

## Article

# Antioxidant Enzyme and Cytochrome P450 Activities Are Involved in Horseweed (*Conyza Sumatrensis*) Resistance to Glyphosate

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**Abstract:** The intensive global use of glyphosate has led to the evolution of glyphosate resistant (GR) weed species, including the economically damaging horseweed (*Conyza sumatrensis*). We evaluated the glyphosate resistance mechanisms of *C. sumatrensis*. While 5-enolpyruvylshikimate-3-phosphate synthase activity was similar between the glyphosate resistant (GR) and nonresistant biotypes, plants from the GR population accumulated lower shikimate levels than susceptible ones, suggesting the absence of target-site resistance mechanisms. Decreases over time in glyphosate concentrations in GR leaves were not accompanied by increases in glyphosate concentrations in their stem and roots, indicating lower glyphosate distribution rates in GR plants. The early appearance of aminomethylphosphonic acid (the main glyphosate metabolite) in leaves, as well as its presence only in the stems and roots of GR plants, suggests faster glyphosate metabolism in GR plants than in susceptible ones. GR plants treated with glyphosate also showed greater antioxidant (ascorbate peroxidase [APX] and catalase [CAT]) and cytochrome P450-enzyme activities, indicating their great capacity to avoid glyphosate-induced oxidative stress. Three non-target mechanisms (reduced glyphosate translocation, increased metabolism, and increased antioxidant activity) therefore confer glyphosate resistance in *C. sumatrensis* plants. This is the first time that APX, CAT and P450-enzyme activities are related to GR in *C. sumatrensis*.

**Keywords:** aminomethylphosphonic acid; degradation; herbicide; shikimate; weed



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## 1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine], the active ingredient in numerous trade formulations used for total vegetation control, is a postemergence, broad-spectrum, non-selective systemic herbicide [1]. After the introduction of glyphosate-resistant (GR) commercial crop plants, glyphosate became the most widely used herbicide in the world [2]. Its herbicidal effect is due to the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), inhibiting the biosynthesis of the amino acids phenylalanine, tyrosine, and tryptophan [3].

Glyphosate is intensively used in Brazil for weed control in areas cultivated with GR crops [4] as preemergence herbicides are rarely used in that country and other postemergence treatments increase weed control costs [5]. With the wide use of that herbicide, increased concentrations of glyphosate have been observed in environments and deleterious effects of the herbicide have been observed in non-target organisms, like algae, aquatic

insects and plants [6–9]. Moreover, as a result of its indiscriminate use, GR weed biotypes are emerging very quickly [4]. The annual cost of glyphosate resistance in soybean crops alone in Brazil is estimated to be between \$3.7 and 6.0 billion Brazilian Reals, reaching R\$9.0 billion when considering the 5% productivity losses due to competition from GR weeds [10]. The first case of weed glyphosate resistance (in *Lolium rigidum*) was reported in 1996, twenty years after the introduction of the herbicide—which was considered an indicator of the slow development of weed resistance [11]. By 2022, however, the International Survey of Herbicide Resistant Weeds reported 340 cases of glyphosate resistant weeds in 29 countries, involving a total of 51 species, eleven of which occur in Brazil (*Amaranthus palmeri*, *A. hybridus*, *Chloris elata*, *Conyza bonariensis*, *Conyza canadensis*, *Conyza sumatrensis*, *Digitaria insularis*, *Echinochloa crus-galli* var. *crus-galli*, *Eleusine indica*, *Euphorbia heterophylla*, and *Lolium perene* ssp. *multiflorum*) ([www.weedscience.com](http://www.weedscience.com); access on 22 May 2022). Horseweeds (*Conyza* spp.) are among the most common weeds found growing among perennial and annual crops [12]. Its presence in soybean crops reduces yields by up to 90% in relation to areas cultivated in the absence of those weeds [13,14]. Horseweed has been mainly controlled in soybean and corn plantations by the use of glyphosate [15], although horseweed control using that herbicide is no longer satisfactory due to the growing resistance of some biotypes [12,15,16]. *Conyza* weed plants are highly adaptable, extremely competitive, and easily dispersed [17]. Considering the particularly successful acquisition of herbicide resistance by horseweed, and the economic losses associated with its presence among crops, a better understanding the mechanisms involved in the weed resistance to glyphosate has become increasingly important for defining new strategies for its management.

