

Assessment the Phytotoxic Effects of Prosulfuron on Wild Sunflower (*Helianthus annuus* L.) in Laboratory and Nursery Conditions

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Abstract

This study evaluated the effect of the herbicide prosulfuron on two wild sunflower populations from northern Tamaulipas, México, which differed in their history of herbicide exposure. Population C12 originated from a farm with documented history of routine prosulfuron applications, meanwhile, C36 had no prior exposure. A bioassay was conducted under both laboratory and nursery conditions, evaluating visual damage, plant height, leaf necrosis, and electrolyte leakage over 28 days after application (DAA). Prosulfuron induced foliar chlorosis in apical bud within seven days, which progressed to growth inhibition and necrosis, with more pronounced symptoms in susceptible C36 population. Plant height was significantly reduced in C36, reaching nearly 50% reduction at 14 DAA, while C12 exhibited only at 4% reduction. Electrolyte leakage further supported these findings, showing a 74% solute loss in C36 compared to 14.7% in C12 at 21 DAA. Microscopic analyses revealed cellular disruptions consistent with the action mode of acetolactate synthase (ALS) inhibitors. Visual assessments demonstrated poor control of the C12 population despite previous exposure, suggesting the potential selection of tolerant individuals, whereas C36 was highly susceptible. These results underscore the importance of integrating physiological, morphological and cellular parameters to detect early signs of herbicide resistance. Notably, cellular electrical conductivity proved to be an effective preliminary screening tool for distinguishing tolerant populations, offering a practical alternative to more time-consuming bioassays. Overall, the findings indicate the presence of a potentially tolerant biotype of wild sunflower in northern of Tamaulipas, and highlight the urgent need for integrated weed management strategies to prevent the further development of resistance and to sustain the efficacy of chemical control in sorghum and other cereal crops.

Keywords: broadleaf weed, damage, herbicide, broadleaf weed, selective

1. Introduction

Conventional, cultural and manual weed management practices are labor-intensive, time-consuming, and often economically unsustainable. Consequently, chemical weed control has become a more cost-effective and practical alternative to manual weeding (Kumar et al., 2018). Moreover, the adoption of chemical management strategies has also become increasingly essential (Santosh et al., 2024), particularly in resource-limited agroecosystems where labor shortages are exacerbated by rural-to-urban migration and demographic shifts (Varela et al., 2017), however, the repeated and prolonged use of specific chemical herbicides has contributed to the evolution of resistant weed, for example, glyphosate-resistant weeds (Arispe-Vazquez et al., 2023).

In sorghum (*Sorghum bicolor* Moench) (Poaceae), weeds often germinate earlier than the crop because of its slow initial growth under low temperatures (Emendack et al., 2021), particularly during the autumn–winter cropping cycle in northern of Tamaulipas, México. Consequently, if weed control is not implemented at the appropriate time, competition throughout the growing season can severely reduce cereal yields, including sorghum (Bararpour et al., 2019).

Rising labor have further impacted sorghum production in northeastern Tamaulipas (Alejandro et al., 2020). Moreover, the effectiveness of weed management strategies depends on several factors, including farm size, production system, weed species composition, and the phenological stage of weeds. During the autumn–winter agricultural cycle in this region, the predominant weed is wild sunflower (*Helianthus annuus* L.) (Asteraceae), locally known as “polocote” (Rosales et al., 2011), an annual species native to the area that reproduces exclusively by seed area (Kantar et al., 2015). Weed control in sorghum production typically relies on non-selective preplant herbicides, such as glyphosate (Espinosa & Cisneros, 2022). For post-emergence management, selective herbicides like as prosulfuron (NIH, 2024) or hormonal herbicides such dicamba (Mishra et al., 2016) are commonly applied. Additionally, the combined use of prosulfuron and glyphosate in preplant applications has been reported as an effective strategy for managing weeds in spring cereals, including wheat, barley, and oats (Soltani et al., 2014).

