.66 **	5536 **	1.5 ns
	CO_2	1	195.51 **	0.48ns	65.24 ns	1.45 **	0.24 ns	0.002 ns	5236 **	13.5 **
	Herbicide rates (H)	5	355.04 **	8.62 **	75.24 ns	15.98 **	0.95 ns	2.67 **	40,269 **	142.1 **
	T × CO_2	1	21.57 ns	0.0001 ns	72.48 ns	0.003 ns	0.03 ns	0.13 ns	4 ns	0.37 ns
	T × H	5	2.7 ns	0.038 ns	74.00 ns	0.022 ns	0.20 ns	0.01 ns	214 ns	0.12 ns
	CO_2 × H	5	0.82 ns	0.047 ns	74.3 ns	0.07 ns	0.35 ns	0.002 ns	3220 **	1.92 **
	T × CO_2 × H	5	1.63 ns	0.041 ns	73.59 ns	0.043 ns	0.12 ns	0.001 ns	101 ns	0.3 ns
	Error	72	9.33	0.175	74.28	0.18	0.58	0.083	407	0.51
B. tectorum	Temperature (T)	1	20.18 ns	0.069 ns	0.43 **	0.15 ns	2.25 **	0.53 **	1953 **	2.04 *
	CO_2	1	0.35 ns	0.53 **	0.43 **	1.92 **	0.11 ns	0.44 **	244 ns	3.37 **
	Herbicide rates (H)	5	557.38 **	5.25 **	3.82 **	17.99 **	0.48 ns	4.37 **	33,342 **	202 **
	T × CO_2	1	6.42 ns	0.13 ns	0.083 ns	0.005 ns	1.17 *	0.21 *	667 ns	0.04 ns
	T × H	5	9.77 ns	0.0068 ns	0.019 ns	0.04 ns	0.05 ns	0.04 ns	163 ns	0.64 ns
	CO_2 × H	5	13 ns	0.079 ns	0.042 ns	0.21 **	0.04 ns	0.08 ns	998 **	2.57 **
	T × CO_2 × H	5	4.54 ns	0.046 ns	0.003 ns	0.03 ns	0.12 ns	0.01 ns	292 ns	0.24 ns
	Error	72	6.52	0.067	0.048	0.07	0.26	0.05	266	0.38
C. album	Temperature (T)	1	14.4 *	0.49 ns	0.15 *	0.001 ns	156 ns	6600 ns	1365 ns	0.042 ns
	CO_2	1	61.3 **	0.48 ns	0.42 **	0.63 **	146.37 ns	6384 ns	155 ns	9.375 **
	Herbicide rates (H)	5	320.1 **	8.62 **	3.47 **	22.86 **	122.58 ns	6810 ns	38,040 **	194.4 **
	T × CO_2	1	1.2 ns	0.0001 ns	0.0008 ns	0.04 ns	116.99 ns	6828 ns	45 ns	1.5 ns
	T × H	5	3.52 ns	0.038 ns	0.0138 ns	0.002 ns	121.42 ns	6725 ns	207 ns	0.34 ns
	CO_2 × H	5	4.92 ns	0.047 ns	0.0334 ns	0.07 ns	119.07 ns	6688 ns	3386 **	2.07 **
	T × CO_2 × H	5	1.19 ns	0.041 ns	0.0011 ns	0.005 ns	119.47 ns	6709 ns	591 ns	0.25 ns
	Error	72	2.38	0.14	0.0274	0.03	119.01	6736	349	0.51
E. crus-galli	Temperature (T)	1	38.76 **	0.012 ns	0.026 *	0.002 ns	1.13 **	0.57 **	661 ns	3.37 **
	CO_2	1	34.41 **	0.136 **	0.161 **	0.59 **	0.29 **	1.16 **	2185 ns	15.04 **
	Herbicide rates (H)	5	281.71 **	0.686 **	0.494 **	2.34 **	0.11 **	12.02 **	54,925 **	124.2 **
	T × CO_2	1	0.019 ns	0.0054 ns	0.0030 ns	0.06 ns	0.02 ns	0.032 ns	1218 ns	0 ns
	T × H	5	1.105 ns	0.0017 ns	0.0028 ns	0.004 ns	0.023 ns	0.137 *	1181 ns	0.87 *
	CO_2 × H	5	1.961 ns	0.0058 ns	0.011 **	0.030 ns	0.015 ns	0.308 **	1010 ns	1.99 **
	T × CO_2 × H	5	0.414 ns	0.0014 ns	0.0031 ns	0.008 ns	0.017 ns	0.091 ns	702 ns	0.8 ns
	Error	72	1.411	0.0058	0.0041	0.015	0.030	0.042	635	0.35

df (degree of freedom), HE (herbicide efficacy), ALS (acetolactate synthase), RV (root volume), R/S (root/shoot ratio), RdW (root dry weight), SdW (shoot dry weight). ** significant at p = 0.01; * significant at p = 0.05; ns not significant.