The mechanisms commonly involved in herbicide resistance acquisition by weeds include changes in herbicide absorption, vacuolar herbicide sequestration (and consequent reduced herbicide movement to the target sites), rapid metabolic herbicide detoxification, herbicide target site alterations, and gene amplification [12,16,18–21]. There is specific evidence that glyphosate induces oxidative stress [1,22,23] and inhibits cytochrome P450 activity (P450) [24,25] in susceptible plants—raising suspicion that GR weeds have high antioxidant capacities and low P450 sensitivities. While antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) may keep reactive oxygen species (ROS) levels under control (avoiding oxidative damages) in plants, P450 activity may contribute to herbicide detoxification [22,26]. Investigations of oxidative metabolism and P450 activity in GR weeds exposed to glyphosate have nonetheless been very scarce.

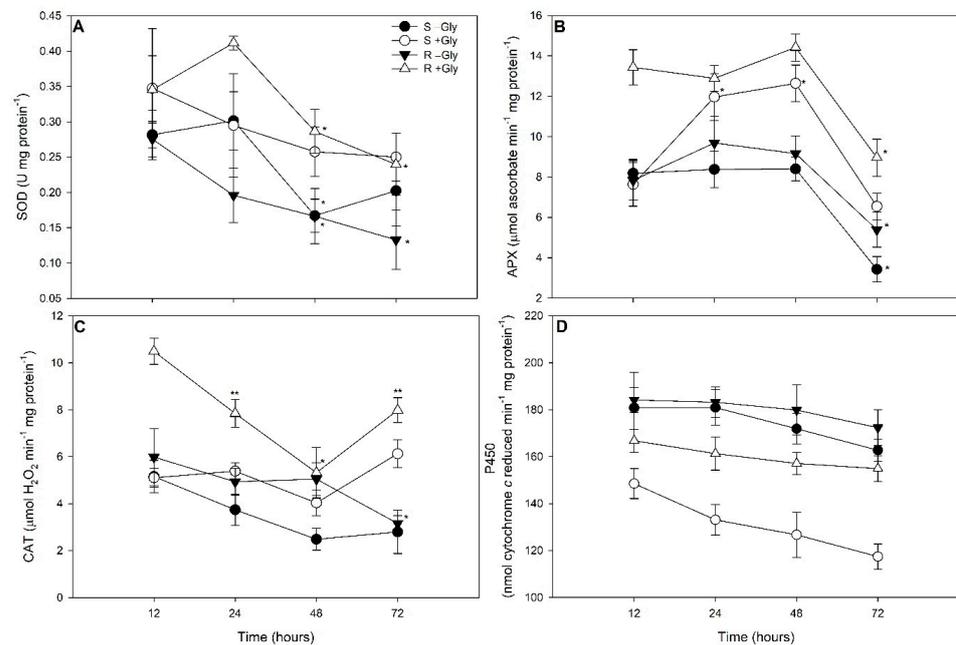
We therefore examined the non-target site mechanisms associated with glyphosate resistance in a *Conyza sumatrensis* biotype by evaluating the translocation and metabolism of that herbicide as well as its effects on the shikimate pathway, oxidative metabolism, and cytochrome P450 activity in both resistant and susceptible horseweed biotypes. We aimed to understand the physiological mechanisms related to the plant biotype resistance to glyphosate. We specifically sought to elucidate the roles of antioxidant enzymes and P450 in glyphosate resistance and thus contribute to the development of techniques (such as biology engineering) for horseweed management when glyphosate has lost its efficiency for weed control.