Prosulfuron ($C_{15}H_{16}F_3N_5O_4S$) is a selective sulfonylurea herbicide primarily used for post-emergence weed control (NIH, 2024). Sulfonylurea herbicides, first discovered in 1975, rapidly established a new benchmark in chemical weed management due to their high efficacy at remarkably low application rates—typically 10 to 100 times lower than those required for conventional herbicides (Brown, 2010). Their widespread adoption has been further driven by their favorable environmental profile, reduced toxicological risks, and versatility across a wide range of production systems, currently, 18 active ingredients within this chemical family are registered for use in more than a dozen major crops worldwide (NIH, 2024).

These herbicides act by inhibiting acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched-chain amino acids, including valine, leucine, and isoleucine (Brown, 2010; Zulet et al., 2013). The disruption of this pathway prevents protein synthesis, leading to growth inhibition and tissue necrosis in susceptible plants species (Qingyan et al., 2007). These compounds are highly potent, requiring very low application rates—typically 2 to 75 g ha⁻¹—while maintaining strong herbicidal efficacy (Sarmah & Sabadie, 2002; Barros et al., 2016; Wei et al., 2017). Sulfonylureas are rapidly absorbed through both foliage, roots, and efficiently translocated throughout the plant’s vascular system (Imig et al., 2023). However, the widespread use of ALS-inhibiting herbicides has accelerated the evolution of herbicide-resistant weed populations (Hatami et al., 2016). Within the sulfonylurea chemical family, prosulfuron has been particularly associated with a higher number of resistant weed species compared to other herbicide groups (Torra et al., 2021). The objective of this research was to evaluate the weed control efficacy and phytotoxic effects of prosulfuron on two populations of wild sunflower (“polocote”) in northeastern México.

2. Method

2.1 Location and Origin of Biological Material

The northern region of Tamaulipas, which borders the United States, comprises municipalities such as Nuevo Laredo, Reynosa, Matamoros, Río Bravo, and Miguel Alemán. This region, including Ciudad Río Bravo, is characterized by a hot and extreme climate, with an average annual temperature of 24 °C, and temperature ranges from average highs of 36.8 °C to lows of 9.7 °C. The area has semi-arid climate, receiving annual precipitation between 440 to 533 mm.

For this experiment, seeds were collected from wild sunflower populations and assigned collection numbers, from which two representative populations were selected for evaluation: C12, obtained in San Fernando, Tamaulipas, at the locality “La Estación” on May 19th, 2023 (coordinates 24°73'88"N, -97°97'67"W), corresponding to a rainfed production area; and C36, collected in Río Bravo, Tamaulipas, from lot B1 within the experimental area of Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) on May 12th, 2023 (coordinates 25°96'54"N, -98°01'25"W), corresponding to an irrigated production area.

2.2 Experiment Conduction

The experiment was conducted as a bioassay following the methodology described by Bolaños et al. (2018), using open-air pots and consisting of two distinct phases: a laboratory stage and a nursery stage.

2.2.1 Laboratory Stage

In the first stage, five grams of wild sunflower seeds from each collection underwent a 24 h water pre-treatment to break seed dormancy. On January, 2024, 30 seeds per Petri dish were plant, with a total of 20 Petri dishes per collection. The dishes were placed in a germination chamber at 5 °C for seven days, to promote stratification and synchronize germination. Subsequently, they were transferred to laboratory conditions at 25 °C and maintained until radicle emergence was observed (Cisneros et al., 2025).

2.2.2 Nursery Stage

In the second stage, 10 pre-germinated seeds were transplanted into per rigid plastic pot (20 × 16 cm; capacity 3.2 kg). The substrate consisted of a 70:30 soil-to-sand mixture, which was pre-hydrated prior to transplantation. The transplanting process was performed on November, 2023. After transplantation, the pots were irrigated as needed to maintain optimal moisture conditions, and seedlings were subsequently thinned to three plants per pot to ensure uniform growth and development.

Treatments were applied on March, 2024, when plants had reached a height of 8-10 cm and developed 6-7 true leaves. The commercial formulation of prosulfuron was applied at a rate of 17.1 g a.i. kg⁻¹. The adjuvant Bionex (containing tridecyl polyoxyethylene alcohol 200 g L⁻¹, polyoxyethylene nonylphenol 50 g L⁻¹, and propylene glycol 54 g L⁻¹) was applied at 1 mL L⁻¹ using potable water (pH of 6.7, EC of 0.026 dS m⁻¹). Applications were performed with a manual backpack sprayer with a flat fan nozzle (8003E), operating at a pressure of 40 PSI and delivering a spray volume of 200 L ha⁻¹.

