

Relationship between Vegetation Indices and Pinoxaden Toxicity in Two Populations of *Avena fatua* L.

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ABSTRACT

Objective: To study the relationship between vegetation indices and the toxicity of the herbicide pinoxaden on two populations of *Avena fatua* L.

Design/methodology/approach: A completely randomized design with a 2×4 factorial arrangement was used in the laboratory, with factor A being the two populations of *Avena fatua* and factor B being the four concentrations of the pinoxaden herbicide (0, 30, 60 and 120 g a.i. ha⁻¹). The percentage of control, plant height and the GA and GGA vegetation indices were evaluated. The data were analyzed with an ANOVA and a comparison of means was performed with the Tukey test ($\alpha=0.05$). The relationship between the control percentage and vegetation indices was determined by Pearson correlation analysis.

Results: There was a higher percentage of control, plant height, GA index and lower GGA index in the *Avena fatua* population from alfalfa compared to the wheat population, indicating that pinoxaden has greater phytotoxicity for the alfalfa population. A negative correlation was observed between the control percentage and the GA index for the two populations regardless of the evaluation time, a similar negative correlation was found for the GGA index in both populations. This indicates that the GA and GGA indices decrease as the control percentage increases.

Findings/conclusions: The GA and GGA indices were inversely correlated with the control percentage of the herbicide pinoxaden. The GA and GGA indices obtained through digital camera images are feasible to estimate the toxicity levels of the herbicide pinoxaden.

Keywords: Pinoxaden, vegetation index, wild oats, digital image.

Citation: González-Ruiz, A., Ail-Catzim, C. E., López-Urquidez, G. A., López-Orona, C. A., Medina-López, R., & Estrada-Acosta, M. D. (2024). Relationship between Vegetation Indices and Pinoxaden Toxicity in Two Populations of *Avena fatua* L. *Agro Productividad*. <https://doi.org/10.32854/agrop.v17i11.13119>

Academic Editor: Jorge Cadena Iniguez

Associate Editor: Dra. Lucero del Mar Ruiz Posadas

Guest Editor: Daniel Alejandro Cadena Zamudio

Received: May 02, 2024.

Accepted: September 17, 2024.

Published on-line: December XX, 2024.

Agro Productividad, 17(11) supplement. November. 2024. pp: 73-82.

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INTRODUCTION

Wild oats (*Avena fatua* L.) are considered one of the major phytosanitary problems in wheat cultivation worldwide (Tidemann, 2021). This weed reduces wheat yield due to its high competition and, in extreme cases, causes total crop loss (Jäck *et al.*, 2017). Management of *Avena fatua* in this crop is primarily conducted using ACCase-inhibiting herbicides (Scursoni *et al.*, 2011; Sasanfar *et al.*, 2017).

Therefore, it is crucial to study the susceptibility of *Avena fatua* to ACCase inhibitors. These studies are conducted both in the field and in the laboratory, typically using the visual scale of the European Weed Research Society (Qasem, 2007; Scursoni *et al.*, 2011). However, this method is unreliable, as visual observation depends on the observer's perception, which makes the results subjective. For this reason, it is necessary to have more reliable, highly sensitive, and consistent methodologies to differentiate between plants treated with herbicides.

Recent studies involve the use of multispectral imaging to measure herbicide toxicity (Yao *et al.*, 2012; Huang *et al.*, 2016), but these measurements are performed using specialized equipment (spectroradiometers) that are generally not accessible to producers. On the other hand, the use of conventional digital cameras can be a useful tool in detecting herbicide toxicity. Digital cameras allow for high-resolution images, and through these images, vegetation indices can be determined based on the saturation, intensity, and tonality of the image (Casadesus and Villegas, 2014).

Vegetation indices allow for the quantification of plant characteristics such as leaf area, leaf senescence, grass cover, and turf quality (Li *et al.*, 2005; Lati *et al.*, 2011). The use of digital cameras could represent an additional alternative for herbicide toxicity studies by determining vegetation indices, such as Green Area (GA) and More Green Area (GGA) using Breedpix[®] 0.1 software. This would estimate weed control based on the increase or decrease in vegetation (Velasco-López *et al.*, 2020). Therefore, this study aimed to investigate the relationship between vegetation indices and the toxicity of the herbicide pinoxaden on two populations of *Avena fatua* L.

MATERIALS AND METHODS

Biological Material

Two populations of *Avena fatua* were collected: one from alfalfa crops and the other from wheat crops, in the Mexicali Valley, Baja California. Seeds from both populations were germinated on filter paper moistened with 10 mL of distilled water in Petri dishes. They were kept at 4 °C for 48 hours. Subsequently, they were incubated at room temperature (23 ± 2 °C) in darkness for 24 hours. Finally, they were placed in a growth chamber at a temperature of 21.1 °C, with a photoperiod of 16 hours light and 8 hours darkness.