## 2. Results

### 2.1. Oxidative Stress Markers

Significant interactions between biotypes, glyphosate concentrations, and times of exposure were observed for SOD and APX activities (Table S1). Exposure for 24 h to glyphosate resulted in increased SOD activity in GR plants (R + Gly) in relation to non-resistant plants (Figure 1A). At 48 h, high SOD activity was observed in all plants exposed to glyphosate, regardless of their biotype (S/R + Gly) (Figure 1A). At 72 h, glyphosate exposure increased SOD activity in resistant biotype plants (R + Gly) as compared to GR plants without glyphosate treatment (R–Gly) (Figure 1A). Regardless of the glyphosate

treatment (0 or 1080 g ae ha<sup>-1</sup>), SOD activity at 48 and 72 h was lower in the GR biotype (R -/+ Gly) in relation to 12 and 24 h of exposure (Figure 1A).



**Figure 1.** Activities of superoxide dismutase (A), ascorbate peroxidase (B), catalase (C), and NADPH-cytochrome P450 reductase (D) in glyphosate susceptible (S) and resistant (R) *Conyza spp.* plants exposed to 0 (-Gly) or 1080 g glyphosate (a.e.) ha<sup>-1</sup> (+Gly) for 72 h. Values are represented as the mean  $\pm$  standard error of five replicates. \*, \*\* indicates significant differences between times with the same biotype and glyphosate concentrations, by the Contrast test (considering  $p < 0.05$ ).

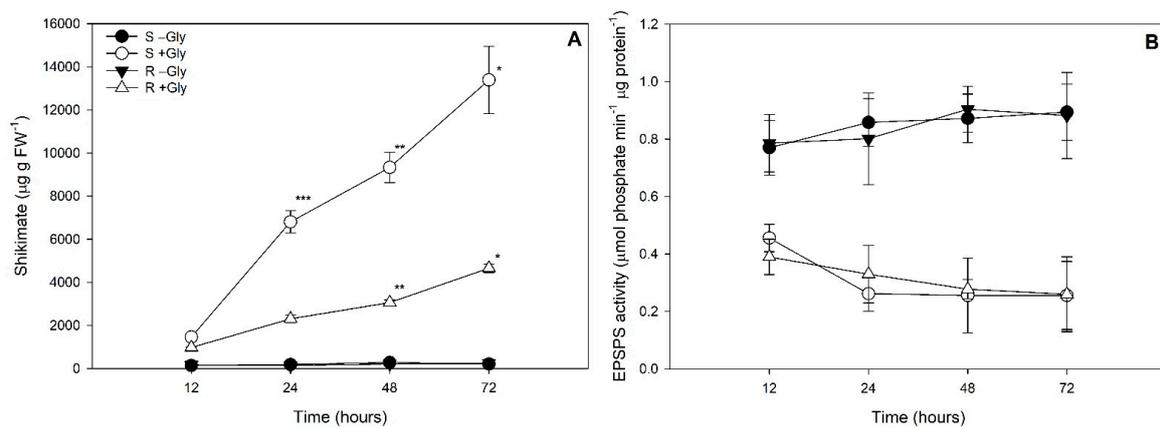
Regardless of the biotype, glyphosate exposure (S/R + Gly) resulted in increased APX and CAT activities after 24 h of exposure; however, enzyme activity increases were observed earlier (after 12 h) in GR-resistant plants (R + Gly) as compared to the susceptible biotype (S + Gly) (Figure 1B,C). With the exception of susceptible plants exposed to glyphosate (S + Gly; which did not show significantly different APX activities between 12 h and 72 h), APX activity decreased at 72 h regardless of the biotype and glyphosate exposure (Figure 1B). CAT activity was greater at 72 h in relation to 48 h of exposure in plants of both biotypes when exposed to glyphosate (S/R + Gly; Figure 1C).

## 2.2. NADPH-Cytochrome P450 Reductase Evaluations

Significant interactions between biotypes and glyphosate concentrations were observed in terms of NADPH-cytochrome P450 reductase (P450) activity (Table S1). Regardless of the biotype, P450 activity was greater in plants without any exposure to glyphosate (Figure 1D). When treated with glyphosate, greater P450 activity was observed in GR plants than in the susceptible biotype (Figure 1D).