2.3 Treatments and Experimental Design

Four treatments were established: C12P and C36P, representing plants treated with prosulfuron, and C12T and C36T, serving as untreated controls. In the laboratory, the treatments were arranged in a completely randomized design (CRD), with 10 replicates per treatment, whereas in the nursery they were arranged in a randomized complete block design (RCBD), also with 10 replicates per treatment.

2.4 Weed Control

2.4.1 Evaluation of Herbicide Effect in the Nursery

Weed damage was assessed through four visual evaluations conducted at 7, 14, 21, and 28 days after application (DAA). Herbicide phytotoxicity was estimated visually using the European Weed Research Society (EWRS) scale (Table 1) (Champion, 2000). The data were expressed as percentages (Metcalf et al., 2023).

At each sampling date, plant height (cm) was measured from the base of the stem to the apical growth point. Additionally, the number of dead plants was recorded and expressed as a percentage of the total plants per pot, providing an estimate of herbicide-induced mortality.

Table 1. European Weed Research Society (EWRS) visual scale for assessing herbicide-induced weed control

Value	Control (%)	Effect on Weed
1	99.0-100.0	Death
2	96.5-99.0	Very Good Control
3	93.0-96.5	Good Control
4	87.5-93.0	Sufficient Control
5	80.0-87.5	Medium Control
6	70.0-80.0	Regular Control
7	50.0-70.0	Poor Control
8	1.0-50.0	Very Poor Control
9	0.0-1.0	No Effect

2.4.2 Evaluation of Herbicide Effect in Laboratory

Electrolyte leakage is widely used criterion to evaluate the effects of herbicides on cell membrane permeability (Prendeville & Warren, 2006). The physiological impact of herbicide toxicity was quantified by measuring the loss of solutes from the leaves between 7 and 21 DAA. No measurements were taken at 28 DAA due to extensive leaf deterioration in the susceptible population.

The assay was performed by determining electrical conductivity, following protocols adapted from Hatsugai & Katagiri (2018) and El-Mahrouk et al. (2024). From five plants per treatment, two fully expanded leaves were marked, and two leaf discs (5×5 mm) were excised from each plant (one disc per leaf). The samples were initially placed in Ziploc bags for one h and subsequently processed in the laboratory. Prior to measurement, the discs were washed with distilled water for 1 min and transferred to Falcon tubes containing 25 mL of deionized and sterile water kept at 25 °C for 20 h. After this period, leaf tissues were removed with tweezers, and the electrical conductivity ($\mu\text{mhos/cm}$) of the solution was measured. The conductivity meter sensor was rinsed with sterile water between samples. Leaf tissues were preserved at 5 °C in Falcon tubes containing sterile distilled water for subsequent analyses.

For microscopic examination, fresh leaf samples were analyzed without prior treatment to observe external changes on the adaxial (upper) surface, which is normally exposed to the herbicide, and images were captured using a UNIQUE® stereo microscope.

2.5 Statistics and Data Analysis

Statistical analyses were performed to compare means at each sampling date using Student's t-test performed independently for each time point, using SAS 9.4 (SAS, 2016). Additionally, herbicide-induced damage symptoms were assessed descriptively and visually to complement the quantitative evaluations.

3. Results and Discussion

The results revealed statistically significant differences in control degree achieved with prosulfuron between the two wild sunflower collections throughout the during the evaluation period (Table 2). Collection C12, originates from San Fernando, is from an area where local farmers and agronomic technicians have reported herbicide escapes following standard prosulfuron applications. Under the controlled bioassay conditions of this study, the observed response in C12 may indicate potential resistance to the commercial dose commonly used for this weed (Rosales et al., 2011).

