

ATRAZINE PERSISTENCE APPLIED AS COMMERCIAL AND XEROGEL FORMULATIONS IN OXISOL¹

Persistência do Atrazine em Formulações Comercial e Xerogel em Latossolo Vermelho Distroférico

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ABSTRACT - The intensive use of pesticides have contaminated the soil and groundwater. The application of herbicides as controlled release formulations may reduce the environmental damage related to their use because it may optimize the efficiency of the active ingredient and reducing thus the recommended dose. The objective of this study was to evaluate the persistence of the herbicide atrazine applied as commercial formulation (COM) and as controlled release formulation (xerogel - XER) in Oxisol. The experimental design used was split-plot randomized-blocks with four replications, in a (2 x 6) + 1 arrangement. The two formulations (COM and XER) were assigned to main plots and different atrazine concentrations (0, 3.200, 3.600, 4.200, 5.400 and 8.000 g atrazine ha⁻¹) were assigned to sub-plots. Persistence was determined by means of dissipation kinetics and bioavailability tests. The methodology of bioassays to assess the atrazine availability is efficient and enables to distinguish the tested formulations. The availability of atrazine XER is higher than the commercial in two different periods: up to 5 days after herbicide application and at the 35th day after application. The XER formulation tends to be more persistent in relation to COM formulation.

Keywords: residual herbicides, controlled release, half-life.

RESUMO - O uso intensivo de defensivos agrícolas pode ocasionar problemas de contaminação de solo e águas subterrâneas. A aplicação de herbicidas em formulações de liberação controlada pode minimizar os danos ambientais relacionados ao seu uso por otimizar a eficiência do ingrediente ativo e, com isso, diminuir a dose recomendada. O objetivo deste trabalho foi avaliar a persistência do atrazine aplicado como formulação comercial (COM) e em formulação de liberação controlada (xerogel - XER) em Latossolo Vermelho. O delineamento utilizado foi o de blocos ao acaso, em parcelas subdivididas, com quatro repetições, em um esquema (2 x 6) + 1. Nas parcelas principais foram alocadas as formulações de atrazine (COM e XER) e, nas subparcelas, as concentrações dos herbicidas (0, 3.200, 3.600, 4.200, 5.400 e 8.000 g de atrazine ha⁻¹). A persistência foi avaliada por ensaios de biodisponibilidade e de cinética de dissipação. A metodologia de bioensaios para avaliação da disponibilidade de atrazine foi eficiente e permitiu diferenciar as formulações testadas. A disponibilidade de atrazine XER foi superior à da COM em dois períodos distintos: até cinco dias após a aplicação e também aos 35 dias depois da aplicação do herbicida. A formulação XER tende a ser mais persistente em relação à formulação COM.

Palavras-chave: herbicidas residuais, liberação controlada, meia-vida.

¹ Recebido para publicação em 14.7.2012 e aprovado em 6.2.2013.

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INTRODUCTION

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), herbicide of the s-triazine family is one of the most commonly used worldwide, due to its broad spectrum of weed control, low cost and by presenting residual effect on soil (Fleck & Vidal, 2001).

Atrazine (ATZ) is classified as moderately persistent, and contaminates water and foods. The analysis on residues of atrazine in soils has received a special attention because its persistence increases its pollutant potential and can damage subsequent crops (Delmonte et al., 1996).

Controlled release is defined as a process in which the rate and period of release are predetermined to fulfill the necessary effects (Moura, 2008). The use of controlled release ATZ can be an alternative to minimize the environmental impact caused by its widespread use in agriculture. In vitro studies indicated that the release of ATZ from xerogels followed a kinetics slower than the commercial herbicide (Ávila et al., 2009). Moreover, the affinity of ATZ for soil matrix was lower when applied in the form of xerogel, suggesting a low dissipation of the herbicide via sorption in relation to the product applied in the form of suspension. Nevertheless, information on the behavior of controlled-release ATZ applied in the field is still lacking.