Seven *Avena fatua* plants with 1 cm of hypocotyl were transplanted into 14 cm diameter \times 10.57 cm depth (1.2 L volume) plastic pots. A substrate composed of a mixture of organic matter and loamy soil in a 5:3 v/v ratio was used. The plants were fertilized from the first leaf stage to the 3-4 leaf stage with commercial Poly Feed (19-19-19+M.E) fertilizer at a dose of 10 g L⁻¹ of water.

Experimental Design

The experiment involved evaluating three concentrations (30, 60, and 120 g a.i. ha⁻¹) of the herbicide pinoxaden and a control (water only) on the two populations of *Avena fatua*. The experiment was conducted under controlled laboratory conditions (temperature 23 ± 2 °C and a 16:8 h light photoperiod), using a completely randomized

design with a 2×4 factorial arrangement. Factor A corresponds to the two populations of *Avena fatua*, while Factor B represents the three herbicide concentrations and the control. Three replications were included, each represented by a pot containing seven *Avena fatua* plants.

The 3-4 true leaf stage was considered the criterion for applying the herbicide treatments. The herbicide solution was prepared with distilled water, and 3 mL L⁻¹ of the adjuvant Inex A (Fatty Alcohol Ethoxylate and Polydimethylsiloxane) was added. The herbicide solutions were applied with a hand sprayer fitted with a flat fan nozzle (Teejet 8002), adapted and connected to an air compressor, calibrated at a pressure of 35 PSI and a water flow rate of 400 L ha⁻¹.

Evaluations of the treatments on both *Avena fatua* populations were conducted at 6, 12, and 18 days after application (DAA). Herbicide toxicity was assessed by estimating the percentage of visual control for each experimental unit using the scale proposed by the European Weed Research Society (EWRS) (Ciba-Geigy ag, 1992). Plant height was measured using a graduated ruler in centimeters (Burril *et al.*, 1977). Digital images were captured with a 12.1-megapixel digital camera (SONY Cyber-shot DSCW200) and processed with Breedpix[®] 0.1 software to estimate the GA and GGA vegetation indices.

Statistical Analysis

The data from the toxicity experiment, plant height, and vegetation indices were analyzed using ANOVA with a completely randomized design and a 2×4 factorial arrangement using PROC GLM. Least squares means were compared with Tukey's test at a significance level of 0.05 (SAS, 2001). The relationship between toxicity and vegetation indices was analyzed using Pearson correlation with PROC CORR (SAS, 2001).

RESULTS AND DISCUSSION

The analysis of variance indicated that the effects of the factors population of *Avena fatua*, pinoxaden concentration, and their interactions were significant for percentage of control and plant height in both populations of *Avena fatua* at 6 DAA (F=102.46; df=3; p=0.0001; F=0.001; df=3; p=0.0001, respectively) and 12 DAA (F=35.34; df=3; p=0.0001; F=8.54; df=3; p=0.0013, respectively). However, for the evaluation at 18 DAA, the interaction of these factors was not significant (F=3.03; df=3; p=0.0597; F=2.88; df=3; p=0.0683, respectively). For the variables GA and GGA, the analysis of variance indicated that the effect of the interaction between *Avena fatua* population and herbicide concentration was not significant at 6 DAA (F=0.52; df=3; p=0.6811; F=0.81; df=3; p=0.0510, respectively), 12 DAA (F=2.26; df=3; p=0.1204; F=2.44; df=3; p=0.1022, respectively), and 18 DAA (F=2.44; df=3; p=0.1034; F=1.51; df=3; p=0.2483, respectively). However, the main effects of the factors were significant for the GA and GGA indices in both *Avena fatua* populations.

In general, the concentrations of 30, 60, and 120 g a.i. ha⁻¹ of pinoxaden applied to the alfalfa population exhibited a higher percentage of control compared to when applied to the wheat population (Table 1) at 6 DAA (F=732.30; df=1; p=0.0001), 12 DAA (F=63.18; df=1; p=0.0001), and 18 DAA (F=6.20; df=1; p=0.0242).

The wheat population showed a higher percentage of control as the concentration of pinoxaden increased (Table 1) at 6 DAA ($F=599.24$; $df=3$; $p=0.0001$), 12 DAA ($F=352.39$; $df=3$; $p=0.0001$), and 18 DAA ($F=79.94$; $df=3$; $p=0.0001$). In contrast, in the alfalfa population, the three herbicide concentrations resulted in similar percentages of control at 12 and 18 DAA (Table 1).