## 2.3. Shikimate Concentrations and EPSPS Activities

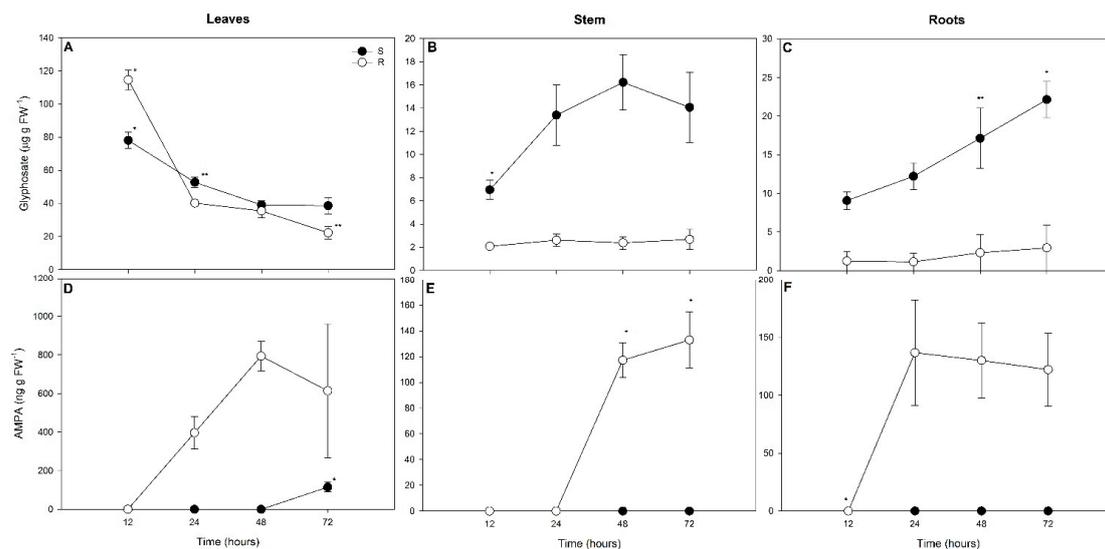
Significant interactions between biotypes, glyphosate concentrations, and times of evaluation were observed in terms of shikimate concentrations in plant leaves (Table S1). In plants not exposed to glyphosate, leaf shikimate concentrations did not differ between the two biotypes or in terms of experimental times (Figure 2A). Leaf shikimate concentrations increased over time in susceptible plants treated with glyphosate, while GR plants did not demonstrate significantly different shikimate concentrations after 48 or 72 h of glyphosate exposure (Figure 2A). Leaf EPSPS activities did not significantly differ between biotypes, but were greater in plants not exposed to glyphosate treatments in relation to those exposed to that herbicide (Figure 2B).



**Figure 2.** Shikimate concentrations (A) and 5-enolpyruvylshikimate-3-phosphate synthase activities (B) in glyphosate susceptible (S) and resistant (R) *Conyza* spp. plants exposed to 0 (-Gly) or 1080 g glyphosate (a.e.)  $\text{ha}^{-1}$  (+Gly) for 72 h. Values are represented as the mean  $\pm$  standard error of five replicates. \*, \*\*, \*\*\* indicates significant differences between times with the same biotype and glyphosate concentrations, by the Contrast test (considering  $p < 0.05$ ).

#### 2.4. Glyphosate and AMPA Concentrations

No glyphosate or AMPA were found in plants not exposed to glyphosate treatments. Significant interactions between biotypes and times of evaluation were observed for glyphosate and AMPA concentrations in all plant organs (Table S2). While leaf glyphosate concentrations were greatest after 12 h of exposure, those concentrations were lower after 24 and 72 h in GR plants in relation to susceptible plants (Figure 3A). Leaf glyphosate concentrations decreased over time in both biotypes (Figure 3A). Stem and root concentrations of glyphosate were greater over time in susceptible plants than in GR plants (Figure 3B,C); glyphosate concentrations in the stems and roots of susceptible plants increased after 24 h and 48 h of exposure, respectively, but did not significantly differ after different times of exposure among GR plants (Figure 3B,C).