The evaluation of processes such as adsorption, leaching and persistence, as well as plant uptake, allows determining the bioavailability of herbicides to plants (Schroll et al., 1992). The presence of residues of herbicides is commonly determined by physical, chemical and biological methods. Any organism sensitive to actions of certain toxins can be subjected to bioassays. In general, the bioassay is a widely used tool to estimate the damage caused by xenobiotics, and is used by IBAMA in methods adopted for registration of pesticides (Peres, 2003).

Owing their mode of action, triazines are absorbed by roots, and exclusively transported via xylem. ATZ is also absorbed by leaves, thus is only transported from the point of application to the end part of the plant where it was applied (Silva et al., 2007). Plants present necrosis and

curling of leaves due to the herbicide action (Fleck & Vidal, 2001), which are rapidly identified from the phytotoxicity assessment, which is a parameter frequently used in bioassays (Gazziero et al., 1997; Ulbrich et al., 1998, Nunes and Vidal, 2009).

This work hypothesized that controlled-release atrazine has a longer persistence time in soil, compared with commercial formulation. The present study aimed to investigate the persistence of atrazine applied as xerogel in oxisol and to compare the behavior presented by the liquid commercial formulation. The results will enable to assess the agronomic feasibility of using atrazine as xerogel.

MATERIAL AND METHODS

A field experiment was set up in the Experimental Area of the Federal Technological University of the Paraná State, Campus Pato Branco, located in the municipality of Pato Branco, Paraná State (26°07'S and 52°41'W). The soil is classified as oxisol (Embrapa, 2006), and has clayey texture, allic character (Bhering et al., 2008). The climate according to the classification of Köppen, is humid subtropical with hot summer (Cfa) (Pandolfo et al., 2002).

The experiment had a split-plot randomized-blocks design, with four replications, in a (2 x 6) + 1 arrangement. Formulations (commercial ATZ - *COM* and xerogel atrazine - *XER*) were assigned to main plots, and ATZ concentrations (0, 3200, 3600, 4200, 5400 and 8000 g ha⁻¹), were assigned to subplots, with addition of four weeded control plots. The commercial product (*COM* formulation) was applied in the form of water dispersible granule (WG) and called GENIUS®. The ATZ xerogel (*XER* formulation) was synthesized in the Chemistry Institute of the Federal University of the Rio Grande do Sul State, according to the methodology described by Hirsch (2011).

Subplots consisted of five rows of corn, with 0.7 m spacing and 7 m length for the *COM* treatment (24.5 m²) and 3 m length (10.5 m²) for the *XER* treatment. The useful area of each subplot was made up of the three central rows, excluding 0.5 m from the ends of each row.



Herbicides were applied pre-emergence, soon after sowing the crop. Corn (Pioneer 30R50H) was sowed on October 13rd, 2009, using precision seed drill aiming the density of 67000 plants per ha⁻¹. Seeds were previously treated with thiametoxan + fipronil (1.75 + 0.5 g kg⁻¹ seeds). The fertilization was performed following the recommendation obtained of the fertility evaluation and used 24, 60 and 60 kg ha⁻¹ N, P₂O₅ and K₂O, respectively, with the formulation 8-20-20. For topdressing were used 65 kg N ha⁻¹, as urea, next to the planting line of corn, between V₂ and V₃ stages (Ritchie et al., 1993). At the time of topdressing application it was applied insecticide methamidophos (300 mL to 100 L water) at 20 days after sowing.

The herbicide in the *COM* formulation was sprayed to the moist soil after planting corn, with backpack sprayer pressurized with CO₂, kept at constant pressure, with fan type nozzles 110.02, spaced 0.50 m from each other in the 2.5 m wide bar, totaling a spray volume of 200 L ha⁻¹. The herbicide in the *XER* formulation was manually applied to the soil. Since this formulation is in powder and is difficult to dilute in water, for its application in the plots, the *XER* product was mixed to a sample of soil from the experimental area, previously dried, ground and sieved, at a ratio of 2 kg per subplot. Later, the herbicide contained in the mixture *XER*+soil was applied by broadcasting to the soil.