Table 3. The main effects of temperature regimes, CO_2 concentrations, and herbicide rates on weed control efficacy.

Species		Height (cm)	SdW (g pot^{-1})	RdW (g pot^{-1})	Biomass (g pot^{-1})	R/S	RV (cm^3)	ASL Activity	HE (%)
	Temperature °C								
A. retroflexus	30/20	22.07 ± 2.14 a	ns	ns	ns	2.70 ± 0.28 a	1.04 ± 0.01 b	252.20 ± 33.5 b	ns
	34/24	18.02 ± 1.45 b	ns	ns	ns	1.94 ± 0.36 b	1.30 ± 0.02 a	267.39 ± 14.54 a	ns
B. tectorum	30/20	ns	ns	0.97 ± 0.07 b	ns	ns	ns	186.58 ± 17.7 b	1.16 ± 0.24 b
	34/24	ns	ns	1.10 ± 0.01 a	ns	ns	ns	195.60 ± 31 a	1.31 ± 0.11 a
C. album	30/20	15.27 ± 2.19 a	ns	0.50 ± 0.08 b	ns	ns	ns	ns	ns
	34/24	14.49 ± 2.01 b	ns	0.58 ± 0.05 a	ns	ns	ns	ns	ns
E. crus-galli	30/20	17.06 ± 0.84 a	ns	0.30 ± 0.02 b	ns	1.48 ± 0.29 a	ns	ns	ns
	34/24	15.79 ± 1.28 b	ns	0.34 ± 0.02 a	ns	1.26 ± 0.2 b	ns	ns	ns
	CO_2 (ppm)								
A. retroflexus	400	18.62 ± 2.02 b	ns	ns	1.89 ± 0.12 b	ns	ns	ns	ns
	700	21.47 ± 1.6 a	ns	ns	2.14 ± 0.08 a	ns	ns	ns	ns
B. tectorum	400	ns	1.46 ± 0.18 b	0.97 ± 0.02 b	ns	ns	ns	ns	ns
	700	ns	1.61 ± 0.32 a	1.10 ± 0.07 a	ns	ns	ns	ns	ns
C. album	400	14.08 ± 1.33 b	ns	0.47 ± 0.05 b	1.09 ± 0.13 b	ns	ns	ns	ns
	700	15.68 ± 0.61 a	ns	0.60 ± 0.03 a	1.25 ± 0.33 a	ns	ns	ns	ns
E. crus-galli	400	15.82 ± 1.11 b	0.39 ± 0.04 b	ns	0.67 ± 0.03 b	1.43 ± 0.17 a	ns	ns	ns
	700	17.02 ± 2.82 a	0.46 ± 0.04 a	ns	0.83 ± 0.05 a	1.32 ± 0.12 b	ns	ns	ns
	Herbicide rates (g ha^{-1})								
A. retroflexus	25	19.75 ± 2.57 c	1.40 ± 0.11 b	ns	1.96 ± 0.17 b	ns	1.10 ± 0.08 c	ns	ns
	37.5	20.96 ± 0.79 bc	1.41 ± 0.08 b	ns	2.04 ± 0.11 b	ns	1.31 ± 0.03 b	ns	ns
	50	15.73 ± 1.43 d	0.72 ± 0.02 c	ns	1.11 ± 0.20 c	ns	0.79 ± 0.07 d	ns	ns
	62.5	22.98 ± 1.55 b	1.60 ± 0.06 b	ns	2.35 ± 0.23 b	ns	1.43 ± 0.1 b	ns	ns
	75	14.01 ± 1.09 d	0.61 ± 0.01 c	ns	0.93 ± 0.04 c	ns	0.64 ± 0.02 d	ns	ns
B. tectorum	0	26.85 ± 2.2 a	2.66 ± 0.12 a	ns	3.71 ± 0.16 a	ns	1.73 ± 0.03 a	ns	ns
	25	19.89 ± 3.2 c	1.36 ± 0.08 c	0.87 ± 0.02 cd	ns	ns	1.11 ± 0.08 c	ns	ns
	37.5	21.44 ± 0.29 bc	1.48 ± 0.27 bc	0.92 ± 0.04 c	ns	ns	1.16 ± 0.04 bc	ns	ns
	50	17.18 ± 1.21 d	1.10 ± 0.26 d	0.75 ± 0.06 de	ns	ns	0.89 ± 0.03 d	ns	ns
	62.5	22.63 ± 2.87 b	1.6 ± 0.07 b	1.08 ± 0.02 b	ns	ns	1.31 ± 0.03 b	ns	ns
	75	15.41 ± 1 d	1.06 ± 0.08 d	0.60 ± 0.03 e	ns	ns	0.72 ± 0.07	ns	ns
	0	32.20 ± 0.9 a	2.63 ± 0.04 a	1.97 ± 0.04 a	ns	ns	2.21 ± 0.22 a	ns	ns
C. album	25	14.51 ± 1.66 c	ns	0.32 ± 0.01 cd	0.59 ± 0.01 c	ns	ns	ns	ns
	37.5	15.12 ± 0.79 bc	ns	0.43 ± 0.02 bc	0.90 ± 0.03 b	ns	ns	ns	ns
	50	11.64 ± 1.22 d	ns	0.28 ± 0.03 d	0.51 ± 0.03 c	ns	ns	ns	ns
	62.5	16.15 ± 0.85 b	ns	0.52 ± 0.06 b	1.07 ± 0.01 b	ns	ns	ns	ns
	75	9.390 ± 0.79 e	ns	0.21 ± 0.02 d	0.41 ± 0.03 c	ns	ns	ns	ns
	0	22.46 ± 1.88 a	ns	1.46 ± 0.09 a	3.56 ± 0.35 a	ns	ns	ns	ns
E. crus-galli	25	15.06 ± 1.05 d	0.34 ± 0.02 d	ns	0.59 ± 0.04 d	1.45 ± 0.18a	ns	195.8 ± 13.79 c	ns
	37.5	16.37 ± 1.33 c	0.42 ± 0.01 c	ns	0.72 ± 0.03 c	1.38 ± 0.07ab	ns	247.8 ± 24.45 b	ns
	50	13.21 ± 0.69 e	0.27 ± 0.04 e	ns	0.46 ± 0.05 e	1.46 ± 0.11a	ns	185.8 ± 9.8 d	ns
	62.5	17.76 ± 2.17 b	0.52 ± 0.03 b	ns	0.94 ± 0.06 b	1.28 ± 0.24b	ns	264.7 ± 30 b	ns
	75	12.22 ± 1.4 f	0.22 ± 0.09 e	ns	0.38 ± 0.07 e	1.39 ± 0.05ab	ns	174.5 ± 15.5 d	ns
	0	23.93 ± 1.96 a	0.79 ± 0.03 a	ns	1.42 ± 0.02 a	1.26 ± 0.15b	ns	327.4 ± 28.8 a	ns

