



Relationship of the NDVI index and the leaf greenness index SPAD to pinoxaden phytotoxicity in populations of *Avena fatua* of wheat and alfalfa crops from the Mexicali Valley in the laboratory

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Abstract

The visual method is used to evaluate the effectiveness of ACCase inhibitor herbicides on *Avena fatua*. Digital technologies can also be used to evaluate the efficacy of herbicides and detect damage to the photosynthetic apparatus. Given limited laboratory research on these methods, this study investigated the correlation between the Normalized Difference Vegetation Index (NDVI) and chlorophyll content in relation to pinoxaden phytotoxicity in two *A. fatua* populations. A completely randomized 2 × 4 factorial design included two populations and four pinoxaden concentrations. Measurements included percentage of control (%C), weed control index (WCI), dry matter (DM) production, NDVI vegetation indices, and SPAD units. A repeated-measures analysis with a factorial time arrangement was conducted, and least-squares means were compared using Tukey's test. The percentage of control and vegetation indices were correlated using Pearson's correlation. The relationship between the percentage of control and vegetation indices was determined using Pearson's correlation. In contrast to the wheat population, the alfalfa population showed increases in %C and weed control index. In contrast, the alfalfa population exhibited lower dry matter, NDVI, and SPAD values, suggesting that pinoxaden is more phytotoxic to alfalfa. The NDVI, SPAD, and control percentages were negatively correlated in both *A. fatua* populations, suggesting that the vegetation indices decline as the control percentage increases. It is possible to estimate the toxicity of the herbicide pinoxaden in laboratory settings using instruments that quantify chlorophyll content and NDVI.

Keywords Wild oats · Spectral indices · Digital sensor · ACCase · Resistance

Introduction

The global wheat and barley crops are significantly affected by wild oats (*Avena fatua* L.), a weed that can reduce yields by up to 70% when competing with cereal crops (Beckie et

al. 2012). The economic significance of wild oats in wheat cultivation has led farmers to prefer chemical herbicides for control, particularly ACCase enzyme inhibitor herbicides (Sasanfar et al. 2017), as they can control weeds quickly (Zand et al. 2010). Weed management can vary across an agricultural region; farmers may make repeated applications, increase the dose, or change the herbicide's mode of action when control is considered inadequate (Nath et al. 2024). This produces variable degrees of susceptibility to chemical herbicides across weed populations.

Therefore, it is justified to conduct studies to determine the susceptibility of different *A. fatua* populations to ACCase inhibitor herbicides. Various studies have been conducted to evaluate the biological effectiveness of chemical herbicides in the field and laboratory (Qasem 2007; Scursoni et al. 2011). Typically, the conventional approach is predicated

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on quantitative variables, such as plant height, plant density (m^{-2}), visual control evaluation using the European Weed Science Society scale, and, in certain instances, crop yield (Burril et al. 1977; Rosales-Robles et al. 2013).

Alternatively, there are reports of research that employs digital technologies to diagnose physiological stress in plants (Mohammed et al. 2019). This technology can detect herbicide damage to weeds. Thus, infrared technology is an efficient tool for monitoring plant reflectance based on the spectral response induced by herbicides (Huang et al. 2018). Reflectance values are used to estimate the Normalized Difference Vegetation Index (NDVI), which has shown an inverse correlation with symptoms induced by various herbicide groups (Alvarenga et al. 2023; Travlos et al. 2021). Similarly, relative chlorophyll index (SPAD) and chlorophyll fluorescence meters can be used to evaluate herbicide efficacy and detect damage to the photosynthetic apparatus (Ali and Honermeier 2016; Dayan and Zaccaro 2012; Maxwell and Johnson 2000), so they could help measure the toxicity caused by ACCase-inhibiting herbicides (Cobb and Reade 2010).

The assessment of herbicide susceptibility is one of the many biotic and abiotic stresses being diagnosed in agriculture through the widespread use of digital technologies. However, there is a dearth of research on the application of these technologies in the laboratory for herbicides. To date, no research has been conducted linking pinoxaden toxicity to NDVI and chlorophyll content. This could provide complementary information in the visual assessment of the toxicity of ACCase-inhibiting herbicides. Consequently, we initiated a laboratory study to investigate the phytotoxicity of pinoxaden and the correlation between NDVI and chlorophyll content in two populations of *Avena fatua*.