Based to the EWRS visual scale, the control of C12 I considered very poor, with less than 50% control at 28 DAA (Bolaños et al., 2018). In contrast, the population from CERIB-CIRNE, which had no prior exposure prosulfuron, exhibited medium control (85.5%) at 14 DAA, sufficient control at 21 DAA, and very good control by the end of the evaluation period (Table 2). These results suggest that previous exposure history may strongly influence the efficacy of prosulfuron in controlling wild sunflower populations.

Table 2. Control of the herbicide prosulfuron in wild sunflower (*H. annuus*) from the north of Tamaulipas, México

Contrast	Days After Application			
	7	14	21	28
	----- Damage to the Plant (%) -----			
C12P vs. C36P	8 vs. 12 *	11.3 vs. 85.5 **	17.0 vs. 92.7 **	25.5 vs. 98.7 **

Note. Average data per collection. C12P = San Fernando Collection; C36P = Río Bravo Collection. Statistical significance according to t Student *P < 0.05; **P < 0.01.

One of the more notable biometric responses of wild sunflower to herbicide application is a reduction in plant height (Table 3). This effect is attributed to the inhibition of branched-chain amino acid synthesis caused by prosulfuron, which disrupts the activity of ALS, consequently inhibiting protein synthesis and cell division. Susceptible plants exhibit stunted growth (Qingyan et al., 2007), in some cases, mortality (Prendeville & Warren, 2006), as observed in this experiment, where the C36 collection showed high susceptibility (Table 3).

In herbicide resistance studies, the identification of susceptible biotypes is essential to confirm resistance through dose–response assays (Ritz et al., 2015; Bolaños et al., 2018). In both populations (C12 and C36), with or without herbicide treatment, significant differences in plant height were detected (Table 3). The greatest reduction was observed in the susceptible population (C36) at 14 DAA, showing an almost 50% decrease in height at 14 DAA compared to the untreated control. Conversely, the more tolerant population (C12) exhibited only a 4% reduction, and this minimal response remained consistent up to 28 DAA.

Table 3. Effect of prosulfuron on plant height in wild sunflower (*H. annuus*) from the north of Tamaulipas, México

Contrast	Days After Application			
	7	14	21	28
	----- Plant height (cm) -----			
C12P vs. C12T	13.1 vs. 15.0 *	13.6 vs. 16.0 **	14.1 vs. 17.3 **	15.5 vs. 18.9 **
C36P vs. C36T	14.2 vs. 16.0 *	8.3 vs. 16.6 *	6.4 vs. 18.3 **	6.3 vs. 19.2 **

Note. Average data per collection. C12P and C36P, with the herbicide Prosulfuron and C12T and C36T= control without herbicide. Statistical significance according to t Student *P < 0.05; **P < 0.01.

The visual damage symptoms caused by prosulfuron were evident seven days after application (Figure 1) The first symptom observed was growing-point chlorosis in both populations, C12 and C36 (Figures 1B, 1C), compared with their respective untreated controls (Figures 1A, 1D). However, plants within each population exhibited variable responses; some individuals were more severely affected by the herbicide than others (Figures 1C and 1F).

The severity of herbicide-induced symptoms was greater (C36) than in the more tolerant population (C12). The initial symptom observed was chlorosis of the apical bud at 7 DAA, which was more intense in the C36 population (Figure 1F). By 14 DAA, symptoms had intensified C36, leading upper leaf deformation and significant growth suppression compared with C12.



Figure 1. Initial symptoms of prosulfuron's effect on wild sunflower weeds seven days after application. Collection 12 from San Fernando (A, B, and C); Collection 36 from Río Bravo (D, E, and F), both originating from northern Tamaulipas, México. A and D = Controls. B = No visible symptoms; C = Some plants exhibited slight visual symptoms, such as leaf blinding without chlorosis, while the most susceptible individuals showed signs of decline and wilting; E = Chlorosis at the growing point, visually estimated at 30% damage; F = The most susceptible plants in the pot exhibited decline and begin to wither.

At 21 DAA, extensive necrosis was observed, and several plants in C36 had already died. In surviving individuals, leaf necrosis progressed to the stems by 28 DAA (Figure 2B), preventing recovery from herbicide damage (Qingyan et al., 2007; Brown, 2010), in contrast, plants from the tolerant C12 population (Figure 2A).

