PERSISTENCE OF THE BIOCIDE ACTIVITY OF ATRAZINE IN SOILS OF THE SOUTHEAST OF BUENOS AIRES PROVINCE1

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RESUMO

Persistência do herbicida atrazine em solos do Sudeste da Provincia de Buenos Aires

O objetivo do presente trabalho foi estudar a persistência da atrazine em solos do sudoeste da Província de Buenos Aires, Argentina, por meio de um bioensaio com aveia. Estudaram-se doses de atrazine de 0; 0,58; 1,16 e 2,32 µg.g $^{-1}$ de solo seco (SS), os quais foram aplicados em vasos que continham solos de Balcarce, A. Gonzáles Chaves e San Cayetano. O conteúdo de matéria orgânica (MO) dos solos foram 5,70; 5,15 e 3,84 % para Balcarce, A. Gonzáles Chaves e San Cayetano, respectivamente. *Avena sativa* cv. Millauquén foi semeada em vasos e cultivada sob condições de casa-de-vegetação até o desenvolvimento completo da segunda folha. A porção aérea das plantas foi colhida e determinado o peso da matéria seca. Com os dados obtidos calculou-se o peso relativo da matéria seca (PRMS), com relação ao tratamento sem herbicida. Após um período de tempo curto de descanso, repitiu-se o processo anterior, até totalizar quatro períodos sucessivos. A fitotoxicidade da atrazine foi expressa em termos de 50 % de redução do PSR (GR_{50}), nos solos avaliados. Aos 42 dias da aplicação obtiveram valores de GR_{50} de 0,30; 0,64 e 0,90 µg.g^{-1} SS nos solos de San Cayetano, Balcarce e A. G. Chaves, respectivamente. A persistência de atrazine na dose recomendada (1,16 µg.g-^{1}), considerando uma redução de 80 % do PSR, foi de 100, 143 e 221 dias desde a aplicação para A. G. Chaves, Balcarce e San Cayetano, respectivamente. Estes resultados são consistentes com o menor conteúdo de MO e capacidade de troca catiônico, assim como o maior pH para o solo de San Cayetano, com relação aos solos restantes estudados.

Palavras chave: Bioensaio, aveia, GR50, persistência, fitotoxicidade, Argentina.

ABSTRACT

Atrazine persistence in soils of the southeast of Buenos Aires Province, was studied by an oat bioassay. Atrazine doses of 0.58, 1.16, and 2.32 µg.g $^{-1}$ dry soil weight (DSW) were applied to pots containing soils from Balcarce, A.Gonzáles Chaves and San Cayetano sites, whose organic matter (OM) content of soils were 5.70, 5.15, and 3.84 %, respectively. *Avena sativa* cv. Millauquén plants were grown in the pots under greenhouse conditions at different times after atrazine application. Shoots were evenly cut above the soil and dry weight determined as a measure of plant growth. Plants grown in non-sprayed soils were used as controls. Relative dry weight (RDW) of shoots was calculated as percentage of control. Atrazine phytotoxicity was expressed in terms of 50 % plant growth reduction (GR_{50}) in the soils under study. Herbicide persistence was expressed in terms

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of days after treatment (DAT) needed for the plant to achieve 80 % of RDW. Atrazine GR_{50} values of 0.30, 0.64, and 0.90 µg.g $^{-1}$ DSW in soils from San Cayetano, Balcarce and A. G. Chaves, were respectively obtained at 42 DAT. Herbicide persistences at the recommended dose (1.16 µg.g^{-1}) were 100, 143, and 221 DAT for A. G. Chaves, Balcarce and San Cayetano soils, respectively. San Cayetano soil had both the lowest OM content and cation exchange capacity (CEC), as well as the highest pH, of all the soils studied here. These results were consistent with both the lowest GR50 and the highest persistence obtained for atrazine in this soil.

Key words: Oat bioassay, GR50, phytotoxicity, Argentina.

INTRODUCTION

The study of environmental pollution is a subject of vital interest in developed countries and it has forced international authorities to control the presence of pesticide residues in waters, soils and food (Liégeois *et al.,* 1992).

In general, organo-phosphorated insecticides and triazine herbicides are the most widely used pesticides in the world today; thus they have been considered in several countries as indicators of environmental contamination due to agricultural activity (Moltó *et al.,* 1991; Schneider & Hammock, 1992).

The analysis of herbicide residues in soils has received particular consideration as their persistence added to their potential polluting aspect may damage the subsequent crops (Liégeois *et al.,* 1992). In this aspect, atrazine is important for three main reasons: 1) it is the most used herbicide (Moltó *et al.,* 1991); 2) it is moderately persistent (Graham *et al.,* 1992) and, 3) it may contaminate water and food (Bushway *et al.,* 1992a; Bushway *et al.,* 1992b; Hahn *et al.,* 1992) if it is used without knowing its persistence and mobility, factors which, on their turn, are influenced by environmental conditions and the particular characteristics of the soil (Weber, 1991; Rocha & Walker, 1995). Hence, the study of residues of atrazine in waters and soils is a subject of current attention (Bushway *et al.,* 1992a; Felding, 1992; Moltó *et al.,* 1991; Francioso *et al.,* 1992; Graham *et al.,* 1992; Hahn *et al.,* 1992; Smith, 1992). To our knowledge, however, there are no references in the literature about research carried out in this field in Argentina.