Materials and methods

Location of the experiment

The Parasitology Laboratory of the Institute of Agricultural Sciences at the Autonomous University of Baja California, situated at Carretera a Delta S/N Ejido Nuevo León, 50 km south of Mexicali, Baja California, Mexico ($32^{\circ}24'44.16''$ N, $115^{\circ}11'56.87''$ W), was the site of the study. The laboratory is situated at an altitude of 12 m above sea level and was used from December to June 2021.

Handling of plant material

Two populations of *A. fatua* were collected in the Mexicali Valley, Baja California; one population was collected from alfalfa crops (cultivar CUF-101) produced under

a conventional system with gravity irrigation in raised beds, located in the Ejido Saltillo ($32^{\circ}25'8.48''$ N, $115^{\circ}3'48.19''$ W). Weed control was achieved using selective post-emergence systemic herbicides from the imidazolinone (imazamox and imazethapyr) and cyclohexanedione (clethodim and sethoxydim) chemical groups. Herbicide applications were performed twice during the production cycle, depending on the presence of new generations of narrow-leaf or broadleaf weeds. The other population was collected from wheat crops, cultivar Cirno, grown using a conventional gravity-irrigated system in raised beds, located in the Ejido Paredones (Latitude: 32.587889 N and Longitude: -114.938361 W). For narrow-leaf weed control in this crop, selective post-emergence systemic herbicides from the sulfonylurea chemical group (iodosulfuron-methyl-sodium, flucarbazone sodium), aryloxyphenoxypropanoates (fenoxaprop-p-ethyl, clodinafop-propargyl), and phenylpyrazolines (pinoxaden) were used. The application frequency of these herbicides in this crop ranged from once to twice during the wheat production cycle, within the first 30 to 35 days after emergence.

Seeds from both populations were placed on blotting paper moistened with 10 mL of distilled water in Petri dishes. They were kept at 4°C for 48 h, then kept in the dark at $23 \pm 2^{\circ}\text{C}$ for 24 h. After that, the seeds moved to a growth chamber set at $21 \pm 1^{\circ}\text{C}$ with a 16-hour light and 8-hour dark cycle.

Seven *A. fatua* plants with 1 cm of hypocotyl were transplanted into plastic pots (14 cm diameter, 10.57 cm in depth, 1.2 L volume) containing a 5:3 v/v mixture of organic matter and silt soil. Plants were fertilized from the first leaf stage to the 3–4 leaf stage with commercial Poly-Feed GG fertilizer (19 N-19P-19 K + M.E., Haifa, Mexico) at a dose of 10 g L^{-1} of water.

Experimental design

The experiment involved the evaluation of 0.5x, 1.0x, and 2.0x the commercial concentration of pinoxaden (60 g.a.i ha^{-1}) for the control of *A. fatua* in the field. These concentrations correspond to 30, 60, and 120 g.a.i ha^{-1} of the herbicide pinoxaden, respectively, and include a control (water only) in the two populations of *A. fatua*. The experiment was conducted under controlled laboratory conditions (temperature $23 \pm 2^{\circ}\text{C}$ and a 16:8 h light: dark photoperiod), using a completely randomized design with a 2×4 factorial arrangement. Factor A corresponded to the two populations of *A. fatua*, while factor B represented the three concentrations of the herbicide and the control. Three replicates were included for each treatment, each represented by a pot containing seven *A. fatua* plants.

Herbicide treatments were applied at the 3–4 true-leaf stage of *A. fatua*. Solutions were prepared with distilled water and 3 mL L⁻¹ of Inex A (ethoxylated fatty alcohol and polydimethylsiloxane) adhesive. Applications used a manual sprayer with a Teejet 8002 flat-fan nozzle, connected to an air compressor at 35 PSI and delivering 400 L ha⁻¹.

Treatment evaluation

The effects of treatments on both *A. fatua* populations were evaluated at 3, 9, 15, and 21 days after application (DAA). Herbicide toxicity was measured by estimating the percentage of visual control for each experimental unit according to the European Weed Research Society (EWRS) scale (Rosales-Robles et al. 2013). The weed control index for each treatment at 21 DAA was calculated using Eq. 1, which is based on dry matter production (Lal et al. 2017).

$$WCI = \frac{\text{Dry weight of weeds in the control} - \text{Weed dry weight in the treatment plot}}{\text{Dry weight of weeds in the control}} \times 100. \quad (1)$$

The GreenSeeker™ Handheld® portable sensor (Ntech Industries Inc.) was employed to collect Normalized Difference Vegetation Index (NDVI) measurements at a height of 0.60 m above the plant canopy. Relative chlorophyll content was measured at the midpoint of the leaf immediately above the flag leaf of each *A. fatua* plant using an SPAD 502 Plus portable chlorophyll meter (Konica Minolta Inc.).