**Figure 3.** Glyphosate and AMPA concentrations in the leaves (A,D), stems (B,E), and roots (C,F) of glyphosate susceptible (S) and resistant (R) *Conyza* spp. plants exposed to 0 (-Gly) or 1080 g glyphosate (a.e.)  $\text{ha}^{-1}$  (+Gly) for 72 h. Values are represented as the mean  $\pm$  standard error of five replicates. \*, \*\* indicates significant differences between times with the same biotype and glyphosate concentrations, by the Contrast test (considering  $p < 0.05$ ).

AMPA was not detected in either the stems or roots of susceptible plants (Figure 3E,F). AMPA was detected earlier in the leaves of GR plants than in susceptible plants, being detected after 24 h of exposure in GR plants and at 72 h in the susceptible biotype (Figure 3D); at 72 h, leaf AMPA concentrations were greater in GR than in susceptible plants (Figure 3D).

### 3. Discussion

The glyphosate resistance of the GR horseweed populations examined in the present study was confirmed by the lower shikimate concentrations observed in their leaves in relation to those found in susceptible plants after herbicide exposure (Figure 2A). Shikimate accumulation tests, however, do not reveal which type of mechanism [target-site resistance (TSR) or non-target site resistance (NTSR)] conferred glyphosate resistance [27]. To exclude the involvement of TSR mechanisms (such as EPSPS mutations or EPSPS gene amplification) [28], herbicide target enzymes assays are necessary [29]. The enzymatic activity of EPSPS were similar between the two horseweed biotypes, whether exposed or not to glyphosate (Figure 2B), thus excluding the involvement of TSR mechanisms in glyphosate resistance [28]. We therefore investigated biochemical responses as well as glyphosate distribution and metabolism in plants, aiming to elucidate possible NTSR mechanisms involved in their GR tolerance.

NTSR mechanisms have been related to glyphosate weed resistance [30]. Reduced glyphosate absorption and translocation, as well as its increased metabolism, conferred resistance in saltmarsh aster (*Aster squamatus*) [28]. In *Echinochloa colona*, aldo-keto reductase metabolizes glyphosate and confers resistance [31]. The antioxidant system activity of *Amaranthus palmeri* was found to be related to glyphosate resistance [32]. Glyphosate resistance in *C. canadensis* was related to impaired glyphosate translocation and its metabolism [33], while lower distribution rates between plant organs assured glyphosate resistance in *C. bonariensis* [15]. Similarly, the GR *C. sumatrensis* population investigated here evidenced differential glyphosate translocation and metabolism in relation to the sensitive population. In contrast to susceptible plants, in which glyphosate concentrations decreases over time were followed by increased glyphosate concentrations in the stems and roots (Figure 3), in GR plants, no increases in glyphosate concentration were observed in their stems and roots over time (Figure 3)—indicating that glyphosate translocation differs between the biotypes, being lower in GR plants. One could argue that decreased leaf uptake of that herbicide could result in glyphosate resistance in the GR biotype, although the higher glyphosate concentrations at 12 h observed in GR leaves in relation to susceptible plants eliminates that hypothesis (Figure 3A). Interestingly, the rate of glyphosate disappearance in the leaves of GR plants was not followed by its increased translocation. We therefore became interested in investigating herbicide metabolism. One of the main glyphosate by-products is AMPA, which has been found in the tissues of plants exposed to glyphosate [34]. The early appearance of AMPA in the leaves of GR plants, as well as the presence of that metabolite in their stems and roots, indicate that this biotype can metabolize glyphosate much faster than susceptible plants. Therefore, similar to previous findings [15,31], we observed that impaired glyphosate translocation and heightened glyphosate metabolism are mechanisms related to glyphosate resistance in *C. sumatrensis* plants.