In order to evaluate the persistence in soil of ATZ in the two formulations were held periodical samplings of soil in plots that received 8000 g ha⁻¹ atrazine.

After removing the straw covering the previous crop (rvegrass) with a trowel, soil samples were collected in the 0 – 5 cm layer of the profile at 1, 5, 10, 14, 27 and 35 days after application (DAA) of herbicides. Samples were stored in plastic pots with capacity of 300 cm³, and stored at -5 °C.

In the period May 3-24, 2010 was performed the bioassay in greenhouse with collected samples, using previously germinated seedlings of oat (*Avena sativa*), indicative of herbicide persistence. Soil was thawed 24 hours before starting the bioassay and four previously germinated seeds were introduced

into each pot. Irrigation was performed daily, by hand, in order to provide good water availability to plants.

It was evaluated the phytotoxicity and height. The phytotoxicity of ATZ to bioindicator plants was evaluated from 18 to 21 days after transplantation (DAT), for soil samples that have been collected at different time intervals. For this, were assigned scores ranging from 0% (no effect) to 100% (lethal effect to plants) (Frans et al., 1986). The height was examined from 18 to 21 DAT, by measuring the distance between the base of the plant and the end of the leaf with a millimeter ruler.

For assessing the ATZ biodegraded in soil, we first constructed a dose-response curve, with oat plants seeded in 300 cm³ pots containing samples of soil (< 2 mm) of the experiment. *COM* and *XER* ATZ formulations were applied on the surface at concentrations corresponding to 0, 15, 30, 50, 65, 80 and 100% recommended dose (4000 g ha⁻¹), equal to 0, 600, 1200, 2000, 2600, 3200 and 4000 g p.a ha⁻¹. Values of performed evaluations (phytotoxicity and height) determined in the same periods of bioassays were related to the ATZ dose of each formulation.

The amount of ATZ biodegraded in samples of soil of the experiment was estimated for each formulation, by replacing the values of height and phytotoxicity obtained in the evaluation of persistence in the dose-response curve obtained with indicator plants.

The dissipation kinetics of ATZ in *COM* and *XER* formulations was determined by the first-order kinetics model, using the equation 1.

$$\ln([c_i]/[c_f]) = -kt \quad (\text{eq. 1})$$

where [c_i] and [c_f] is the ATZ concentration at initial and final time, respectively, calculated by the bioassay; *k* is the dissipation constant of ATZ; and *t* is the time. A [c_i] was considered in the time in which the maximum amount was detected in soil, after applying the herbicide. The *t_i* presented in the table of results of dissipation kinetics was considered the time after application in which occurred the higher availability of herbicide, detected by the bioassay method.



The half-life ($t_{1/2}$) of ATZ in the soil solution was calculated by equation 2.

$$t_{1/2} = 0.693/k \quad (\text{eq. 2})$$

Data of biotested ATZ were converted to percentage in relation to control without herbicide. Data of dose-response curve, bioavailability and dissipation kinetics were subjected to analysis of variance by the F-test. Mean values were compared by DMS test at 5% probability of experimental error ($p \geq 0.05$). The fit of calibration curves was carried out by polynomial regression, using the statistical software Winstat.

RESULTS AND DISCUSSION

Persistence

For phytotoxicity to oat plants, a triple interaction was verified between formulation, periods of collecting soil samples and period of evaluation of variables.