Analysis of results

A repeated-measures analysis with a PROC MIXED factorial arrangement over time (SAS 2001) analyzed experimental data for visual control percentage, NDVI, and chlorophyll content. Tukey's test compared the least-squares means. Dry matter production and weed control index variables were analyzed using a completely randomized design with a 2 × 4 factorial arrangement. Tukey's PROC GLM (SAS 2001) compared least-squares means. PROC CORR in SAS (2001) conducted a Pearson correlation analysis to determine the relationship between NDVI and chlorophyll content.

Results

Herbicide toxicity and weed control index

The results indicate a significant disparity in the sensitivity of *A. fatua* populations depending on their crop origin. The herbicide pinoxaden resulted in a higher percentage of control ($F_{(1,16)} = 25.05$, $p = 0.0001$) for the *A. fatua* population collected in the alfalfa crop compared to the population from the wheat crop (Table 1). Specifically, populations associated with alfalfa cultivation exhibited higher susceptibility, with control levels exceeding 88% 9 days after application of the three evaluated pinoxaden doses. In comparison, populations from wheat cultivation showed a delayed response, requiring concentrations of 60 and 120 g a.i. ha⁻¹ to achieve 81% efficacy nine days after application (Table 1).

The three pinoxaden concentrations produced significant differences in *A. fatua* control ($F_{(3,16)} = 103.79$, $p = 0.0001$). In the wheat population, control increased with increasing herbicide concentration at 9, 15, and 21 days after application. In the alfalfa population, the concentrations did not differ significantly. This group achieved over 48% control three days after application, and all concentrations reached 100% control from day 15 onward (Table 1). These findings confirm greater and faster susceptibility in the alfalfa population.

The biomass of *A. fatua*, expressed as dry weight, showed a significant response to the applied pinoxaden dose. In alfalfa cultivation, a reduction of up to 88.99% was observed with the minimum dose. These results indicate that wild oat plants grown in alfalfa cultivation exhibited reduced growth due to pinoxaden's greater phytotoxicity. In contrast, the wheat population showed greater tolerance, requiring the maximum dose to achieve biomass inhibition comparable to 84.39%. Regarding the weed control index, the alfalfa crop population exhibited high susceptibility, with control levels of 88%, and no significant variation among the evaluated doses. However, in the wheat population, control was achieved using a high dose, which reached the levels of efficacy observed in alfalfa (Table 2).

Table 1 The least squares mean of the percentage control of two populations of *Avena fatua* that were exposed to three concentrations of pinoxaden and a control under laboratory conditions

Concentration (g.a.i. ha ⁻¹)	Population of Avena fatua							
	Wheat DAA				Alfalfa DAA			
	3	9	15	21	3	9	15	21
0.00	0.00dAx	0.00dDx	0.00dDx	0.00dCx	0.00dBx	0.00dBx	0.00dBx	0.00dBx
30.00	10.00dAx	33.33cCy	53.33cCy	59.83cBy	48.09cAy	92.38aAx	99.47aAx	100.00aAx
60.00	20.00dAx	81.67bBy	85.00bBy	88.26aAz	50.00cAy	88.81aAx	99.90aAx	100.00aAx
120.00	20.00dAx	91.67aAy	99.33aAy	100.00aAy	50.00cAy	97.56aAx	99.95aAx	100.00aAx

The capital letters A-D indicate groups defined by population, concentration, and assessment time, shown in columns. The lowercase letters a-d refer to different groupings based on populations, concentrations, and assessment times. The letters x-z represent groups in which population, concentration, and assessment time interact linearly

Table 2 The mean of the least squares of dry weight and weed control index (WCI) in two populations of *Avena fatua* that were exposed to three concentrations of pinoxaden and a control under laboratory conditions

Concentration (g.a.i ha ⁻¹)	Dry weight (g)		WCI (%)	
	Wheat	Alfalfa	Wheat	Alfalfa
Control	4.10aA	3.390bA	0	0
30	2.00cB	0.373dB	50.44bC	88.12aA
60	1.63cB	0.376dB	61.19bB	88.01aA
120	0.64dC	0.510dB	84.01aA	83.75aA

The grouping of interactions between a population and concentrations, represented in columns, for dry weight and WCI, is denoted by capital letters A through C. The lowercase letters a-d are used to indicate interactions between distinct populations and concentrations

The higher percentage of control (Table 1), lower dry weight, and higher weed control index (Table 2) observed for the *A. fatua* population collected in the alfalfa crop confirm that it was more susceptible to pinoxaden than the population collected in the wheat crop.

