PHYTOTOXIC STUDY OF THE *E*/*Z* ISOMERS OF THE HERBICIDE ALLOXYDIM IN WHEAT

Sevilla-Morán B.[1], Santín-Montanyá M.I.[2], Villarroya-Ferruz M.[1], Cubero-Torrado N.H.[1], Alonso-Prados J.L.[1], Sandín-España P.[1]*

[1]*DTEVPF - Unit of Plant Protection Product, Instituto Nacional de Investigación Agraria y Alimentaria (INIA), 28040 Madrid, Spain.*

[2]*Department of Plant Protection, Instituto Nacional de Investigación Agraria y Alimentaria (INIA), 28040 Madrid, Spain.*

** sandin@inia.es*

Summary: Alloxydim is an herbicide marketed as the *E*-isomer applied at early stages of grass weeds. In the field, sunlight and temperature can induce the isomerization reaction of *E*-alloxydim to form the mixture of *E* and *Z* isomers. A bioassay has been performed to compare the herbicidal activity of *E*-alloxydim and its corresponding *E*/*Z* isomeric mixture in wheat plants. The IC_{50} values calculated from the root lengths of wheat for E-alloxydim and alloxydim mixture were 0.37 and 0.70 mg L^{-1}, respectively. The *Z* isomer of alloxydim in the isomeric mixture has no phytotoxic effect on the wheat germination whereas *E*-alloxydim inhibited the seed germination at low concentrations (from 0.0 to 4.0 mg L^{-1}).

Keywords: Degradation, cyclohexanodione oxime, isomerization, germination bioassay.

Resumen: ***Estudio fitotóxico de los isómeros E/Z del herbicida alloxidim en trigo.*** Aloxidim es un herbicida comercializado como el isómero *E* que se aplica en los estadios tempranos de las malas hierbas. En el campo, la luz solar y la temperatura pueden inducir las reacción de isomerización de *E*-aloxidim para formar la mezcla de los isómeros *E* y *Z*. Se ha llevado a cabo un bioensayo para comparar la actividad herbicida de *E*-aloxydim y su correspondiente mezcla de isómeros *E*/*Z* en trigo. Los valores de IC_{50} calculados a partir de la longitud de raíz de trigo de *E*-aloxidim y la mezcla 0,37 y 0,70 mg L^{-1}, respectivamente. El isómero *Z*-aloxidim en la mezcla isomérica no tiene efecto fitotóxico sobre la germinación de trigo, mientras que *E*- aloxidim inhibe la germinación de semillas a bajas concentraciones (de 0,0 a 4,0 mg L^{-1}).

Palabras clave: Degradación, ciclohexanodiona oxima, isomerización, bioensayo de germinación.

INTRODUCTION

The herbicide alloxydim is marketed in Japan as the *E* isomer for the post-emergence control of gramineous weeds in sugar beet, vegetables and other broadleaf crops. Once applied, this herbicide degradates to different by-products under various environmental conditions. One of the main reactions is a rapid isomerization to the *Z* isomer (Figure 1). In our group, different studies have shown that *E*-isomer suffers isomerization under different irradiation intensities and temperatures in environmental waters in few minutes (Sevilla-Morán et al., 2008; Sandín-España et al. 2013). The identification of the *Z*-isomer was performed by HPLC-QToF-MS spectrometry (Sevilla-Morán et al., 2008). Nowadays, one of the major challenges in environmental analytical chemistry is the identification of unknown by-products of pesticides generated in different degradation processes in the environment. This task is highly interesting since some of the degradation products present different properties to the parent compound. In fact, some of them have more persistence and/or toxicity/phytotoxicity (Osano et al., 2002; Boxall, 2009). In the case of alloxydim, to the best of our knowledge, there are no previous studies in the scientific literature on the phytotoxic activity of the different isomers of alloxydim.

In the present work we compare the phytotoxic effects of the herbicide alloxydim (*E* isomer) and its corresponding isomeric *E*/*Z* mixture in wheat by means of seed germination bioassays. These studies are also interesting in order increase knowledge on the efficacy and phytotoxicity of different isomer of pesticides.