In addition to these already described mechanisms, we report here, for the first time, evidence for the involvement of antioxidant and P450 enzymes in glyphosate resistance in *C. sumatrensis* plants. The higher activities of antioxidant enzymes in GR as opposed to sensitive *A. palmeri* populations assured lower lipid peroxidation (an oxidative stress burst) and was related to plant glyphosate resistance [31,35]. Similarly, we observed greater APX and CAT activities in GR leaves exposed to glyphosate in relation to susceptible plants. APX and CAT are important antioxidant enzymes involved in H<sub>2</sub>O<sub>2</sub> scavenging, and therefore in the avoidance of oxidative bursts caused by the accumulation of reactive oxygen species. Additionally, the increased activities of those enzymes have been reported in cases of plant tolerance to xenobiotics [36], including glyphosate [9]. In contrast to H<sub>2</sub>O<sub>2</sub> scavenging enzymes (APX and CAT), SOD appears not to be involved in glyphosate resistance, as

enzyme activity differed between the glyphosate exposed biotypes only at 24 h (Figure 1A). In addition to antioxidant enzyme activities, the biotypes differed in terms of the responses of P450 to glyphosate (Figure 1D). Glyphosate is a known inhibitor of cytochrome P450 activity (P450) [24,25], but P450 activity in the leaves of GR plants was less sensitive to glyphosate than in the sensitive biotype.

Cytochrome P450 monooxygenase constitutes the largest family of enzymes in plant metabolism [37] and is responsible for detoxifying herbicides that are not chemically similar, such as ACCase- and ALS-inhibitors [30]. In *Lolium* spp., for instance, resistance to pinoxaden and iodosulfuron-mesosulfuron was related to the insensitivity of its P450 enzymes, assuring herbicide degradation in resistant biotypes [38]. Similarly, the GR population examined here showed lower sensitivity to glyphosate, which is a known P450 inhibitor in both plants [24,25] and animals [39,40]. However, in contrast to ACCase- and ALS-inhibitors, there have been no reports implicating P450 in glyphosate metabolism. In addition to C-P lyase (which degrades glyphosate into sarcosine and inorganic phosphate), glyphosate oxidoreductase (GOX) activity (which produces AMPA) has been identified as the main mechanisms of glyphosate degradation in plants [41]. The conversion of glyphosate to AMPA by GR horseweed plants indicates that that biotype possesses a GOX-like enzyme, as do other weeds [41–43]. The higher P450 activity observed in GR horseweed biotypes may therefore not be related to herbicide degradation. P450, however, is involved in protection against stress in plants through the biosynthesis and regulation of hormones, fatty acids, sterols, cell wall components, biopolymers, and other defense compounds (such as terpenoids, alkaloids, flavonoids, furanocoumarins, glucosinolates, and allelochemicals) [44]. Additionally, cytochrome P450 has critical roles in maintaining redox homeostasis and protecting the organism from toxic ROS accumulations [45]. Silencing the P450 genes in *Apis cerana cerana*, for example, resulted in the enhanced activities of peroxidase and CAT. Similarly, the overexpression of P450 genes in tobacco resulted in decreased oxidative bursts (due to decreased ROS accumulations) when plants were submitted to drought conditions [46]. Those findings suggest the role of P450 in cell antioxidant defenses. In this context, in addition to inhibiting amino acid synthesis, the ability of glyphosate to kill plants has been related to its capacity to induce oxidative stress [1,22,47,48], and the maintenance of high antioxidant activities may therefore contribute to herbicide resistance of horseweed plants.

Adverse environmental effects associated with herbicide applications have emerged in the form of increases in resistant weed populations [49]. Changes in light, temperature and precipitation regimes, for instance, may collaborate to select plant species able to deal with ROS stress through the activation of antioxidant systems. In this scenario, climate changes may favor glyphosate resistance in weeds with the natural ability to cope with ROS formation and accumulation. The established relationship between antioxidant capacity and GR in *C. sumatrensis* therefore suggests avoiding glyphosate applications during warm weather or during periods of high light intensity-when antioxidant activities tend to increase in plant cells [50].