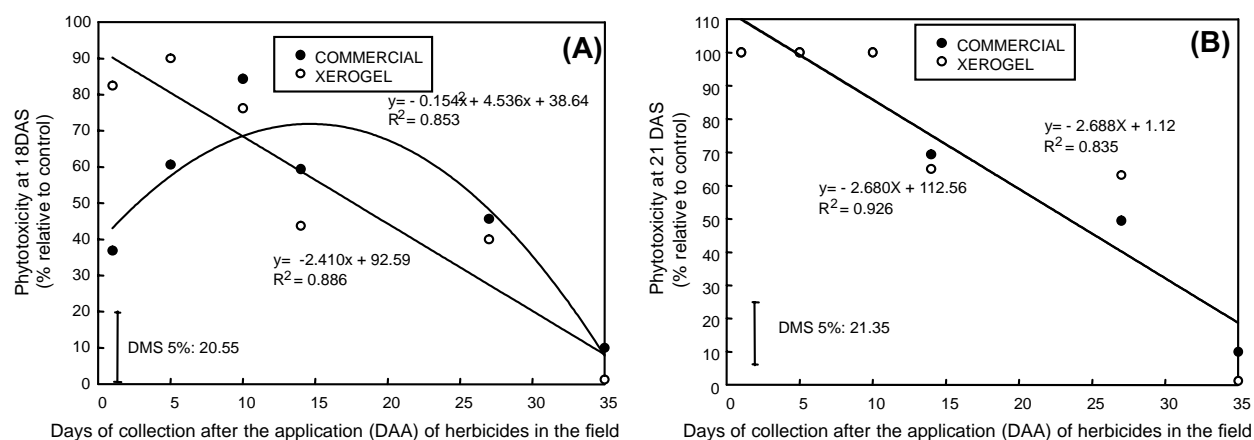
At 18 DAS, the phytotoxicity caused by ATZ in the XER formulation has linearly reduced over time after applying the herbicide (Figure 1A), suggesting that in 35 days occurs the reduction in the herbicide availability. For the COM ATZ formulation, the phytotoxicity increased from the 1 to 10 DAA, with the maximum injury occurring around the 15 DAA

and decreasing thereafter until the 35 DAA (Figure 1A). For being applied on ryegrass straw, part of the COM formulation had no contact with soil immediately after application. Between the 2 and 5 DAA rainfall (accumulation of 69 mm in the period) removed the ATZ to the soil, increasing its concentration in collected samples, and consequently its phytotoxicity until the 15 DAA. In the XER formulation, this has not occurred since it was mixed with soil.

The herbicide retention in the straw can be considerable, as observed by Nunes and Vidal (2008) for ATZ in oat straw cover. The mobilization of the herbicide to the soil depends on the retention mechanism (sorption or physical impediment) and rainfall intensity.

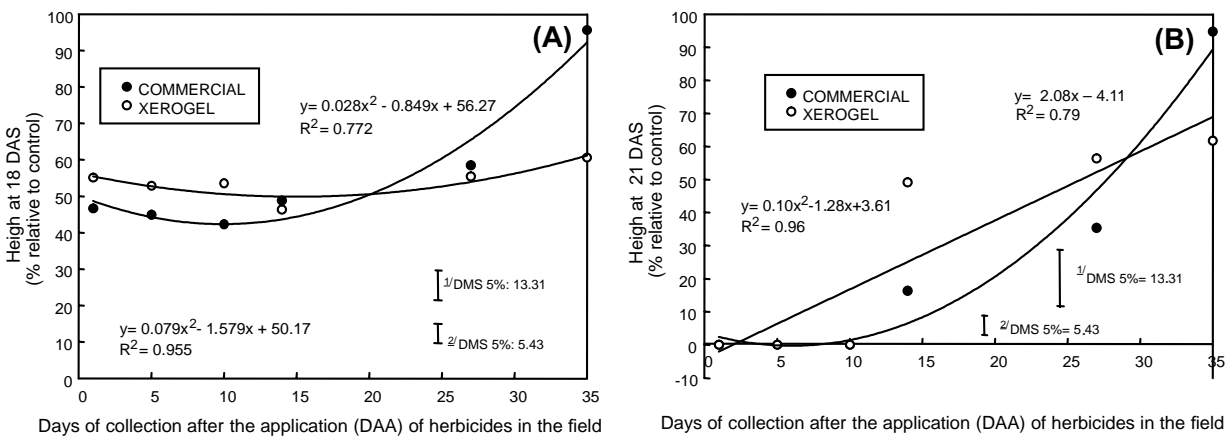
In the assessment held at 21 DAS, the phytotoxicity has linearly reduced with DAA for both formulations (Figure 1B), and given the lack of significant differences, a regression analysis was run for the data set. This behavior was probably due to the intensification of ATZ dissipation in soil (degradation, leaching and sorption) which had not been different between formulations.