This difference in susceptibility between these two populations could be related to the control strategies employed in each crop. In wheat, ACCase-inhibiting herbicides (fenoxa-prop-p-ethyl, clodinafop-propargyl, and pinoxaden) are primarily used, while in alfalfa, weed control is achieved through a variety of herbicide options (imazamox and imazethapyr). This suggests that the *A. fatua* population from wheat experiences greater selection pressure for ACCase-inhibiting herbicides and therefore exhibits lower susceptibility.

Determination of vegetation indices

Regarding NDVI, the population of *A. fatua* derived from alfalfa exhibited a significant and sustained decrease, with values ranging from 68.42% to 73.68% across the three doses evaluated and remained consistent throughout the experimental period. In contrast, the wheat biotype showed a progressive decrease in the index, with doses of 60 and 120 g a.i ha⁻¹ causing reductions of 47.36% to 75% between 9 and 21 days after application (Table 3).

Chlorophyll content in *A. fatua* plants originating from alfalfa cultivation was significantly reduced, as determined by SPAD measurements. All three application rates consistently inhibited chlorophyll synthesis between 9 and 21 days after application, with reductions ranging from 50.84% to 81.40%. In contrast, native wheat populations exhibited decreased greenness only at 60 and 120 g.a.i. ha⁻¹. At 15 days after application, reductions were 57.47% and 80.48%, while at 21 days after application, reductions reached 72.23% and 90.94%, respectively (Table 4).

Pearson's correlation analysis revealed a significant association between the physiological deterioration of *A. fatua* and the time elapsed since herbicide application, indicating that NDVI and SPAD units are reliable indicators of pinoxaden efficacy. Additionally, the findings indicate that physiological damage progresses gradually and becomes fully established by the conclusion of the evaluation period (Fig. 1).

Response surface analysis confirmed a significant inverse relationship between the physiological indices evaluated

Table 3 Means of NDVI minimum squares in two populations of *Avena fatua* that were exposed to three concentrations of pinoxaden and a control under laboratory conditions

Concentration (g.a.i ha ⁻¹)	Wheat DAA				Alfalfa DAA			
	3	9	15	21	3	9	15	21
0.00	0.13Aby	0.19Aax	0.19Aax	0.20Aax	0.18Aax	0.19Aax	0.19Bax	0.19Aax
30.00	0.11Abx	0.13Bbx	0.13Bbx	0.11Bbx	0.06Bcx	0.06Bcx	0.05Bcx	0.05Bcx
60.00	0.11Abx	0.10Bbx	0.10Bbx	0.10Bbx	0.06Bcx	0.06Bcx	0.05Bcx	0.05Bcx
120.00	0.11Abx	0.08Ccy	0.05Ccz	0.05Ccz	0.06Bcx	0.05Bcx	0.05Bcx	0.05Bcx

The interactions between a population, concentrations, and an assessment time are represented by capital letters A-D and are displayed in columns. Lowercase letters a-d are used to represent the interaction of concentrations, populations, and assessment times. Letters x-z indicate the interaction between a population, a concentration, and assessment times linearly

Table 4 The least squares mean of chlorophyll content (SPAD units) in two populations of *Avena fatua* that were exposed to three concentrations of pinoxaden and a control under laboratory conditions

Concentration (g.a.i. ha ⁻¹)	Wheat DAA				Alfalfa DAA			
	3	9	15	21	3	9	15	21
0.00	34.76Aax	35.12Aax	35.39Aax	37.87Aay	32.26Aax	34.73Aax	36.12Aaw	38.07Aaw
30.00	37.67Aay	39.17Aax	29.33Bbx	24.41Bb	36.55Aaw	25.02Bbx	11.51Bcy	9.67Bcy
60.00	36.23Aaw	26.75Bbx	15.05Ccy	7.39Cdz	37.39Aaw	17.07Cbx	8.85Cdy	7.08Cdy
120.00	36.94Aaw	20.21Bcx	7.66Ddy	3.43Ddz	37.58Aaw	14.85Cbx	11.97Bcy	11.15Bcy

The interactions between a population, concentrations, and an assessment time are denoted by capital letters A-D and are presented in columns. Lowercase letters a-d represent the interaction between populations, concentrations, and assessment times. The linear interaction between a population, a concentration, and assessment times is denoted by the letters w-z

and herbicide efficacy (Fig. 2). Specifically, maximum weed control index values were associated with the lowest NDVI and SPAD levels, indicating a negative physiological response to the treatment. Furthermore, although the alfalfa population showed greater susceptibility, wheat exhibited progressive chlorophyll degradation. These results validate both sensors as non-destructive, accurate, and reliable tools for real-time monitoring of herbicide-induced phytotoxicity.

