Colegio de Postgraduados

Evaluation of Grass Weed Control in Sugarcane Using Phosphite Applied as Phosphorous Acid

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ABSTRACT

Objective: To evaluate the effect of Phi application on the control of a grass weed population composed predominantly of stargrass (*Cynodon nlemfuensis* and *C. plectostachyus*) and native grasses (*Axonopus* spp., *Cynodon* spp., and *Paspalum* spp.) in sugarcane cultivation.

Design/methodology/approach: Three 2×2 m plots were established to evaluate foliar spraying with 0, 400, and 800 mM Phi. The trial was conducted in a one-year-old ratoon sugarcane field. Toxicity level, damaged leaf area, and percentage of weed population controlled were assessed through image analysis using ImageJ software. Additionally, antioxidant activity and total chlorophyll concentration in leaf tissue were measured. Data were processed using RStudio version 1.2.5033 (analysis of variance and Tukey's test).

Results: Phi exhibited moderate toxicity at 400 mM and high toxicity at 800 mM. The predominant undesired vegetation included stargrass species (*Cynodon nlemfuensis* and *C. plectostachyus*) and native grasses of the genera *Axonopus*, *Cynodon*, and *Paspalum*. Despite clear foliar damage at both Phi concentrations, most weeds recovered within two to three weeks after application, indicating an efficient physiological strategy to counteract Phi-induced stress. This represents a challenge for the use of Phi as an herbicidal agent. Damaged leaf area reached 20.38% at 400 mM and nearly 51% at 800 mM Phi. Antioxidant activity increased with higher Phi concentrations, whereas total chlorophyll content declined as Phi dosage increased.

Limitations on study/implications: This study focused solely on the effects of phosphorous acid-derived Phi on grass weeds in one-year-old sugarcane. Further research is needed to assess its impact throughout the crop cycle and on a broader range of grass weed species.

Findings/conclusions: Foliar applications of 400 and 800 mM Phi caused moderate and severe phytotoxic effects, respectively, but were not sufficient to effectively control Poaceae weed populations in sugarcane.

Keywords: Weeds, undesired vegetation, sugarcane agroecosystems, phosphite, alternative herbicides.

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INTRODUCTION

Phosphorous acid (PhA) and its conjugate base, phosphite (Phi), can be used as an alternative to glyphosate for controlling undesired vegetation, since its application at high concentrations can induce phytotoxicity and plant death (Li *et al.*, 2025).

In Mexico, sugarcane (*Saccharum* spp. hybrids) is the second most important agroindustrial crop economically, following maize (*Zea mays* L.), with estimated values of 4.4 and 5.4 billion USD, respectively (Statista, 2025). Currently, sugarcane is cultivated on approximately 800,000 hectares across 15 states and 290 municipalities, generating around 500,000 direct jobs and approximately 2 million indirect jobs (PRONAC, 2020; CONADESUCA, 2024).

Although this crop is highly efficient at capturing solar energy and converting it into biomass and sugar, its productivity relies heavily on the intensive use of fertilizers and herbicides (Patel *et al.*, 2024). Considering this, a growing trend is the development of sustainable alternatives for weed management in agriculture to reduce or replace the use of glyphosate (Gandhi *et al.*, 2021; Turek *et al.*, 2022). Among these alternatives, phosphite is gaining attention as an ion with herbicidal potential against numerous weed species in strategic crops worldwide (Achary *et al.*, 2017; Trejo-Téllez *et al.*, 2024; Li *et al.*, 2025).

Phosphorus (P) is an essential element for higher plants, primarily absorbed as inorganic phosphate (Pi), in the form of $H_2PO_4^-$ and HPO_4^{2-} anions (Puga *et al.*, 2024). In the plasma membrane of plant cells, Pi uptake is facilitated by specific transporter proteins (PHT, Phosphate Transporter Family), which are also capable of absorbing other inorganic phosphorus forms such as the phosphite anions (Phi: $H_2PO_3^-$ and HPO_3^{2-}).

Phi is an isostere of Pi, differing by the replacement of one oxygen (O) atom bonded to phosphorus with a hydrogen (H) atom. Despite this structural similarity, Phi cannot substitute Pi as a source of phosphorus once inside the plant cell and can even attenuate phosphate starvation responses (PSR), leading to phytotoxic effects if not properly managed (Achary *et al.*, 2017; Trejo-Téllez *et al.*, 2024). This occurs because Phi triggers a false sufficiency signal for Pi, thereby suppressing the molecular pathways involved in phosphate deficiency responses (McDonald *et al.*, 2001).