HE (herbicide efficacy), ALS (acetolactate synthase), RV (root volume), R/S (root/shoot ratio), RdW (root dry weight), SdW (shoot dry weight). Means with the same letters are not significantly different from others ($p < 0.05$) according to the Tukey HSD test. For the treatments whose interaction effects were significant, the mean comparison of the main effects is not given in this table, and, for them, the comparison of the means is shown in Figures 1–4. ns not significant.

For *B. tectorum*, a C_3 plant, the lowest root volume was observed at 30–20 °C (day/night) and 400 ppm CO_2 concentration (Figure 1A). In contrast, the maximum root/shoot ratio was reached at 30–20 °C (day/night) and 400 ppm CO_2 concentration (Figure 1B). The highest total biomass was obtained with the CO_2 concentration of 700 ppm, which led to an increase of about 15% of the biomass without any herbicide (Figure 1C).

In *C. album*, temperature regimes only influenced plant height and root dry weight. Increasing the temperature from 30/20 °C to 34/24 °C increased the root dry weight of *C. album* by 16.4%. The lowest ALS enzyme activity of *C. album* was observed at 34/24 °C. The CO_2 concentration impacted the height, root dry weight, total biomass, and herbicide efficacy. Herbicide rates also influenced all measured variables of *C. album*, except shoot dry weight, root/shoot ratio, and root volume (Table 2).