For height, normalized in relation to control, a triple interaction was observed between formulation, periods of collection and periods of evaluation (Figure 2).



CV = 32.6%.

Figure 1 - Toxicity of commercial and xerogel atrazine on white oat at 18 (A), 21 (B) days after sowing (DAS). UTFPR, Pato Branco-PR, 2009.



$1/2$ DMS 5%: 13.3% for formulations; $2/2$ DMS 5%: 5.4 for periods of evaluations. Coefficient of variation: 14.7%.

Figure 2 - Height (% relative to control) of white oat according to herbicides (commercial or xerogel atrazine), 18 (A), 21 (B) days after sowing (DAS). UTFPR, Pato Branco-PR, 2009.

When assessed the oat height at 18 DAS, no significant differences were detected between heights of oat plants resulting from the application of different ATZ formulations in any period of evaluation, except for the 35 DAA (Figures 2A), in which the COM ATZ had a lower effect on the height reduction, comparatively to XER ATZ. In this case, the release of the active ingredient in a more controlled way in XER may result in both higher phytotoxicity in later stages (Figure 2A) as also in higher resistance to dissipation in relation to the COM formulation, prolonging its permanence in soil.

In the height assessment at 21 DAS, we observed death of plants in soil samples collected at 1, 5 and 10 DAA, showing intensified symptoms of the herbicide in both formulations over time of evaluation for these systems. In soils collected at 14 and 27 DAA, the presence of the COM formulation has inhibited more intensely the oat height, compared with the XER formulation, but from this date onwards, the availability of both formulations was reduced in comparison with 1, 5 and 10 DAA (Figure 2B).

Longer periods of persistence were identified by other authors. In the study of Blanco et al. (2010), the ATZ persistence evaluated through bioassays (dry weight) in soil classified as Eutrophic Red Latosol reached 56 days after application. Meantime in an

experiment in soils of the South Western Buenos Aires Province with low content of organic matter (between 5.70 and 3.84) in Argentine, the persistence was 143 and 221 days (Delmonte et al., 1996).

ATZ residues in soil can be relatively high, according to Brighenti et al. (2002). In Red Yellow Latosol and Dystrophic Purple Latosol of the Northern Paraná State, ATZ residues were 3 and 6 kg ha⁻¹ at sowing performed 60 days after applying the herbicide in corn crop on the sunflower sowed in succession, results inferred by means of evaluations of persistence and phytotoxicity.

Atrazine biotested over time

Calibration curves obtained from the relationship between parameters of the indicator plant (height and phytotoxicity) and ATZ doses tended to saturation (data not shown) and only the linear part (ATZ dose ≤ 1200 g ha⁻¹) was employed in evaluating the bioavailability of ATZ in samples collected in the field.

For the oat height, a calibration curve was fit at 21 DAS, once evaluations in the experiment were conducted at this time. The lack of significance of formulation has determined the use of the mean of the two formulations to fit the response (Figure 3).

For the variable injury of oat, were adjusted dose-response curves at 15, 18 and 21 DAS.



The equations followed the linear model, with R^2 of 0.79, 0.85 and 0.98, respectively, in the aforementioned periods (Figures 4A, B and C).