Weed recovery after herbicide application exposure depends on multiple factors, including the mode of action, application precision, plant tolerance, and environmental conditions (Krähmer et al., 2021).

By 28 DAA (Figure 2), 94% of plants in the susceptible C36 population were dead, while the remaining 6% exhibited severe damage with minimal likelihood of survival (Figure 2D). In contrast, within the C12 population, only one out of 15 plants died; and the surviving individuals displayed mild to moderate symptoms—primarily chlorosis—without significant growth inhibition. These results confirm that the C12 population retained a higher tolerance to prosulfuron compared with the susceptible C36 population (Figure 2B and Figure 2C).



Figure 2. Symptoms of prosulfuron effect on wild sunflower weed (*Helianthus annuus* L.) at 28 days after application (DAA). A and B = Collection 12 from San Fernando; C and D = Collection 36 from Río Bravo, northern Tamaulipas, México. A and C = Control with herbicide; B = Plants exhibiting slight visual symptoms of chlorosis; D = Plants showing necrosis and severe visual symptoms.

In both collections, the most susceptible plants died

The depletion of key metabolic intermediates, disruption of photosynthesis processes and assimilate, and transport, and impairment of respiratory activity are also associated with plant death induced by ALS-inhibiting herbicides such as prosulfuron (Zulet et al., 2013). Our results suggest that the herbicide was effectively translocated to the plant's growing points, where it inhibited cell division and the initiation of new leaves in susceptible populations (C36), as observed in Figures 1A and 1B. Prosulfuron is primarily absorbed through the foliage and rapidly translocated via the phloem and xylem to actively growing tissues. Depending on the weed species and environmental conditions, the first visible symptoms characterized by chlorosis and tissue discoloration, generally appear within one to three weeks, with younger tissues being affected first (Figure 1B). Plant death typically occurs within two to four weeks after application (Ganie & Jhala, 2020), which aligns with the results observed in this study (Figure 2C).

Electrolyte leakage increased shortly after herbicide application, indicating damage to cell membranes. At 1 DAA, conductivity values ranged between 30–40 $\mu\text{mhos cm}^{-2}$, whereas at 7 DAA the values decreased to 16–26 $\mu\text{mhos cm}^{-2}$, suggesting a partial recovery of tissue integrity. This pattern is consistent with previous findings showing that electrolyte loss is a consequence of compromised cell membrane integrity, a well-established indicator of cell death (Hatsugai & Katagiri, 2022). Early studies with *Asclepias viridis* also reported an initial increase in electrolytic conductivity at 1 DAA followed by a reduction at 7 DAA, suggesting some capacity for recovery (Raja et al., 1985). Overall, these results support that measuring cell membrane permeability is an effective method to differentiate herbicide-resistant from susceptible plants.

Changes in electrolyte leakage from leaves 21 days after prosulfuron application are presented in Table 4. No significant differences were observed between populations C12P and C12T, with exhibited similar levels of electrolyte leakage. In the C12P population, values remained relatively stable over time, ranging from 278.36 to 242.50 $\mu\text{mhos cm}^{-2}$, with signs of cellular recovery by the end of the study as solute loss decreased. In contrast, the susceptible C36 population displayed a markedly different response. While C12 gradually lost leaf solutes, recovery was evident by 21 DAA. The C36 population, however, showed progressive solute loss, reaching 74% by 21 DAA, compared with only 14.7% in C12—approximately five times lower.

The intensive use of ALS inhibitor herbicides has led to the evolution of resistance in several weed species, including *Papaver rhoeas*, *Glebionis segetum*, and *Veronica persica* (Marshall et al., 2010). Herbicides such as prosulfuron, a member of the sulfonyleurea family, act by inhibiting the ALS enzyme, resulting in progressive plant deterioration. Typical symptoms include stunted growth due to disrupted protein synthesis following

herbicide application (Mishra et al., 2016; Zulet et al., 2013). These physiological effects alter cell membrane permeability (Hatsugai & Katagiri, 2018) and, in severe cases, lead to cell necrosis (Figure 3D) and eventual plant death, as observed in the susceptible C36 population. symptoms typically develop gradually as the active ingredient is absorbed and translocated throughout the plant (Torra et al., 2021).