E-alloxydim Z-alloxydim

Figure 1. Chemical structure of E-alloxydim and Z-alloxydim.

MATERIAL AND METHODS

Chemical reagents

The analytical standard of *E*-alloxydim was acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany) (98% purity). As the *Z*-isomer was not commercially available, it was obtained as a mixture of *E*/*Z* isomers from *E*-alloxydim (see next section).

Ultrapure water was obtained from a Millipore system (Milli-Q-50 18 mΩ) and MeOH (HPLC grade) were supplied by Labscan (Stillorgan, Co., Dublin, Ireland).

Obtaining of isomeric mixture of alloxydim

To obtain the mixture of *E*/*Z*, a thermic isomerization reaction of *E*-alloxydim was performed as follows; a methanolic solution of *E*-alloxydim (20 mg L^{-1}) was exposed to 80 ºC for 40 min in a thermostatic bath. Thereafter, the solvent was removed with the use of a vacuum centrifuge (Eppendorf AG, Hamburg, Germany) and the residue was dissolved in the appropriate ultrapure water volume to obtain a final concentration of 20 mg L^{-1}.

This procedure allows obtaining an *E*/*Z* alloxydim mixture with a ratio of 42/58.

Seed germination bioassays

The study was conducted with a wheat species (*Triticum aestivum* L. cv Castan) in a growth chamber with a cycle of 16 h of light (illumination 100 µE m^{-2} s^{-1}) at 22 ± 1 ºC and 8 h of darkness at 16 ± 1 ºC.

Twenty-five seeds wheat were distributed in a Petri dish (13 cm diameter) on double-layered Whatman No. 1 filter paper. The seeds were then treated with 15 mL of either *E*-alloxydim or with *E*/*Z* alloxydim mixture at ten different concentrations (0.0; 0.2; 0.4; 0.6; 0.8; 1.0; 1.2; 1.6; 2.0; 4.0 mg L^{-1}).

The experimental work followed a completely randomized design by to replicates and appropriate control systems containing no herbicide (with ultrapure water) were included in each experiment.

The effects of *E*-alloxydim and *E*/*Z* alloxydim mixture were measured by the main germination parameters of the seeds (root length and coleoptile length) recorded 3 days after seeding. The ratio of E/Z alloxydim was stable during the whole experiment.

Statistical analysis

The IC_{50} values (herbicide concentration required to cause a 50% inhibition of root growth) for alloxydim and *E*/*Z* alloxydim mixture were determined by means of direct plots of root length *vs.* the concentration of these compounds (Figure 2) assuming a non-linear regression given by the log-logistic equation (Seefeldt et al., 1995). The equation of these curves is:

$$Y = C + \frac{D - C}{1 + e^{b \cdot [\log(X) - \log(IC_{50})]}} \qquad (1)$$

where Y is the root length (cm), X is the herbicide concentration (mg L^{-1}), D is the upper asymptote (maximum root growth of plants), C is the lower asymptote (minimum root growth of plants), b is the slope of the curve around the IC_{50}, and IC_{50} is the concentration giving 50% root length inhibition.

RESULTS AND DISCUSSION

Root and coleoptile lengths were measured to evaluate the effects of *E*-alloxydim and *E/Z* alloxydim mixture in the germination bioassays. The root length of wheat was significantly affected at low concentrations of *E*-alloxydim regarding the concentrations assayed (Figure 2a) and *E/Z* alloxydim mixture (Figure 2b). Coleoptile was less sensitive to both compounds, therefore only the effects on root lengths were considered.