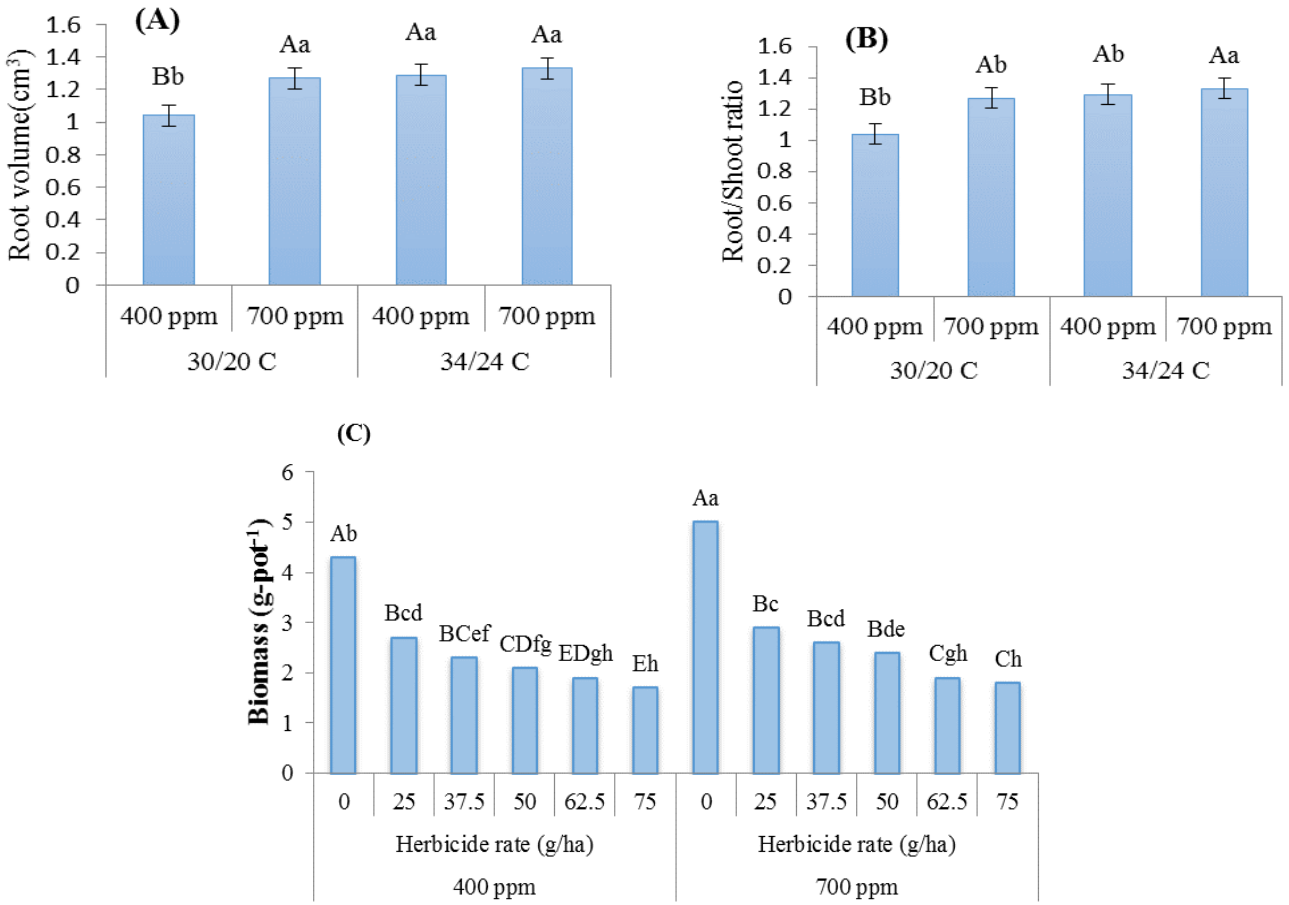

Figure 1. Effects of different CO_2 concentrations, temperatures, and herbicide (sulfosulfuron 75% + metsulfuron methyl 5%) rates on (**A**) root volume, (**B**) root/shoot ratio, and (**C**) biomass of *Bromus tectorum*. The vertical bars represent the standard error. The column letters indicate the differences between the treatments ($p < 0.05$) according to Tukey's HSD test. Different capital letters indicate a significant difference among different CO_2 levels (**A**,**B**) or herbicide rate (**C**), and different lowercase letters indicate a significant difference between the two temperature (**A**,**B**) and CO_2 levels (**C**) ($p < 0.05$).

Regarding *E. crus-galli*, the temperature influenced the plant height, root dry weight, root/shoot ratio, root volume, and herbicide efficacy. Except for ALS activity, CO_2 concentration affected all measured variables for this weed. Different rates of herbicide also had a significant impact on all the traits (Table 2). Overall, the highest root dry weight and volume were observed at the 700 ppm CO_2 concentration, and 34/24 °C temperature regime without herbicide application (Figure 2). Furthermore, herbicide efficacy was affected by the interaction of temperature by herbicide rate, with the maximum herbicide efficacy reached at 75 g ha^{-1} and the lowest temperature regime (30/20 °C) (Figure 3).