The herbicide Semeron[®], which contains rimsulfuron as its active ingredient, also belongs to the sulfonylurea family. It disrupts protein stability, inducing oxidative stress that interferes with mitotic processes and ultimately leads to cell death (Kozhuro et al., 2005).

Electrolyte leakage is considered a reliable early indicator of cell damage. Even after just 12 h of exposure to contact herbicides such as paraquat and diquat, electrolyte leakage can detect cell injury. In contrast, treatments with 2,4-D, 1% X-77 surfactant, or a non-phytotoxic isoparaffinic oil did not alter leaf cell permeability within the same period (Prendeville & Warren, 2006). This response is also associated with the appearance of necrotic leaf areas (Ganie & Jhala, 2020), as observed in this present study (Figure 3). The onset and extent of cell damage depend on the herbicide's mode of action (Parven et al., 2025). The mode and mechanism of action describe how an herbicide interacts with plant physiology until plant death occurs (Pitty, 2018).

Table 4. Cell damage due to the effect of prosulfuron in the wild sunflower (*Helianthus annuus* L.) from the north of Tamaulipas, México

Contrast	Days After Application			Average	
	7	14	21	CP	CT
	----- Loss of Solutes from the Leaf ($\mu\text{mhos cm}^{-2}$) -----				
C12P vs. C12T	278.36 vs. 239 ns	295.74 vs. 238.0 ns	242.5 vs. 236.40 ns	272.0	237.80
C36P vs. C36T	243.3 vs. 166.10 *	476.98 vs. 196.94 *	884.0 vs. 222.54 **	534.76	195.19

Note. Average: average data per collection. C12P and C36P, with the herbicide Prosulfuron and C12T and C36T = control without herbicide, CP = Population with prosulfuron, CT = population without prosulfuron, Means = average of the three readings per treatment and per population. Statistical significance according to t Student **($P < 0.05$); ($P < 0.01$), ns = no significative.