## 4. Material and Methods

### 4.1. Greenhouse Experiments

*Conyza sumatrensis* (Retz.) E.Waker seeds of both the resistant and susceptible biotypes were collected among soybean crops in Paraná State, Brazil. A rapid resistance test was performed to confirm weed susceptibility to glyphosate that involved sowing seeds in polypropylene pots (0.8 L, 14 × 6 cm) containing a sterile substrate (shells of composted pine, vermiculite, peat, and fertilizers [nitrogen, phosphorus and potassium]). The pots were then kept under greenhouse conditions with minimum/maximum temperatures of 25/32 °C and natural light supplemented by sodium vapor lamps to provide a 12 h photoperiod and an average photosynthetic active radiation of 825  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ . When the seedlings were at the rosette stage (4 to 6 leaves), corresponding to the appropriate stage of herbicide application, the two biotypes were treated with 0 (ultrapure water) or

1080 g ae ha<sup>-1</sup> of glyphosate (Roundup Original, 356 g equivalent acid (ea) L<sup>-1</sup>, Monsanto, Brazil), according to the manufacturer's recommendations for horseweed plants, and using an experimental backpack sprayer (CO<sub>2</sub> pressurized, equipped with two flat-fan nozzles) to apply the equivalent of 200 L of the syrup per hectare. After 72 h of exposure to glyphosate, the susceptible (but not the resistant) plants began to show symptoms of intoxication (chlorosis, necrotic spots, and wilting)—symptoms that were easily noted after 12 days of exposure (Figure 4). The tests were conducted until the resistant biotype produced seeds (110 days); no resprouting was observed in susceptible plants exposed to glyphosate.



**Figure 4.** Resistant (A) and susceptible (B) *Conyza* spp. biotypes exposed to 1080 g glyphosate (ea) ha<sup>-1</sup> for 12 days.

After the confirmation of glyphosate resistance/susceptibility of the biotypes, new experiments, under the same conditions described above, were performed using a randomized block design with five pots per biotype (corresponding to replicates) per treatment, in a two (herbicide concentrations) × five (times of evaluation) factorial scheme. The plants were harvested 0, 12, 24, 48 and 72 h after the beginning of the glyphosate treatments. Evaluations were halted after 72 h of exposure (as the susceptible biotype plants treated with glyphosate began showing pronounced symptoms of intoxication, including chlorosis and leaf necrotic spots) to allow evaluations of their metabolic conditions prior to the onset of acute cell damage and plant death.

Five plants from each biotype and each glyphosate treatment were harvested at each evaluation time, thoroughly washed with distilled water, and separated into roots, stems, and leaves. Samples of the fifth and sixth nodes (from the shoot apex), corresponding to fully expanded leaves, were selected for the enzyme assays. All plant samples were immediately frozen in liquid nitrogen and stored at −80 °C until assayed.

#### 4.2. Enzymatic Evaluations of the Leaves

To study the antioxidant enzymes, 0.1 g of leaves were macerated in 1 mL of an extraction buffer containing 100 mM potassium buffer (pH 7.8), 100 mM EDTA, 1 mM *L*-ascorbate, and 2% polivinilpirrolidona (PVP)-40 (*m/v*). The protein contents of the samples were determined using the Bradford method [51]. Superoxide dismutase (SOD; EC 1.15.1.1) [52], catalase (CAT; EC 1.11.1.6) [53], and ascorbate peroxidase (APX; EC 1.11.1.11) [54] activities were evaluated as oxidative stress markers. The measurements of NADPH-cytochrome P450 reductase were performed following [55]. EPSPS (EC 2.5.1.19) was extracted from the leaves according to [56], and its enzymatic activity measured

according to [57], using an EnzCheckQR phosphate assay Kit (Invitrogen, Carlsbad, CA, USA). The specific activities of EPSPS were determined in the absence and in the presence of 100  $\mu\text{M}$  glyphosate (Sigma Aldrich, Brazil), and expressed as phosphate ( $\mu\text{mol}$ ) liberated per  $\mu\text{g}$  of total soluble protein (determined using Bradford method) per minute.