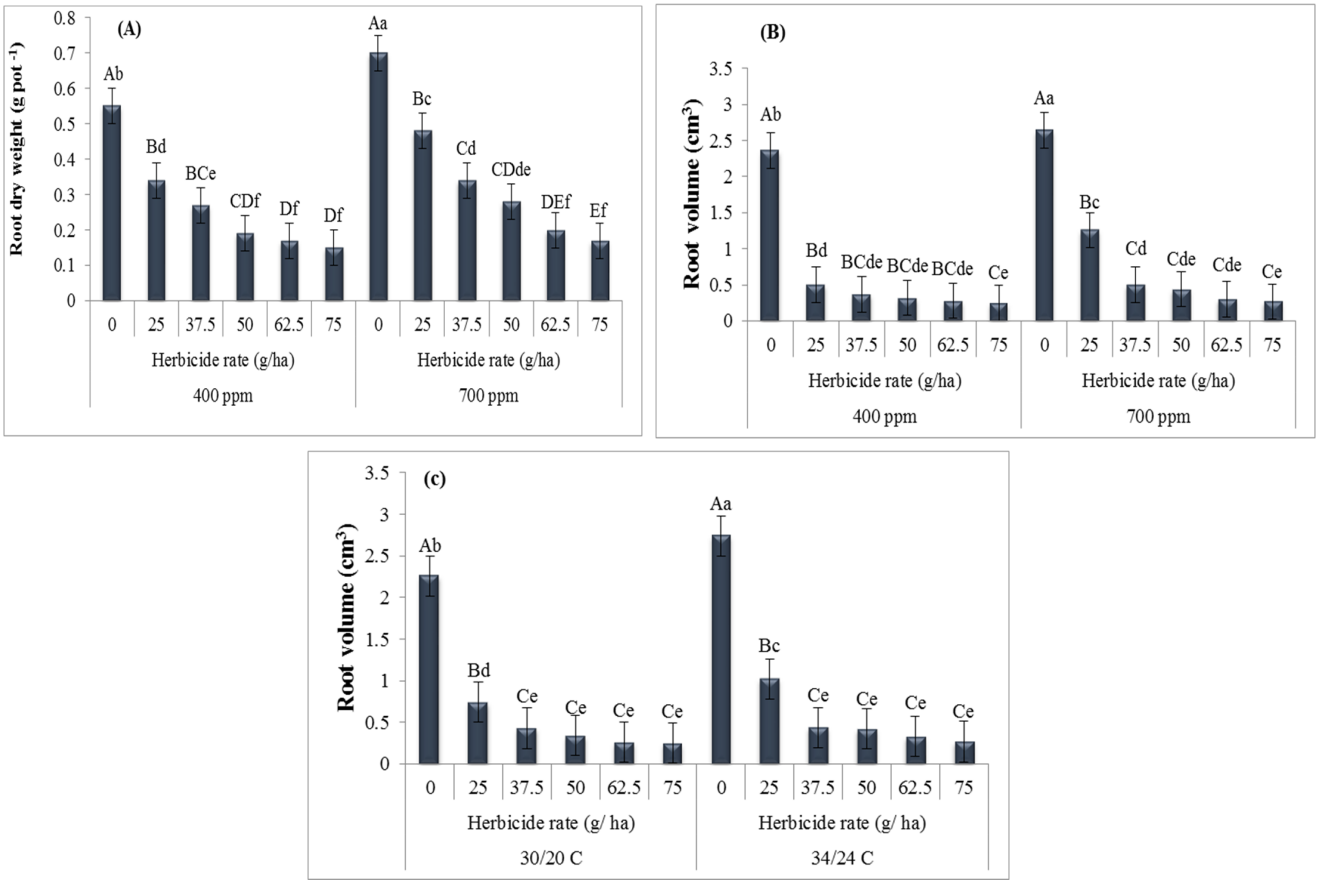

Figure 2. Effects of different CO_2 concentrations, temperatures, and herbicide (sulfosulfuron 75% + metsulfuron methyl 5%) rates on (**A**) root dry weight, (**B**) root volume, and (**C**) root volume of *E. crus-galli.* The vertical bars represent the standard error. The column letters indicate differences among treatments ($p < 0.05$) according to the Tukey HSD test. Different capital letters indicate a significant difference among different herbicide rate inside the CO_2 levels (**A**,**B**) or temperature (**C**), and different lowercase letters indicate a significant difference between the two CO_2 levels (**A**,**B**) and temperature (**C**) ($p < 0.05$).

Herbicide efficacy in all weed species was affected by the carbon dioxide concentration and the herbicide rates ($p < 0.01$). The highest weed control was observed at 700 ppm of CO_2 and the rate of 75 g ha^{-1} of sulfosulfuron 75% + metsulfuron methyl 5%. In general, with the increase of CO_2 concentration, the effectiveness also increased at higher herbicide rates. For example, herbicide efficacy increased by approximately 22% on *A. retroflexus*, 15% on *B. tectorum*, 12% on *C. album*, and 16% on *E. crus-galli* compared to the manufacturer's recommended dose (50 g ha^{-1}).

Except for *E. crus-galli*, CO_2 concentration and herbicide rates affected ALS activity (Table 2). In *A. retroflexus* and *C. album*, the highest inhibition of ALS enzyme activity was obtained at 700 ppm of CO_2 and 50% above the recommended rate (75 g ha^{-1}) of SMM (Figure 4), while, in *B. tectorum*, the lowest enzyme activity was observed at 700 ppm CO_2 and 62.5 g ha^{-1} of SMM (Figure 4).