The results of this study suggest that there is a difference in susceptibility between the two *Avena fatua* populations, indicating that pinoxaden has greater phytotoxicity for the alfalfa population compared to the wheat population (Table 1). The alfalfa population achieved more than 80% control at 6 DAA regardless of the concentration evaluated. At 12 and 18 DAA, it reached more than 97% and 100% control, respectively, at all three tested concentrations, suggesting that pinoxaden has a rapid control action on this *Avena fatua* population. These results are consistent with those reported by Scursoni *et al.* (2021) when evaluating pinoxaden on *Avena fatua*.

On the other hand, the wheat population exhibited less than 60% control with all three evaluated concentrations at 6 DAA. Although the percentage of control increased at 12 and 18 DAA, only the 120 g a.i. ha⁻¹ concentration of pinoxaden achieved 100% control at 18 DAA (Table 3). This indicates that this population shows greater tolerance to this herbicide, likely due to pinoxaden (an ACCase inhibitor) being one of the most commonly used herbicides in the Mexicali Valley for controlling this weed in wheat cultivation (Tafuya-Razo *et al.*, 2017).

As a result, the *Avena fatua* population from wheat may have experienced greater selection pressure for this herbicide, thereby decreasing its susceptibility to pinoxaden (Cruz-Hipolito *et al.*, 2011). In contrast, in alfalfa cultivation, weed control is achieved

Table 1. Effect of three concentrations of pinoxaden on the percentage of control in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treatment (g a.i. ha ⁻¹)	Control (%)	
		Alfalfa	Wheat
6 DAA	Control	0.000fCx	0.000fADx
	30	83.093bBx	18.333eCy
	60	88.807aAx	43.333dBy
	120	90.000aAx	55.000cAy
12 DAA	Control	0.000dBx	0.000dDx
	30	97.380aAx	36.667cCy
	60	97.857aAx	85.000bBy
	120	99.333aAx	97.000aAx
18 DAA	Control	0.00Bx	0.000Cx
	30	100.00Ax	61.67By
	60	100.00Ax	86.67Ax
	120	100.00Ax	100.00Ax

Letters A-D indicate the comparison between concentrations for a population and an evaluation time as columns. Letters a-d represent the interaction of populations and concentrations for an evaluation time. Letters x-y show the comparison between populations for a concentration and evaluation time in a linear manner.

using various herbicides such as carfentrazone, diuron, flumioxazin, hexazinone, imazethapyr, metribuzin, pendimethalin, paraquat, and saflufenacil (Adjesiwor and Prather, 2022). This prevents selection pressure for a specific herbicide group, contributing to the high susceptibility of the *Avena fatua* population from alfalfa cultivation to pinoxaden.

All three concentrations of pinoxaden resulted in shorter plant height compared to the control (no application) in both populations of *Avena fatua* (Table 2) at 6 (F=51.01; df=3; p=0.0001), 12 (F=72.23; df=3; p=0.0001), and 18 days after application (DDA) (F=81.72; df=3; p=0.0001). The alfalfa population exhibited shorter plant height compared to the wheat population at 6 DDA (F=114.01; df=1; p=0.0001), 12 (F=63.51; df=1; p=0.0001), and 18 DDA (F=24.70; df=1; p=0.0001), regardless of the pinoxaden concentration (Table 2).

At 6 days after application (DDA), the concentrations of 30, 60, and 120 g a.i. ha⁻¹ of pinoxaden reduced the height of the alfalfa population by 30.71%, 32.91%, and 40.14%, respectively, compared to the control treatment. For the wheat population, the reduction in height was 45.59%, 54.08%, and 55.21% for the concentrations of 30, 60, and 120 g a.i. ha⁻¹ of the herbicide, respectively (Table 2). Similarly, the reduction in plant height in both populations increased at 12 days after application (DDA), with average percentages of 63.80% and 63.39% for the alfalfa and wheat populations, respectively. A similar pattern was observed at 18 DDA, with average percentages of 77% and 61.39% for the alfalfa and wheat populations, respectively. This reduction in plant height in both *Avena fatua* populations may be related to the death of meristematic tissue caused by pinoxaden (Kukorelli *et al.*, 2013).