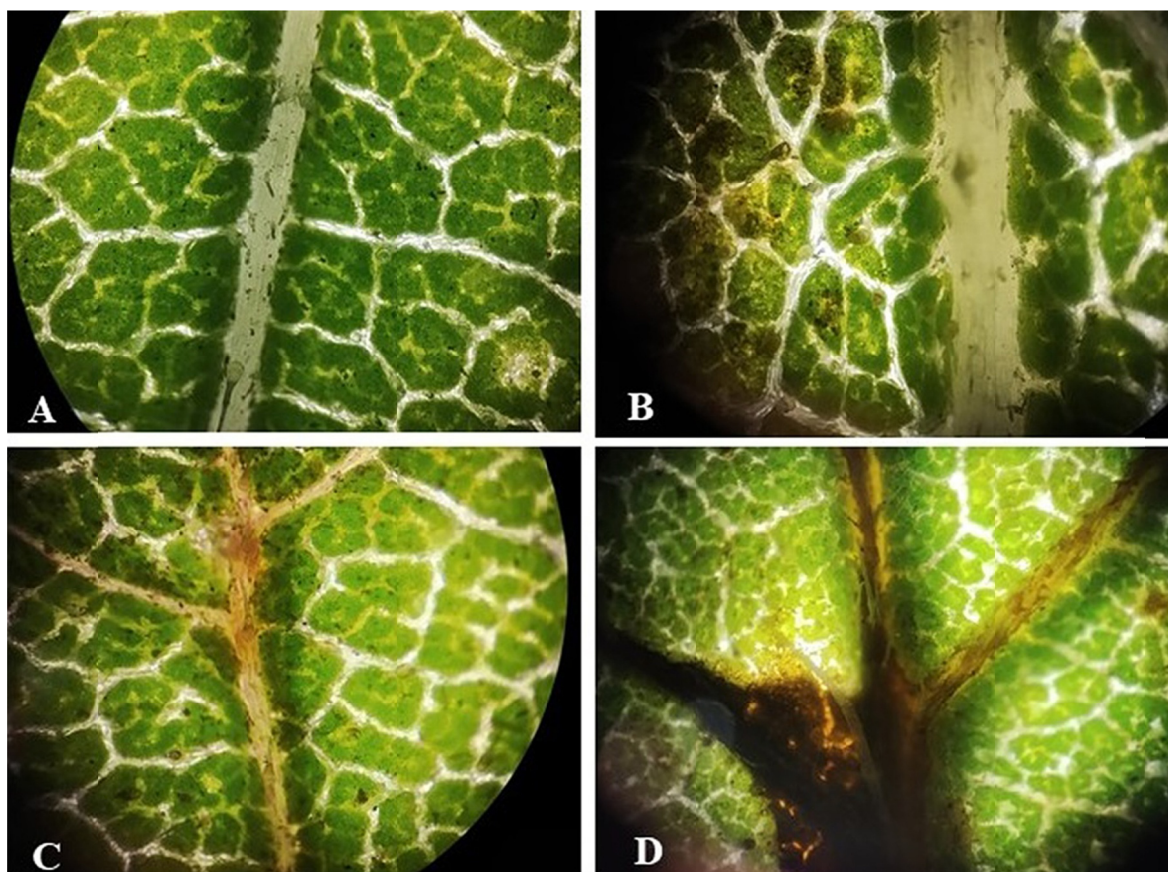


Figure 3. Changes in the external structure of the top layer of the wild sunflower leaf (*H. annuus*) before and after the application of prosulfuron. Progress of necrosis in the collection 36. A = Leaf blade without herbicide, the upper layer was observed to be integrated with its central and secondary veins; B = at 14 DAA necrotic spots are observed in the limbus, in addition deterioration advances in areas near the central rib at 14 DAA (C and D).

The necrotic zone due to cell death is more intense in the central rib and leaf limbus at 28 DAA

These results indicate that measuring cellular electrical conductivity can serve as a preliminary method to identify or discriminate populations with potential herbicide tolerance, offering an alternative to conventional sensitivity bioassays. This approach is particularly useful when handling a large number of collections or suspected redundant populations. Early detection of herbicide resistance in a suspected resistant weed biotype requires a series of tests. Hatami et al. (2016) recommend that such experiments focus on the response of whole plants suspected of harboring resistant biotypes, rather than on susceptible plants.

The origin of the polocote population influenced the degree of control achieved with prosulfuron, as reflected in differences in the speed and severity of damage. The primary symptom of prosulfuron exposure was foliar chlorosis, observed at the apical bud within the first seven days after application. Collection C36, which had no prior exposure to the herbicide, was more susceptible than collection C12, originated from a farm with regular prosulfuron use each agricultural cycle. Plants from collection C12 exhibited low mortality and mild visual symptoms, and even at 28 DAA, control remained poor.

The prosulfuron-susceptible population (C36) showed five times greater solute loss at the end of the trial and exhibited reduced growth, severe chlorosis, and leaf necrosis due to herbicide exposure. These results indicate the presence of a biotype with possible tolerance to prosulfuron in the northern region of Tamaulipas, México.

4. Conclusion

The origin of the polocote population influenced the degree of control achieved with prosulfuron, as reflected in differences in the speed and severity of damage. The primary symptom of prosulfuron exposure was foliar chlorosis, observed at the apical bud within the first seven days after application. Collection C36, which had no prior exposure to the herbicide, was more susceptible than collection C12, originated from a farm with regular

prosulfuron use each agricultural cycle. Plants from collection C12 exhibited low mortality and mild visual symptoms, and even at 28 DAA, control remained poor.

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Authors Contributions

Ma. Eugenia Cisneros-López led the conceptualization of the study, designed the experiments. Miguel Ángel Valdez-Hernández carried out the experiments, collected the data, and performed the statistical analyses. Martín Espinosa-Ramírez assisted with the experiments and managed laboratory materials. José Luis Arispe-Vázquez provided technical and methodological support and reviewed the manuscript. Juan Manuel Covarrubias-Ramírez handled data analysis. Rosendo Hernández-Martínez took charge of drafting the manuscript. All authors read and approved the final manuscript.

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