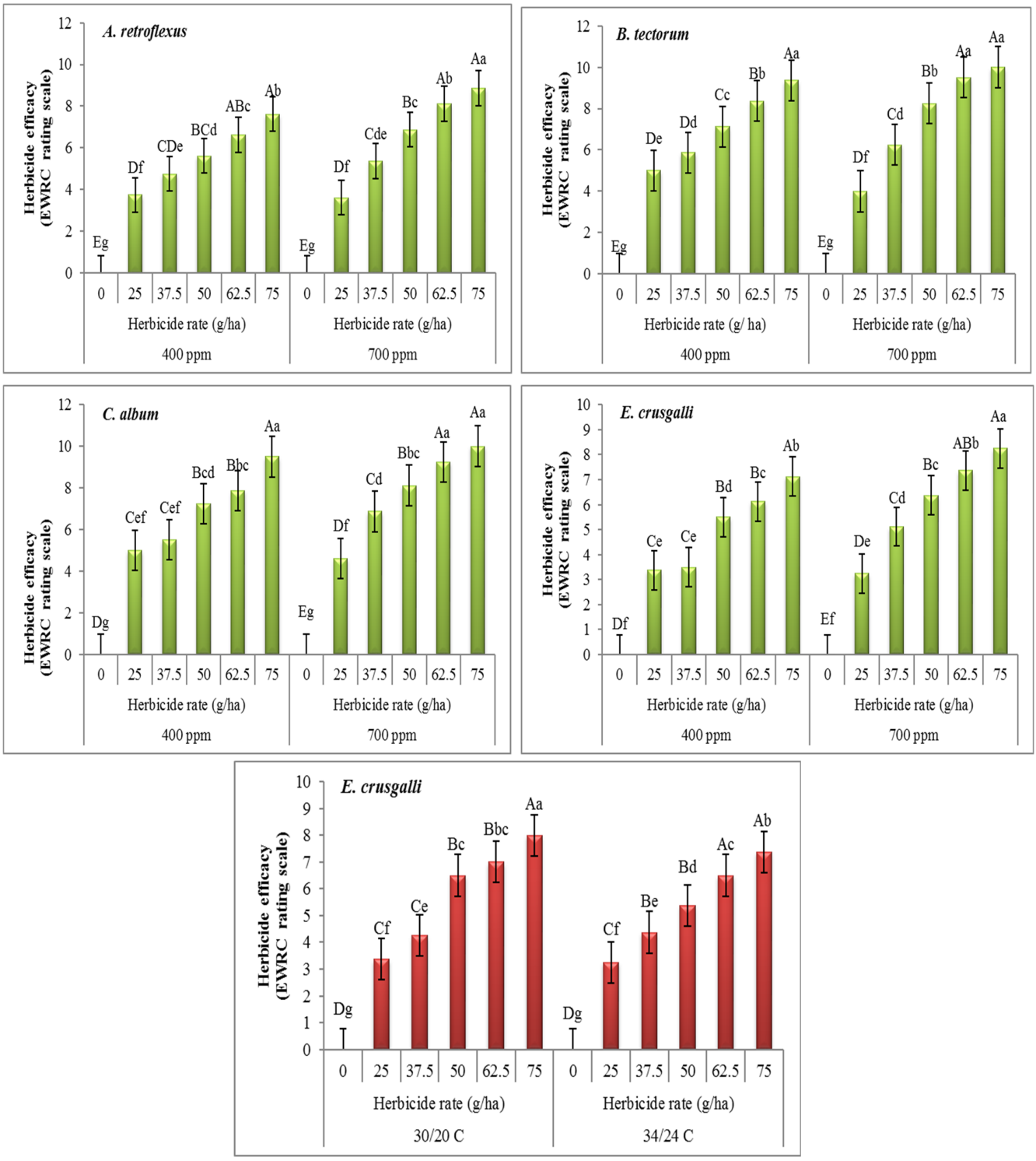

Figure 3. Effects of different CO_2 concentrations and temperatures on the efficacy of (sulfosulfuron 75% + metsulfuron methyl 5%) rates on *A. retroflexus*, *B. tectorum*, *C. album*, and *E. crus-galli*. The vertical bars represent the standard error. The column letters indicate differences among treatments ($p < 0.05$) according to the Tukey HSD test. Uppercase letters indicate main effects and lowercase letters indicate interaction effects between treatments.