Discussion

The evaluation results demonstrated that the *A. fatua* population collected from the alfalfa crop exhibited significantly greater susceptibility to pinoxaden compared to the population from the wheat crop. This conclusion is supported by a higher control percentage (Table 1), lower dry weight, and an increased weed control index (Table 2). The distinct

responses between the two populations were further confirmed when the commercial dose was reduced by 50%.

In contrast, the wheat population showed lower control, higher dry weight, and lower weed control index at 30 and 60 g.a.i. ha⁻¹ of pinoxaden, which indicated that this population had developed greater tolerance to the herbicide. This tolerance likely arose because growers repeatedly applied herbicides with the same mode of action in wheat fields across the study area. That practice increased selection pressure, allowing less-susceptible biotypes to survive, reproduce, and gradually dominate the population. (Tafoya-Razo et al. 2017). Therefore, control failures may result from *A. fatua* being subjected to selection pressure from this herbicide, thereby decreasing its susceptibility to pinoxaden. In the future, populations that are highly resistant to this herbicide may be selected (Espinoza et al. 2009; Cruz-Hipolito et al. 2011; Beckie et al. 2012).

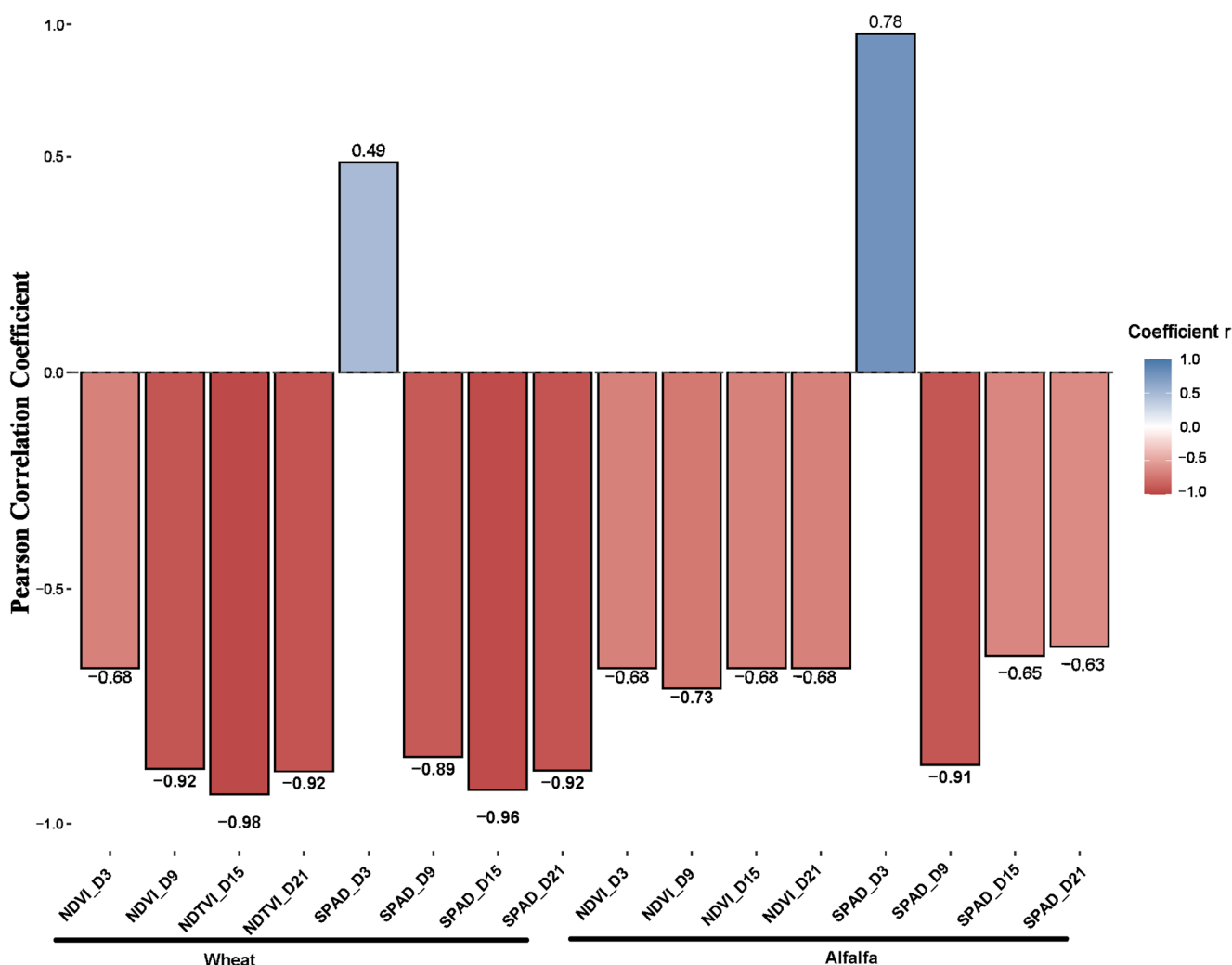
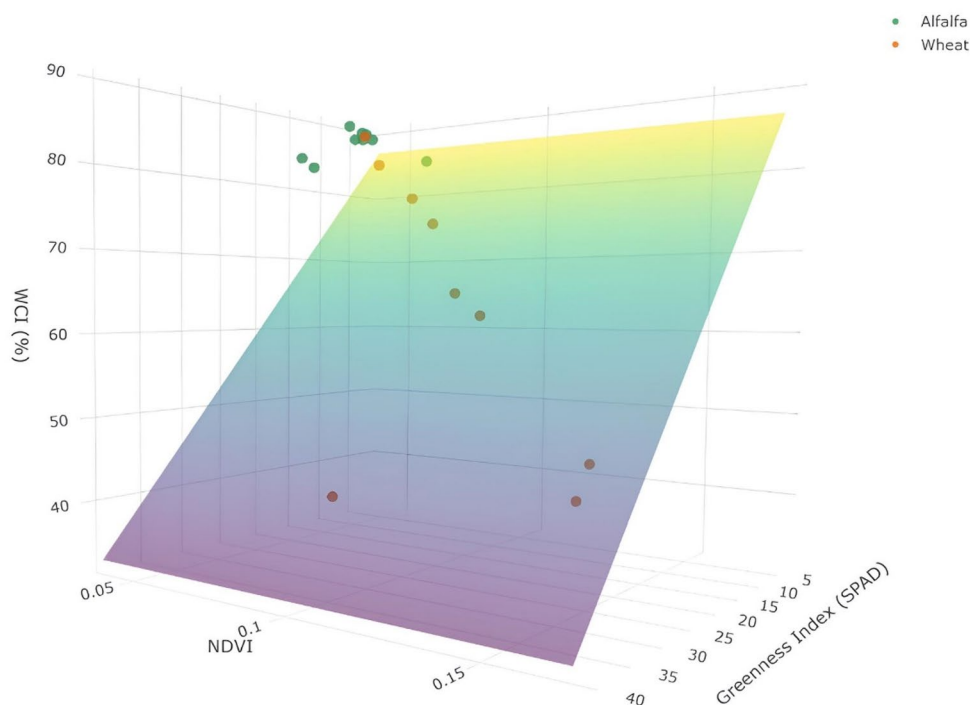


Fig. 1 Pearson correlation between the Normalized Difference Vegetation Index (NDVI) and chlorophyll content (SPAD) in *A. fatua* and pinoxaden control

Fig. 2 Response surface of the efficacy of the herbicide pinoxaden (WCI) as a function of the Normalized Difference Vegetation Index (NDVI) and chlorophyll content (SPAD) in two populations of *A. fatua*



In contrast, the population originating from alfalfa cultivation showed a more susceptible response, registering high control percentages from 9 days after application onwards, exceeding 80%. Additionally, across all three doses assessed, it exhibited complete control at 15 and 21 days after application (Table 2). The behavior of this population indicated that pinoxaden produced toxic effects more rapidly and efficiently, a pattern consistent with the findings of Scursoni et al. (2021) in their evaluation of the herbicide's efficacy against *A. fatua*.