Table 2. Effect of three concentrations of pinoxaden on plant height in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treatment (g a.i. ha ⁻¹)	Plant height (cm)	
		Alfalfa	Wheat
6 DAA	Control	17.797bAy	37.193aAx
	30	12.330cBy	20.233bBx
	60	11.940cBy	17.080bBx
	120	10.653cBy	16.657bBx
12 DAA	Control	30.673bAy	55.687aAx
	30	11.760cBy	29.033bBx
	60	10.700cBy	16.783cCx
	120	10.837cBy	15.353cCx
18 DAA	Control	48.01Ax	55.63Ax
	30	11.34By	31.63Bx
	60	10.65By	17.73Cx
	120	9.77By	15.07Cx

Letters A-D indicate the comparison between concentrations for a population and an evaluation period in column format. Letters a-d show the interaction of populations and concentrations for a specific evaluation period. Letters x-y denote the comparison between populations for a concentration and evaluation period in a linear fashion.

The inhibition of lipid synthesis, which constitutes the membranes of these growing cells, leads to cell death (Kaundun, 2014), which reduces the growth of new leaves in the treated plants (Kukorelli *et al.*, 2013), and consequently decreases plant height in both studied populations.

Although there is a significant reduction in plant height due to pinoxaden in both populations, the effect was less pronounced in the wheat population across all three evaluation dates and herbicide concentrations. The plant height of the wheat population was significantly greater than that of the alfalfa population, with the concentration of 30 g a.i. ha⁻¹ showing that the height of the wheat population exceeded that of the alfalfa population by 39.06%, 59.49%, and 64.15% at 6, 12, and 18 days after application, respectively. This indicates that this concentration was not sufficient to cause the death of the meristematic tissue. Additionally, it is possible that the wheat population plants, due to their higher tolerance, might metabolize the herbicide molecule and recover from the toxic effects of pinoxaden, leading to greater growth. Another aspect that could explain this result is that the concentration of 30 g a.i. ha⁻¹ is half of the recommended concentration for controlling *Avena fatua* in wheat cultivation (60 g a.i. ha⁻¹), which may also have contributed to the lesser reduction in plant height. This aligns with the findings of Scursoni *et al.* (2021), who reported that reducing the field dose of the herbicide fenoxaprop (an ACCase inhibitor) by 50% results in a lesser effect on biomass production in *Avena fatua*, which could translate to greater plant height, as observed in this study.

The concentration of 120 g a.i. ha⁻¹ of pinoxaden exhibited lower GA compared to the control, 30, and 60 g a.i. ha⁻¹ in both populations of *Avena fatua* (Table 3) at 6 DAA ($F=4.94$; $df=3$; $p=0.0003$). Meanwhile, all three herbicide concentrations showed lower

Table 3. Effect of three concentrations of pinoxaden on the green area (GA index) in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treatment (g a.i. ha ⁻¹)	GA Index	
		Alfalfa	Wheat
6 DAA	Control	0.1600Ax	0.1867Ax
	30	0.1267Ay	0.1833Ax
	60	0.1233Ay	0.1733ABx
	120	0.1033By	0.1433Bx
12 DAA	Control	0.1567Ax	0.2200Ax
	30	0.0533By	0.2000Ax
	60	0.0300By	0.1100Bx
	120	0.0267By	0.0667Bx
18 DAA	Control	0.1167Ay	0.1967Ax
	30	0.0433By	0.1233Bx
	60	0.0233By	0.0933BCx
	120	0.0133By	0.0333Cx

Letters A-D show the comparison between concentrations for a single population and an evaluation time in column format. Letters x-y show the comparison between populations for a concentration and an evaluation time in a linear manner.

GA compared to the control at 12 (F=17.28; df=3; p=0.0001) and 18 DAA (F=13.20; df=3; p=0.0001) in both populations. Lower GA was observed in the alfalfa population compared to the wheat population (Table 3) for the concentrations of 30, 60, and 120 g a.i. ha⁻¹ of pinoxaden at 6 (F=21.67; df=1; p=0.0127), 12 (F=29.38; df=1; p=0.0001), and 18 DAA (F=11.40; df=1; p=0.0039).

For the alfalfa population, the three herbicide concentrations showed lower GGA compared to the control at 12 (F=12.30; df=3; p=0.0002) and 18 DAA (F=9.85; df=3; p=0.0006). At 6 DAA (F=8.29; df=3; p=0.0016), the control and 30 g a.i. ha⁻¹ of pinoxaden had higher GGA compared to the 60 and 120 g a.i. ha⁻¹ herbicide concentrations (Table 4).

For the wheat population, the 60 and 120 g a.i. ha⁻¹ concentrations showed lower GGA values compared to the control and the 30 g a.i. ha⁻¹ pinoxaden concentration at 12 (F=12.30; df=3; p=0.0002) and 18 DAA (F=9.85; df=3; p=0.0006). Lower GGA was observed in the alfalfa population compared to the wheat population for all three pinoxaden concentrations (Table 4) at 6 (F=80.00; df=1; p=0.0001), 12 (F=27.04; df=1; p=0.0001), and 18 DAA (F=15.67; df=1; p=0.0011).