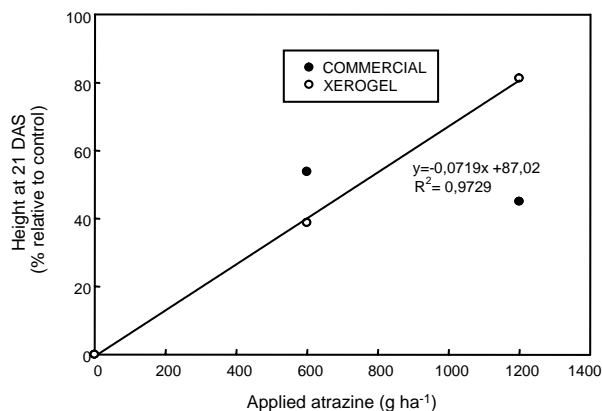


Figure 3 - Height (% relative to control) of white oat according to doses of atrazine, at 21 days after sowing (DAS). UTFPR, Pato Branco-PR, 2009.

As expected, the plant height has decreased and the injury has increased with higher doses, being this effect much more pronounced over the evaluation time. Considering the plant height measured at 21 DAS, the greater amount of ATZ biodegraded occurred up to the 10 DAA, without differences between formulations (Figure 5). From the 14 DAA, the amount of atrazine decreased, and was more marked for *XER* than for *COM*. At 35 DAA, it was no longer registered bioavailability of the *COM* formulation and of the *XER* formulation was around 250.70 g ha⁻¹.

The quantity of ATZ inferred from injury in oat at 15 DAS indicated an amount of ATZ in the collection of 1 DAA equivalent to 7.30% for the *COM* formulation and 15.76% for *XER* formulation of the initial amount applied (Figure 6A). For the 10 DAA, the amount inferred from the injury was 18.29% for the *COM* formulation and 14.43% for the *XER* formulation, showing that the volume of 69 mm rainfall was important to enhance the bioavailability of herbicide in soil. In the evaluation at 18 DAS, the same trend was observed, with the maximum amount of the herbicide identified in the period of 10 DAA, reaching 15.4% for the *COM* formulation and

16.5% for the *XER* formulation. In collections undertaken at 1 and 5 DAA, it was verified in the two periods of evaluation (15 and 18 DAS) that ATZ of the *XER* formulation was at higher concentration in the soil (Figures 6A and B).