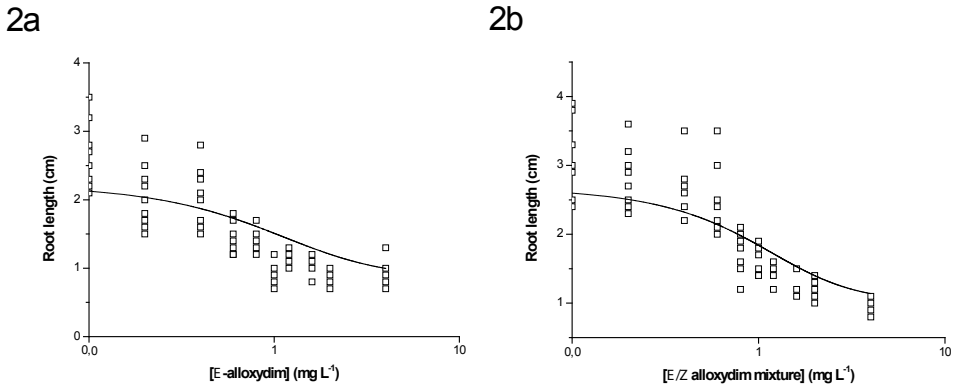

Figure 2. Dose-response curves for E-alloxydim (a) and *E/Z* alloxydim mixture (b) from seed germination bioassays for the root length of wheat.

The IC_{50} values calculated from the coleoptile length were higher than those obtained from the root length, indicating that the roots of the wheat specie *Triticum aestivum* L. cv Castan were more sensitive to *E*-alloxydim and *E/Z* alloxydim mixture.

The log-logistic equations obtained from the experimental data are the following:

E-alloxydim

$$Y = 0{,}86 + \frac{2{,}93 - 0{,}86}{1 + e^{4{,}77\cdot[\log(X) - \log(0{,}37)]}}$$

R^2=77.52

E/Z alloxydim mixture

$$Y = 1{,}03 + \frac{3{,}09 - 1{,}03}{1 + e^{6{,}15\cdot[\log(X) - \log(0{,}70)]}}$$

R^2=83.06

The IC_{50} values calculated from the root lengths of wheat for *E*-alloxydim and *E/Z* alloxydim mixture were 0.37 and 0.70 mg L^{-1}, respectively. The data indicate that *E/Z* alloxydim mixture exert less phytotoxic effect than *E*-alloxydim in the seed germination *T. aestivum* cv Castan. These findings suggest that the *Z* isomer of alloxydim in the isomeric mixture has no phytotoxic effect on the wheat germination, resulting in a minor effectiveness for the control of this cereal if the mixture of both isomers of alloxydim were formed in the field.

CONCLUSIONS

The isomerization of the marketed *E*-alloxydim in the field could lead to a reduction of the efficacy of this herbicide. Furthermore, the easy and rapid formation of the *Z*-isomer generates an increase of xenobiotics in the environment. This isomer has unknown impacts in the environment. Thus, further studies would be desirable to improve the knowledge of the fate of this herbicide.

REFERENCES

BOXALL A (2009) *The handbook of environmental chemistry. Vol. XIV, Part 2P, Transformation products of synthetic chemicals in the environment.* Springer-Verlag, Berlin, Germany.

OSANO O, ADMIRAAL W, KLAMER HJC, PASTOR D & BLEEKER EAJ (2002) Comparative toxic and genotoxic effects of chloroacetanilides, formamidines and their degradation products on *Vibrio fischeri* and *Chironomus riparius. Environmental Pollution* 119**,** 195-202.

SANDÍN-ESPAÑA P, SEVILLA-MORÁN B, CALVO L, MATEO-MIRANDA M & ALONSO-PRADOS JL (2013) Photochemical behavior of alloxydim herbicide in environmental waters. Structural elucidation and toxicity of degradation products. *Microchemical Journal* 106, 212-219.

SEEFELDT SS, JENSEN JE & PATRICK FUERST E (1995) Log-logistic analysis of herbicide dose-response relationships. *Weed Technology* 9, 218-25.

SEVILLA-MORÁN B, SANDÍN-ESPAÑA P, VICENTE-ARANA MJ, ALONSO-PRADOS JL & GARCÍA-BAUDÍN JM (2008) Study of alloxydim photodegradation in the presence of natural substances: Elucidation of transformation products. *Journal of Photochemistry and Photobiology A* 198, 162-168.