This result aligns with Keith et al. (2015), who achieved complete control (100%) of an *A. fatua* population at the edge of a barley crop without prior selection pressure for this herbicide. The study also found that this *A. fatua* population had low tolerance to pinoxaden, indicating it is susceptible to pinoxaden. This information can help track pinoxaden-resistant field populations in the Mexicali Valley.

In contrast to the wheat population, the *A. fatua* population derived from alfalfa exhibited lower NDVI and SPAD values, suggesting that pinoxaden's action caused more physiological damage (Alvarenga et al. 2023). Thomasi et al. (2021) found that the NDVI of three herbicides evaluated in *Avena* spp and decreased from 0.012 to 0.023 at 22 days after application. The findings of this study illustrated that even minor variations in this index could indicate significant physiological changes.

However, the population collected from wheat fields showed clear resistance to pinoxaden. This response aligns with previous reports indicating that *A. fatua* often develops resistance to ACCase-inhibiting herbicides when growers

maintain the same selection pressure for 6 to 10 consecutive years (Takano et al. 2021). In the Mexicali Valley, pinoxaden was used repeatedly to control *A. fatua* in wheat systems. Because of this continuous exposure, the herbicide's efficacy may gradually diminish among local populations (Tafoya-Razo et al. 2017).

The inhibition of lipid synthesis, which provides essential membrane components for dividing cells, reduced NDVI in both *A. fatua* populations treated with pinoxaden compared with the control (Kaundun 2014). This disruption in membrane formation killed meristematic tissue and, in turn, limited the production of new leaves, which explains the lower dry weight recorded in treated plants (Kukorelli et al. 2013). Regarding chlorophyll content, the wheat field population showed higher values than the alfalfa population, particularly at concentrations of 30 and 60 g a.i. ha⁻¹. Swisher and Corbin (1982) noted that susceptible plants lost chlorophyll about 1 week after pinoxaden application, supporting the interpretation that the herbicide caused a greater reduction in pigment levels in the alfalfa population due to its greater susceptibility.

In contrast to allogamous weed species, resistance in *A. fatua* evolved slowly, as observed by Alwarnaidu Vijayarajan et al. (2020). The species's hexaploidy, high self-pollination, and limited genetic variability within populations were blamed for this behavior. Additionally, selection pressure during critical crop stages was reduced because of the difficulty in precisely timing herbicide applications in the field, made worse by high dormancy and irregular germination.

These attributes clarified the distinctive dynamics through which this species acquires resistance in various agroecosystems. This outcome most likely resulted from the *A. fatua* population from wheat fields showing a greater ability to metabolize the herbicide molecule and to recover from the toxic effects of pinoxaden, indicating the presence of a non-target resistance mechanism (Tafoya-Razo et al. 2022). The identified non-target mechanisms are linked to multiple herbicide resistance (MHR) processes. MHR-3 and MHR-4 are involved in intricate metabolic pathways that diminish the efficacy of numerous herbicide modes of action. These mechanisms work in the detection, transport, and detoxification of herbicide compounds. Key enzymes such as glutathione S-transferases also play a role. Burns et al. (2017a, b) report that these mechanisms boost the plant's ability to metabolize or neutralize herbicides before they reach their sites of action. This promotes the survival of biotypes exposed to continuous selection pressure. Several researchers suggest that *A. fatua*'s autogamy accelerates selection by enabling rapid fixation of alleles under sustained herbicide pressure. This process likely favors genetic lines that overcome the limitations of low outcrossing rates and hexaploid inheritance (Alwarnaidu Vijayarajan et al. 2020; Burns et al. 2017a; Nath et al. 2024; Yu et al. 2013). As a result, populations may develop a high frequency of resistant alleles, making resistance difficult to reverse in agricultural systems that rely on repeated chemical control.

Both populations showed a significant correlation between percentage of control and SPAD and NDVI values (Fig. 1). These indicators can therefore assess pinoxaden toxicity and symptoms in *A. fatua* under laboratory conditions. The optical sensors used, particularly those for chlorophyll measurement, proved effective for this purpose, as noted by Thomasi et al. (2021).

However, these same authors asserted that NDVI was ineffective in identifying herbicide damage. This contrasted with the results of the present study, in which NDVI values showed a significant correlation ($r > 0.90$) with pinoxaden toxicity.