The deprotonation of phosphorous acid (PhA; H_3PO_3) and phosphoric acid (PiA; H_3PO_4) generates the Phi and Pi anions, respectively. In both cases, protons are sequentially released, first forming $H_2PO_3^-$ and $H_2PO_4^-$, followed by HPO_3^{2-} and HPO_4^{2-}. However, the loss of a third proton is only feasible for the HPO_4^{2-} anion, since in HPO_3^{2-} the tetrahedral structure is formed by a hydrogen atom directly bonded to phosphorus instead of a fourth oxygen atom, which is present in HPO_4^{2-} (Trejo-Téllez *et al.*, 2024).

Although subtle, these structural differences have important physiological consequences. In practice, the simple substitution of an oxygen atom with a hydrogen atom significantly alters plant responses to the application of either anion or their parent acids. Pi serves as the main readily available source of phosphorus for plants, whereas Phi cannot be metabolized within plant cells, as plant genomes naturally lack genes encoding for the enzyme phosphite dehydrogenase (PTDH or PtxD), which is required to oxidize Phi into Pi and integrate it into metabolic processes (Trejo-Téllez and Gómez-Merino, 2018). As a result, Phi accumulates in the cells, triggering dose-dependent hormetic responses that may be beneficial, neutral, or harmful, depending on Pi availability, Phi concentration, plant genotype, and other factors (Gómez-Merino *et al.*, 2022).

This study evaluated the effect of three concentrations of phosphorous acid —resulting in Phi anions through deprotonation— on the control of grass weeds in sugarcane cultivation. The concentrations tested were 0, 400, and 800 mM Phi, applied to the foliage, with the aim of assessing their herbicidal potential in this crop.

MATERIALS AND METHODS

Experimental site, plant material, and treatments

The study was conducted in sugarcane (*Saccharum* spp. hybrids) fields located at the experimental facilities of the Colegio de Postgraduados, Córdoba Campus, in Amatlán de los Reyes, Veracruz, Mexico (18° 51' 20" N; 96° 51' 37" W; 654 m a.s.l.).

Three treatments corresponding to foliar applications of phosphite (Phi), supplied in the form of phosphorous acid (H_3PO_3), were evaluated at concentrations of 0, 400, and 800 mM. Each treatment was applied to 2×2 m plots, with three replicates per treatment.

To ensure that the observed effects were solely attributable to Phi, no additional weed control methods —manual, mechanical, or chemical— were employed.

Measured variables

Photographs of damaged foliage were taken, and the affected leaf area was quantified using the ImageJ software (Rasband, 2018). Based on this information, the percentage of damaged leaf area, the phytotoxicity level of Phi, and the proportion of controlled vegetation were determined.

Phytotoxicity was evaluated according to the general guidelines of the EPPO PP 1/135 (4) protocol, *Phytotoxicity assessment* (EPPO, 2014), using the percentage of affected leaf area as a quantitative indicator. For comparative purposes, phytotoxicity levels were classified as follows: none or very low (0-5%), slight (>5-20%), moderate (>20-40%), high or severe (>40-70%), and very high or total damage (>70-100%). This classification allowed for a standardized interpretation of the visual effects of phosphite treatments on grass weeds.

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical method, following the procedure described by Silva *et al*. (2024).

The total chlorophyll concentration was determined according to the protocol described by Jiménez-Lao *et al*. (2021).

Experimental design and statistical analysis

A completely randomized design was used, consisting of three treatments and three replicates. Data were processed using the statistical software RStudio version 1.2.5033 (RStudio Team, 2016). An analysis of variance (ANOVA) was performed to determine statistically significant differences at a 95% confidence level ($P \leq 0.05$), followed by Tukey's test for mean comparisons.

RESULTS AND DISCUSSION

Phytotoxicity level, affected leaf area, and weed population control

Foliar application of Phi exhibited a moderately toxic effect at a concentration of 400 mM and a highly toxic effect at 800 mM, as shown in Table 1.