Vegetative indices (GA and GGA) have been used to estimate the green biomass of plants (Casadesús *et al.*, 2007), which is related to the growth of healthy plants. Therefore, these indices can differentiate between plants growing under optimal conditions and plants under stress (Fiorani and Schurr, 2013). In this study, the GA and GGA indices differentiated between *Avena fatua* plants affected by pinoxaden concentrations and untreated (control) plants in both *Avena fatua* populations. Both populations showed a gradual decrease in the vegetative index values as the pinoxaden concentration increased (Tables 3 and 4). These results were consistent across the different evaluation times.

Table 4. Effect of three concentrations of pinoxaden on the greenest area (GGA index) in two populations of *Avena fatua* L. during three evaluation periods after treatment application.

Evaluation Period	Treatment (g a.i. ha ⁻¹)	GGA Index	
		Alfalfa	Wheat
6 DAA	Control	0.1033Ay	0.1500Ax
	30	0.0767Ay	0.1433Ax
	60	0.0667By	0.1400Ax
	120	0.0533By	0.1067Bx
12 DAA	Control	0.0967Ay	0.1667Ax
	30	0.0233By	0.1533Ax
	60	0.0133By	0.0733Bx
	120	0.0100By	0.0367Bx
18 DDA	Control	0.0633Ay	0.1303Ax
	30	0.0200ABy	0.0833ABx
	60	0.0100By	0.0633Bx
	120	0.0057By	0.0097Cx

Letters A-D indicate the comparison between concentrations for a population and an evaluation time as columns. Letters x-y indicate the comparison between populations for a concentration and an evaluation time in a linear manner.

Although both populations of *Avena fatua* exhibited lower levels of GA and GGA, the alfalfa population showed lower values for these vegetative indices compared to the wheat population, regardless of concentration and evaluation time (Tables 3 and 4). This fact could be related to the greater susceptibility of this population to the different concentrations of pinoxaden evaluated in this study. Correlation analyses showed a negative correlation between the percentage of control and the GA index for both *Avena fatua* populations, regardless of the evaluation time. Similarly, both populations showed a negative correlation for GGA (Table 5), indicating that the GA and GGA indices decrease as the percentage of control increases.

Table 5. Pearson correlation between visual control percentage and digital tools in two populations of *Avena fatua* L. from the Valley of Mexicali.

Population	Index	DAA		
		6	12	18
		% Control		
Alfalfa	GA	-0.72141 0.0081	-0.92768 <.0001	-0.86976 0.0003
	GGA	-0.80110 0.0017	-0.95329 <.0001	-0.86555 0.0003
Wheat	GA	-0.57351 0.0512	-0.81531 0.0012	-0.64865 0.0223
	GGA	-0.61926 0.0318	-0.79578 0.0020	-0.59833 0.0399

The alfalfa population showed a stronger correlation compared to the wheat population for the GA index, with correlation coefficients of -0.72141 , -0.92768 , and -0.86976 for 6, 12, and 18 DAA, respectively. Similarly, for the alfalfa population, the GGA index was strongly correlated with the control percentage, with correlation coefficients of -0.80110 , -0.95329 , and -0.86555 for 6, 12, and 18 DAA, respectively.

These results differ from those found by Huang *et al.* (2016), who reported a low correlation between vegetative indices and dicamba toxicity in soybean crops, concluding that vegetative indices are not suitable for estimating the sensitivity to the herbicide dicamba. In contrast, in this study, the high correlation between control percentage and the GA and GGA indices suggests that they are suitable for estimating the sensitivity of *Avena fatua* to the herbicide pinoxaden under laboratory conditions. Additionally, they suggest that these vegetative indices represent a promising tool for assessing herbicide susceptibility in laboratory experiments, providing complementary information or even replacing the visual evaluation of herbicide toxicity. This type of visual assessment is subjective and depends on the operator conducting it, which can result in imprecise outcomes. Therefore, it is crucial to have tools that can determine herbicide toxicity in an objective, repeatable, and reliable manner, such as the GA and GGA indices obtained through digital images using conventional digital cameras.

CONCLUSIONS

According to the results of this research, it is concluded that the values of the GA and GGA vegetation indices were inversely correlated with the herbicide pinoxaden's control percentage (toxicity). Additionally, it is concluded that the GA and GGA indices obtained through digital camera images are feasible tools for estimating the toxicity levels of the herbicide pinoxaden.

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