The findings presented in Figs. 1 and 2 are consistent with previous research documenting the effectiveness of the SPAD meter in identifying the impact of hormonal herbicides, such as 2,4-D dimethylamine, on species of the genus *Echinochloa* (Alridiwersah et al. 2007). The use of this tool facilitated a rapid, non-destructive, real-time assessment of relative chlorophyll content in leaves, a considerable methodological advantage over traditional techniques that require destructive sampling and more complex laboratory analysis (Alridiwersah et al., 2023; Zarzecka et al. 2021). Juneau et al. (2007) provide foundational evidence for the effectiveness of chlorophyll fluorescence methods in ecotoxicological research. When assessed with high-sensitivity

fluorometers, these methods enable non-destructive measurements that maintain the integrity of plant tissue and facilitate the early detection of anomalies in photosynthetic efficiency. This methodology enables the identification of physiological responses that are induced by stress before the appearance of visible damage.

NDVI is an efficient tool for rapidly assessing herbicide efficacy. While not explicitly designed to detect herbicide-induced damage, it helps identify early changes in vegetation growth, vigor, and health following chemical treatments (Alvarenga et al. 2023; Travlos et al. 2021). Because NDVI reflects changes in photosynthetic activity, it serves as an indirect indicator of herbicide-induced stress. Additionally, devices that measure NDVI without removing samples enable continuous, real-time monitoring, which enhances their value in phytotoxicity research and integrated weed management (Kanas et al. 2023; Travlos et al. 2021).

Active devices like GreenSeeker use NDVI measurements to assess how effectively herbicides work in agriculture. Santos et al. (2025) studied soybean weeds and tested five herbicides. GreenSeeker detected changes in plants' spectral responses, and the link between near-infrared and red-light reflectance was especially effective at detecting physiological damage, especially from systemic herbicides.

In general, herbicide efficacy was evaluated by visual assessment of damage to individual leaves or entire plants (Alvarenga et al. 2023). These evaluations, based on pre-defined categories documented over time, are presented as simple and economically viable. However, their accuracy could be affected by the subjectivity of the evaluator, variability linked to their experience, and environmental conditions during the observation process (Campos et al. 2021). Similarly, visual fatigue after long periods of evaluation contributes to increased inaccuracy in estimates (Liakos et al. 2018). In contrast, the use of optical sensors such as the GreenSeeker and the SPAD-502 portable chlorophyll meter provides a supplementary alternative for herbicide toxicity studies, as they facilitate the quantification of NDVI and chlorophyll content (SPAD units). The use of these tools in laboratory experiments facilitates the objective estimation of herbicide susceptibility and can complement, or even replace, traditional visual assessments.

These devices enable the acquisition of precise, rapid, and non-destructive data on plant physiological status, thereby facilitating the development of more effective, timely agronomic decisions. In addition, integrating these techniques with remote sensing technologies, such as uncrewed aerial vehicles, increases their applicability by enabling real-time monitoring at various spatial and temporal scales (Huang et al. 2025). The optimization of resource utilization and the mitigation of plant damage are not the only benefits of this technological collaboration; it also serves as a critical

strategy for developing more sustainable and efficient agricultural systems in the future. The use of sensor-equipped improves cost-effectiveness by optimizing decision-making in complex agricultural environments. Although these expenses necessitate a substantial initial investment, they are mitigated by decreased labor and the selective application of chemical inputs at the appropriate time, particularly in large-scale production systems (Alridiwersah et al., 2023; Gao et al. 2025).

Conclusion

The results indicate that the *Avena fatua* population from alfalfa crops was most susceptible to pinoxaden, likely due to limited use of ACCase-inhibiting herbicides. NDVI values and chlorophyll content measured in SPAD units were inversely related to the control achieved by pinoxaden, highlighting their potential as indicators of the herbicide's toxicity.

The findings confirm that conventional methods remain viable and cost-effective for laboratory herbicide assessment. Instruments measuring NDVI and chlorophyll content provide valuable complementary data for more accurate and efficient evaluation of pinoxaden's phytotoxic effects. Further research should validate these results under field conditions and with other herbicide groups and weed species.

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Author contributions Authors' contributions Andrés González Ruiz (AGR), Guadalupe Alfonso López Urquidez (GALU), Carlos Enrique Ail Catzim (CEAC), and Carlos Alfonso López Orona (CALO) conceived the study; AGR, CEAC, GALU, and CALO were involved in writing—original draft preparation; Henry López López (HLL), Verónica Delgado Pacheco (VDP), Onécimo Grimaldo Juárez (OGJ), AGR, and CEAC were responsible for writing—reviewing and editing.

Data availability All the data supporting the conclusions of this study are available in the article.

Declarations

Competing interests The authors declare no competing interests.

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