The predominant species of unwanted vegetation in the assessed population were *Cynodon nlemfuensis* and *C. plectostachyus* (commonly known as star grasses), along with native grasses from the genera *Axonopus*, *Cynodon*, and *Paspalum*.

In a study conducted by Herrera-Solano *et al.* (2023) on Poaceae weeds in sugarcane (*Saccharum* spp.) fields in the central region of Veracruz, Mexico, various species were identified, including *Brachiaria utica*, *Panicum purpurascens*, *Brachiaria fasciculata*, *Chloris virgata*, *Cynodon dactylon*, *Cynodon plectostachyus*, *Digitaria ciliaris*, *Panicum maximum*, *Rhynchelytrum repens*, *Rottboelia exaltata*, and *Sorghum halepense*.

Despite the evident foliar tissue damage caused by the application of 400 and 800 mM Phi, most grasses exhibited a noticeable recovery within two to three weeks post-treatment. This rapid recovery suggests that these species possess highly efficient physiological mechanisms to counteract the imbalances induced by Phi, posing a significant challenge for their control using herbicidal formulations based on this anion.

In the grass weed population, leaf area was differentially affected depending on the Phi concentration applied. At 400 mM Phi, approximately 20.38% of the foliage exhibited visible symptoms of burn injury, whereas at 800 mM Phi, this value significantly increased to nearly 51% (Table 2).

The application of 400 mM Phi caused phytotoxicity and mortality in approximately 6% of the weed population, while the 800 mM Phi treatment increased this value to 10.2%. Despite these initial effects, as previously noted, most plants exhibited full recovery approximately one month after application, indicating a remarkable resilience capacity of these species to Phi-induced stress (Table 2).

Table 1. Phytotoxicity level of phosphite (Phi) on grass weeds in sugarcane (*Saccharum* spp.) crops in experimental fields in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	0	400	800
Toxicity level in grass weed population	None	Moderate	High or severe
Visual evidence of toxicity level			

Evidence of toxicity documented 48 hours after treatment application.

Table 2. Affected leaf area and controlled weed population in response to the application of phosphite (Phi) in grass weeds in sugarcane (*Saccharum* spp.) cultivation under field conditions in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	Affected leaf area (%)	Controlled population (%)
0	0.00 c	0.00 c
400	20.38 b	5.79 b
800	50.67 a	10.23 a

Data recorded 48 hours after treatment application. Means followed by different letters within each variable are statistically different (Tukey; $P \leq 0.05$).

The stoloniferous grass species evaluated in this study exhibited notable resistance to systemic herbicides, primarily because their leaves absorb only a fraction of the applied compound, which is not efficiently translocated to the roots and stolons (UNL, 2011; Beck *et al.*, 2013). For instance, in German grass (*Echinochloa polystachya*), the application of the herbicides cyhalofop and penoxsulam resulted in only 37% stolon control, while quinclorac achieved just a 12% reduction (Bottoms *et al.*, 2011). In the case of Bermuda grass (*Cynodon dactylon*), stolons that come into contact with the pre-emergent herbicide oxadiazon absorb only minimal amounts of the compound, preserving the root system almost intact, which facilitates rapid regrowth after treatment (Beck *et al.*, 2013).

Antioxidant activity and chlorophyll content

The antioxidant activity recorded in the grass weeds showed a positive correlation with the applied Phi concentration, with significant differences observed among treatments (Table 3). Plants treated with 800 mM Phi exhibited the highest antioxidant activity, with a 65% increase compared to the control. Similarly, the application of 400 mM Phi resulted in a 52% increase in antioxidant activity relative to untreated plants.

In contrast, the total chlorophyll concentration decreased significantly with increasing Phi concentration. The highest mean value was recorded in the control group (3.51 mg g^{-1} fresh weight), whereas treatments with 400 and 800 mM Phi resulted in reductions to 2.19 and 1.23 mg g^{-1} fresh weight, respectively. These reductions correspond to approximately 37.6% and 62.3% relative to the value observed in plants not treated with Phi (Table 3).

Herbicides can induce oxidative stress in plants, thereby activating defense mechanisms such as increased antioxidant activity (Caverzan *et al.*, 2019). This response is associated with the generation of reactive oxygen species (ROS), which accumulate as a consequence of herbicide-induced oxidative damage. In this context, plants with greater herbicide tolerance often exhibit enhanced antioxidant activity as an adaptive strategy (Eceiza *et al.*, 2023). In the present study, total antioxidant activity increased significantly with rising Phi concentrations, suggesting that this compound acted as a stressor that triggered antioxidant responses in grass weed species.