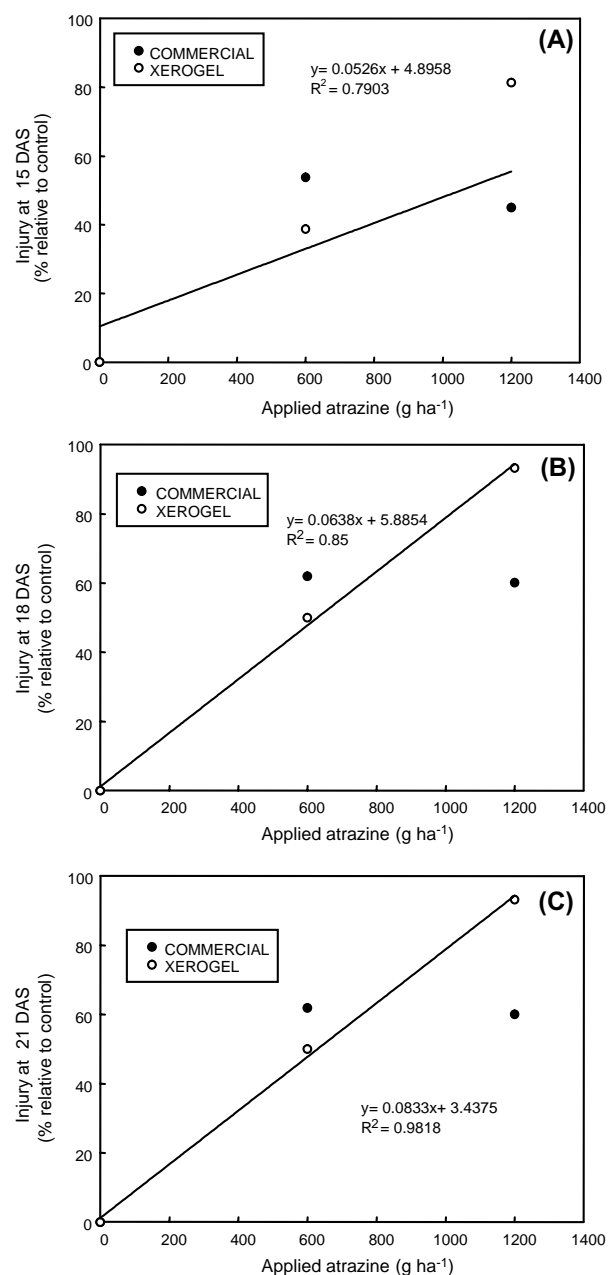


Figure 4 - Toxicity (% relative to control) to white oat of doses of atrazine, 15 (A), 18 (b), 21 (C) days after sowing (DAS). UTFPR, Pato Branco-PR, 2009.

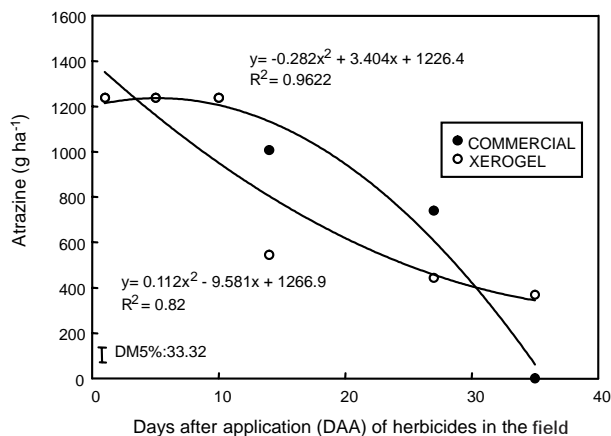


Figure 5 - Atrazine biotected when applied in commercial or xerogel formulations, obtained by the height of white oat, evaluated at 21 DAS in samples of soil collected in the field up to the 35 DAA. UTFPR, Pato Branco-PR, 2009.

This effect can be explained by the distinct way of application between formulations. The *XER* formulation was applied by broadcasting, by mixing it to a portion of soil, which probably has reduced the amount of ATZ attached to the straw of the ground surface present at the time of application, and higher fraction of ATZ dropped directly in the soil. Nevertheless, the *COM* formulation was dissolved in the spray and applied by spray bar, which may have favored the adsorption of part of ATZ applied to the ryegrass straw on the soil.

This fact increased the amount of ATZ biotected in initial times of soil collection

in the *XER* formulation compared with *COM* formulation. The increase of ATZ bioavailability in the *COM* formulation over time until the 15 DAA (Figures 6A and B), was because of rainfall in the first days as previously discussed.

The main hypothesis of this experiment was that the ATZ in the *XER* formulation has a higher persistence than the ATZ in the *COM* formulation. Evidences obtained in bioassays carried out from samples of the field experiment and of ATZ bioavailability data in soil corroborate the initial hypothesis.

The bioassay to evaluate the ATZ persistence, conducted with the white oat species, was effective to determine the period after the herbicide application in which the ATZ availability in soil was kept, with little variation. This method also allowed distinguishing the ATZ formulations tested. For most variables determined in the bioassay with oat (phytotoxicity and height), the period with smaller variation in oat response, since its application, was between 10 and 14 days after the application. This effect on oat plants was reflected in the calculation of herbicide bioavailability, because the ATZ presented a higher availability for adsorption by plants up to 10 days after its application (Figures 5 and 6). After this period, the bioavailability of the herbicide was reduced, for both tested formulations.