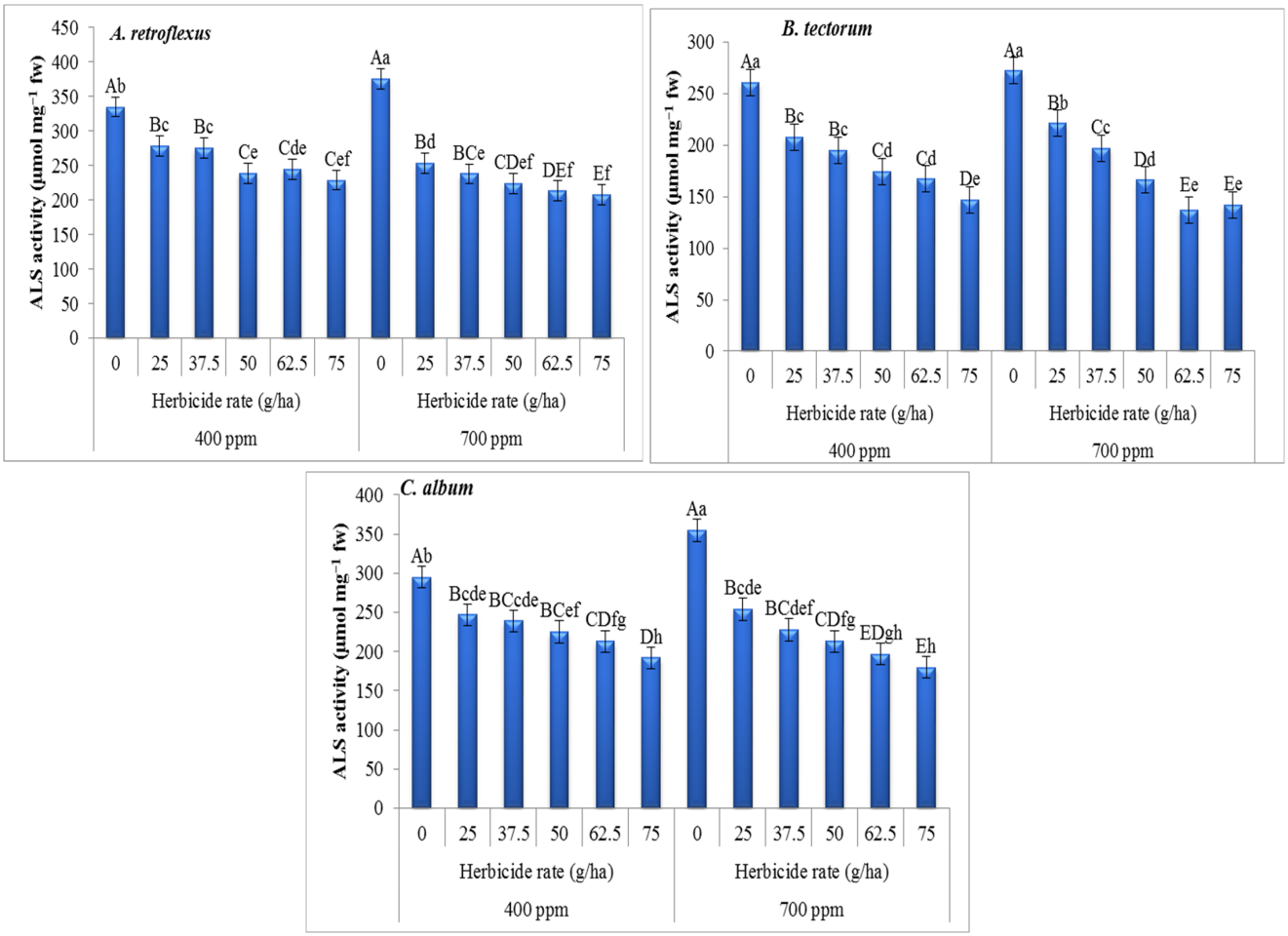

Figure 4. Effects of different CO_2 concentrations and herbicide (sulfosulfuron 75% + metsulfuron methyl 5%) rates on the activity of ALS enzyme in *A. retroflexus*, *B. tectorum*, and *C. album*. The vertical bars represent the standard error. The column letters indicate differences among the treatments ($p < 0.05$) according to the Tukey HSD test. Uppercase letters indicate simple effects and lowercase letters indicate interaction effects between treatments.

4. Discussion

An increase in CO_2 and temperature can cause a change in enzyme activity with a rise in photosynthesis [15], affecting the growth and competitiveness of weeds. The increase in the CO_2 concentration stimulates carboxylation and, thus, reduces photorespiration; commonly, C_3 plants augment their net photosynthesis rates with a higher CO_2 level. Meanwhile, plants with the C_4 photosynthesis pathway have alternate CO_2 fixation mechanisms, so that the CO_2 is initially fixed in the mesophyll cells by phosphoenol pyruvate carboxylase (PEPcase), which has a higher affinity for CO_2 than ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco). CO_2 is regenerated through the 4-carbon organic acid formed in this reaction for fixation by Rubisco in the bundle sheath cells. Due to this internal mechanism, the CO_2 concentration at the Rubisco enzyme activity site augments. As a result, its carboxylation is greater than oxygenation, and its photorespiration is inhibited. Therefore, increasing the CO_2 concentration exerts a lesser effect on the net photosynthetic rate of C_4 plants, unlike in C_3 plants. As a result of the increase in CO_2, many C_3 weeds have shown significant increases in growth and have caused a greater decline in crop yields. For example, an increase of approximately 65% in the biomass of *C. album*, as a C_3 weed, at elevated CO_2 concentrations has been reported by Ziska [3]. This increase in weed growth caused a 39% soybean yield lose. Similarly, increasing the CO_2 concentration caused increases in the competitive ability, biomass, and seed yield of wild rice compared

with those of cultivated rice, which could lead to a further drop in the yield of cultivated rice in the presence of C_3 weeds [16].