#### 4.3. Glyphosate, AMPA and Shikimate Evaluations

The concentrations of glyphosate, aminomethylphosphonic acid (AMPA), and shikimate in the plants were determined using a LC-MS/MS system composed of a XEVO TQD triple quadrupole (Walters) mass spectrometer equipped with an electrospray (ESI) ionization source coupled to an HPLC Varian SYS-LC-240-E equipped with an autosampler, following Gomes et al. [58]. Glyphosate and AMPA concentrations were measured in the roots, stems, and leaves, while shikimate was evaluated only in the leaves. Extractions were performed with 0.4 g of each plant organ, previously thoroughly washed in ultrapure water, using 50 mL of acidified water (pH 2.5) following Matallo et al. [59]. The plant extracts were filtered through C18 SPR cartridges (500 mg/6 mL; Applied Separations, Allentown, PA, USA) previously conditioned with 15 mL of acidified water (pH 2.5) and 5 mL of methanol. The cartridges containing the samples were washed with 3 mL of 50% methanol in water (*v/v*). The eluate was then dried in a SpeedVac machine (RC1010, Thermo), and the residues resuspended in the mobile phase A. Before injection, the samples were filtered through nylon syringe filters (13 mm  $\times$  0.25  $\mu\text{m}$ , Filtrilo Brazil).

An Ascentis<sup>®</sup> C18 column (Sigma-Aldrich, São Paulo, Brazil) was used for chromatographic separation, with a mobile phase consisting of 5 mM of ammonium acetate in water (phase A) and 5 mM of ammonium acetate in methanol (phase B), both pH 7.0. The mass spectrometry analyses were performed in a negative ion mode. Analytical-grade glyphosate, AMPA (Pestanal grade, Sigma-Aldrich, São Paulo, Brazil), and shikimate (analytical standard, Sigma-Aldrich, Brazil) were used to prepare the calibration curves. The six-point calibration curves showed good linearity for the analytes ( $r^2 \geq 0.95$ ;  $p < 0.0001$ ). For quality control, each sample batch included three blanks, three standards, and three fortified samples. The recovery rates of all of the compounds were greater than 89%.

#### 4.4. Statistical Analyses

The experiments consisted of combinations of the two biotypes (sensitive and resistant) and two glyphosate concentrations (0 and 1080 g ea ha<sup>-1</sup>) allotted to the main plots, and four evaluation times (0, 12, 24, 48, 72 h) allotted to the subplots and tested in split-plot design with five replications. Statistical analyses were performed using JMP 7.0 software (SAS Institute Inc.). Data were tested for normality (Shapiro–Wilk) and homogeneity (Bartlett), and then statistically evaluated. For the biochemical evaluations, data were submitted to three-way analyses of variance (ANOVA). Interactions between biotypes, glyphosate concentrations, and times of evaluation were added to the model. For glyphosate and AMPA concentrations, data were evaluated by using two-away ANOVA, with interactions between biotypes and times of evaluation being included in the model. When differences were detected by ANOVA, the means were compared using the Contrast test ( $p < 0.05$ ).

## 5. Conclusions

In addition to the reduced translocation and increased metabolism of glyphosate already described for *C. sumatrensis*, increased APX, CAT, and P450 enzyme activities constitute non-target mechanisms endowing glyphosate resistance. The activities of antioxidant systems and P450 were shown for the first time here to be related to glyphosate resistance in *C. sumatrensis* plants. Our findings open new avenue for studies, establishing cell antioxidant mechanisms as targets for investigating glyphosate resistance in weeds.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/stresses3010005/s1>, Table S1: ANOVA for the interactive effects of biotypes (susceptible and resistant), glyphosate concentrations (0 or 1080 g glyphosate (ae ha<sup>-1</sup>),

and times of exposure (12, 24, 48, and 72 h), Table S2: ANOVA for the interactive effects of biotypes (susceptible and resistant) and times of exposure (12, 24, 48, and 72 h).

**Author Contributions:** G.C.K., R.Z.M. and R.S.A.K. methodology, investigation, formal analysis, review and editing; A.A.M.B. conceptualization, funding acquisition, preparing the review and editing; P.J. and M.P.G. conceptualization, methodology, investigation, formal analysis, preparing the original draft, reviewing & editing, supervision, funding acquisition, and project administration. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data available on request from the authors.

**Conflicts of Interest:** The authors declare that there is no conflict of interest.

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