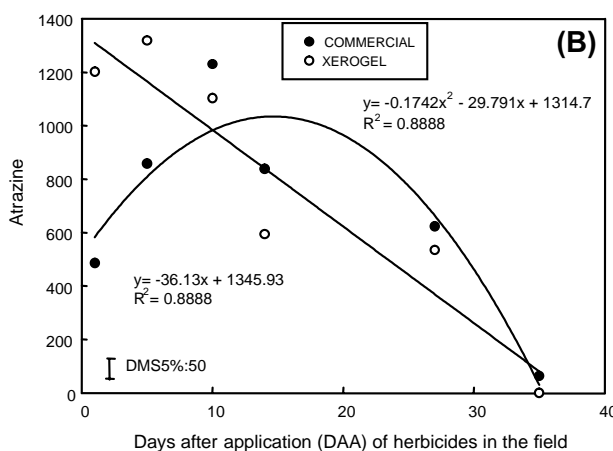
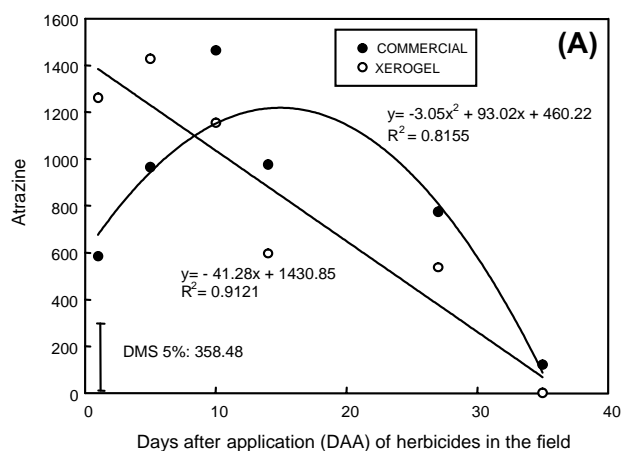


Figure 6 - Atrazine biotected when applied in commercial or xerogel formulations obtained by injuries evaluated at 15 (A) and 18 (B) DAS of white oat, in samples of soil collected in the field up to the 35 DAA. UTFPR, Pato Branco-PR, 2009.



Table 1 - Initial concentration (ci), dissipation constant (k) and half-life (t1/2) of commercial and xerogel atrazine, through phytotoxicity evaluated at 18 DAS. UTFPR, Pato Branco-PR, 2011

Formulation	Ti (days)	ci ± ep (g i.a ha ⁻¹)	k ± ep	r ²	Half-life (t ½) (days)
Commercial	10 ^{ns}	1271.78 ± 210.23 ^{ns}	0.0628 ± 0.024	0.93	21.03 ^{ns}
Xerogel	5	1366.975 ± 195.16	0.0519 ± 0.019	0.91	18.35

^{ns} non-significant by the F-test.

Dissipation kinetics

The results obtained from applying the first-order kinetics to phytotoxicity data for the time of 18 DAS are listed in Table 1. The initial time (ti) was considered as the time after application with the higher availability of the active ingredient of ATZ detected by the bioassay method. The initial concentration (ci) indicated the amount of the active ingredient of herbicide available on the day ti.

Regarding the phytotoxicity data obtained at 18 DAS for *COM* and *XER* formulations, the dissipation constant (k) for the *XER* formulation was 0.0519 and lower than for the *COM* formulation, 0,0628 (Table 1), but there were no differences in half-life (t ½) between formulations.

It is speculated that the numerical difference in t_i of 5 days, although without significant difference between the two formulations, may indicate a promising feature for using the *XER* formulation. Maybe the use of a greater number of repetitions in experiments on the persistence enables to differentiate this attribute between these two formulations.

Our results allow concluding that the ATZ availability of commercial formulation in soil was enhanced by rainfall, while the ATZ of xerogel formulation, due to its form of application, was available more readily after application. For both tested formulations, the peak of availability occurred 10 days after application. The bioassay method for the evaluation of ATZ availability is efficient and enables to discriminate the tested formulations. The xerogel ATZ availability is higher than the commercial one in two different periods: up to 5 days after application and also at 35 days after applying the herbicide. The xerogel formulation tends to be more

persistent in relation to the commercial formulation.

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