It is expected that high concentrations of CO_2 will increase global temperature and extreme temperature events due to greenhouse effects in the future [6,17]. Plants will probably be under high-temperature stress, which could affect their growth rates at different stages. In this work, the degree of photosynthesis stimulation and growth response varied between C_3 and C_4 plants as the temperature increased. In C_3 plants, temperatures above 25 °C increase photorespiration and inhibit CO_2 assimilation [18,19]. Therefore, C_3 weeds could benefit the most from higher concentrations of CO_2, under temperate climates.

On the contrary, the increase in temperature in C_4 plants has little effect on CO_2 assimilation because CO_2 pumping in mesophyll cells decreases the photorespiration rate at all temperatures [20,21], As a result, C_4 plants are better adapted to heat stress and may show rapid canopy growth and root proliferation at high temperatures, compared to C_3 [22]. Weeds may show a wider range of responses to increasing temperatures because of their more extensive gene pool compared with crops, which enables them to adapt to diverse environmental conditions [23]. Due to their rapid growth and establishment, they can easily spread to new territories, and may induce changes in the biodiversity of ecosystems. Since 1998, ALS inhibitors have surpassed all other herbicide classes in terms of the number of weed species for which a resistant population was reported [24]. This resistance may be due to mutations in the ALS enzyme, decreased affinity, synthesis of specific amino acids, and herbicide transfer [25,26]. However, this study showed that increasing the temperature and, especially, increasing the concentration of CO_2 can increase the efficacy of these herbicides and augment the inhibition of ALS activity. The decline in ALS activity for C_3 and C_4 species was consistent with previous works [27–29], and it supported the conclusions reached by Ainsworth et al. [22], that weeds can show a reduction in ALS regardless of their photosynthetic pathway. Raising the temperature and elevating the CO_2 concentration can increase photosynthesis, alter enzymatic activity, and affect the synthesis of amino acids and pigment production [15,30,31]. A number of herbicide action sites have, in turn, been specifically designed to disrupt these biochemical processes. Such herbicides include tribenuron-methyl and sulfosulfuron + metsulfuron methyl (inhibitor of the ALS enzyme), atrazine (Photosystem II inhibitor), and amitrole (pigment inhibitor). Thus, CO_2- or temperature-induced increases in growth could, potentially, increase the efficacy of these herbicides.

The leaf orientation and surface are the first effective factors in absorption and displacement after herbicide application. If the increase in the CO_2 concentration or temperature causes an increase in the leaf surface or in the number of leaves, such a change could increase the absorption and interception of the herbicide. In addition, increasing the temperature could improve the uptake and translocation of the herbicide by affecting the fluidity of the cuticle and the plasma membrane [31]. Increasing CO_2 or temperature can also reduce herbicide absorption through changes in leaf surface characteristics, such as reducing stomatal dimensions, increasing leaf thickness, or changing the cuticular wax's viscosity [32]. An increase in temperature can cause more herbicide absorption and transfer, but, on the other hand, sufficiently high temperatures can reduce the effectiveness of the herbicide by increasing its metabolism [33,34].

5. Conclusions

Climate change, with its severe impacts on crop growth and yield, can endanger food safety. Weed management, as a main practice in crop production, is subject to climate change effects and should also be considered. In this work, a higher CO_2 concentration had a greater effect on C_3 weeds. ALS activity inhibition increased with growing concentrations of CO_2, except for in *E. crus-galli*. The efficacy of the sulfosulfuron 75% + metsulfuron methyl 5% on the *E. crus-galli* decreased when increasing the temperature. As a result, there is a need to adopt methods to enhance the effectiveness of this herbicide, or to find

supplementary control methods in order to exert an acceptable control in the future to prevent crop yield loss in the arable fields infested with this species.

In future climates, existing weed control techniques that rely heavily on herbicide use may have very different effects on weed growth. In spite of comprehensive studies on the possible effects of changing climate variables on various herbicide chemicals, this warrants urgent action. In particular, to research the interactive effects of climate change on weed regulation, it would be important to establish experiments with multiple climate variables. Rather than basing hypotheses on single-factor studies, systematic research efforts from the ecosystem to molecular levels will be required to investigate the interactive effects of different climate variables on plant growth and herbicide efficiency.

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