Herbicides such as iodosulfuron-methyl, clodinafop-propargyl, and 2,4-D can reduce chlorophyll and carotenoid contents while simultaneously inducing oxidative stress by

Table 3. Total antioxidant activity and total chlorophyll concentration in grass weeds in sugarcane (*Saccharum* spp.) in response to foliar application of phosphite (Phi) under field conditions in Amatlán de los Reyes, Veracruz, Mexico.

Phi (mM)	Total antioxidant activity (μg g^{-1} FWB)	Total chlorophylls (mg g^{-1} FWB)
0	448.92 c	3.51 a
400	683.34 b	2.19 b
800	745.12 a	1.23 c

Data recorded 48 h after treatment application. Means followed by different letters within each column are significantly different (Tukey; P≤0.05). FWB: Fresh weight biomass.

increasing hydrogen peroxide (H_2O_2) levels and lipid peroxidation, both in wheat (*Triticum aestivum*) and perennial ryegrass (*Lolium perenne*) (Tarouco *et al*., 2024). In agreement with these findings, the present study also recorded a significant reduction in total chlorophyll concentrations in response to Phi applications, reinforcing the compound's potential phytotoxic effect on leaf tissue.

Plants activate their antioxidant defense system in response to oxidative stress, which is divided into two components: enzymatic and non-enzymatic. The enzymatic system is composed, as the name implies, of enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), among others. The non-enzymatic system includes compounds such as chlorophylls, carotenoids, glutathione, and polyphenols (Rao *et al*., 2025).

Hasan *et al*. (2022) evaluated the efficacy of WeedLock, a broad-spectrum plant-based bioherbicide (*a.i.* EGX-101™, a natural derivative from *Solanum habrochaites*), on ageratum (*Ageratum conyzoides*), goosegrass (*Eleusine indica*), red amaranth (*Amaranthus gangeticus*), and maize. The herbicide caused significant reductions in chlorophyll content and disrupted photosynthetic processes in all treated species. The greatest inhibition of photosynthesis was recorded in *A. conyzoides*, with a 74.88% decrease observed 24 hours after application. In all evaluated species, malondialdehyde (MDA) levels increased by more than 100%, accompanied by elevated proline concentrations —both physiological indicators of oxidative stress. Plants exhibited chlorosis and signs of wilting as early as one hour after treatment application.

In several species of the genus *Urochloa*, increasing doses of glyphosate significantly reduced the quantum efficiency of photosystem II and the electron transport rate, with more pronounced effects under shaded conditions (de Oliveira et al., 2024).

In the present study, foliar application of 400 and 800 mM Phi caused severe damage to the leaf tissue of grass weeds. Nevertheless, most plants were able to recover within two to three weeks after treatment, suggesting that these species possess highly efficient physiological mechanisms to counteract the imbalance induced by Phi.

On the other hand, the sugarcane crop did not exhibit any damage from Phi applications at any of the tested concentrations. It is important to emphasize that the plants were one year old and in the ratoon cycle. Further studies are needed to evaluate the effects of Phi during the early developmental stages of the crop, particularly from the time of planting.

CONCLUSIONS

Although the application of Phi at 400 and 800 mM caused visible damage to the foliar tissue of grass weed populations in sugarcane fields, these weed species exhibited a remarkable recovery capacity, fully reestablishing approximately one month after treatment. Phi application induced oxidative stress in the weeds, as evidenced by a significant increase in antioxidant capacity and a reduction in total chlorophyll concentration. These results indicate that Phi triggers defense mechanisms in weeds but fails to achieve effective control of the grass weed populations tested at the evaluated doses. Therefore, under the conditions and concentrations tested, Phi does not represent a viable herbicidal alternative for managing grass weeds in sugarcane cultivation.

Notably, Phi did not cause phytotoxicity in sugarcane plants when applied at an advanced developmental stage (ratoon crop, one year old), close to harvest. Further studies are recommended to assess its effects during earlier stages of the crop cycle, in order to more clearly establish its safety and potential implications during critical growth phases.

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