Most of the methods used to determine the presence of atrazine in soils are complicated, expensive and require specialized personnel and devices (Felding, 1992; Francioso *et al.,* 1992; Smith, 1992). On the other hand, the detection of traces of the herbicide does not represent a direct measure of its phytotoxic effect, which is crucial to know before performing a crop rotation. A biotest using vegetal species sensitive to the pesticide as control may satisfy both requirements.

The objective of this work was to evaluate the biocide persistence of atrazine in three representative soils of the southeast of Buenos Aires Province, using a sensitive vegetal species in biotests at the greenhouse.

MATERIALS AND METHODS

Soil samples were collected from 0-15 cm depth at three different locations in Buenos Aires Province. All sample sites had no previous history of atrazine application. Each soil sample was previously homogenized, air dried, and passed through a 2-mm sieve before use. The properties of these soils are given in Table 1.

Soil samples were distributed into pots, in aliquots of 400 g dry weight (DSW), each one constituting an experimental sample. An aqueous flowable formulation of atrazine at 50 % active ingredient (a.i.), commercially distributed as Trac 50 FL (Atanor S. A., Argentina), was used. The herbicide was applied to soil in pots using a hand sprayer fitted with a 11002 nozzle tip, to achieve a.i. concentrations of 0.58, 1.16, or 2.32 µg.g^{-1} DSW. These correspond to 1, 2, and 4 kg.ha^{-1} a.i. doses at the field, respectively. Each sample was thoroughly mixed to allow a homogeneous distribution. Non-sprayed soil samples were used as controls. Treatments were included in a complete randomized design and they had a factorial arrange of three soil types and three atrazine doses. Each treatment was replicated six times.

Soils were evaluated for herbicidal activity using an oat bioassay (Crafts & Dreyer, 1960; Sheets *et al.,* 1962; Marriage, 1975; Horowitz, 1976). After atrazine was added to the soils, thirteen oat seeds *(Avena sativa,* cv. Millauquén) were seeded per pot by pressing the basipetal end of each one into the soil up to approximately three quarters of its size. After seedling establishment, plant number was thinned to ten per pot. The seed bed was moistened daily with distilled water to 80 % of field capacity and fertilized with a triple superphosphate (46 % $P_2 0_5$) water solution, to achieve a final concentration of 116 $\mu g.g^{-1}$ DSW. This superphosphate concentration corresponds to 200 $kg.ha^{-1}$, which covers any possible P deficiency in the soils used here. The plants were grown at the greenhouse.

Once the untreated control plants had a completely developed second leaf, approximately in 30 days according to the time of the year, the growth period was considered to have come to an end. Plants were counted and evenly cut above the soil surface, placing all the harvest at 65°C until constant weight. Only live plants were harvested. Once weighed, plants were milled and returned to their corresponding pots, with a fallow period of about twenty days. Next, oat was again seeded in the way previously described. This process was repeated until there were neither external symptoms of phytotoxicity in the plants nor differences among treatments (four periods).

Relative dry weight (RDW) per plant was calculated as a percentage of the untreated control. The analysis of variance was done for all soils and means were compared by Duncan's Multiple Range Test.

Plant inhibition in relation to herbicide concentration in soil was calculated as the percent of reduction of shoot dry weight with respect to the control (Upchurch, 1958; Weber, 1977), and expressed in terms of growth reduction (GR). Doses diminishing growth in 50 % (GR_{50}) for the first growth period (42 days) and for each one of the soils, were calculated according to Sheets *et al.* (1962).

RESULTS AND DISCUSSION

Atrazine is well known by its capacity to percolate thorough soils, which makes it a herbicide of concern in relation to groundwater contamination (Felding, 1992). However, the risk decreases by favourable soil properties such as adsorption capacity and biological and chemical degradation.

Deethylatrazine and hydroxyatrazine have been reported as major degradation products of atrazine (Adam & Thurman, 1991), whose phytotoxicity has also been stressed (Kaufman & Kearney, 1970). In spite of this, few studies have been conducted regarding to atrazine phytotoxic metabolites in soil (Baluch *et al.,* 1993). Radiolabelling, chemical analysis and more recently, immunochemical techniques, have been the main strategies used to study atrazine and its main metabolites in soils (Baluch *et al.,* 1993; Koskinen *et al.,* 1992; Hahn *et al.,* 1992; Schneider & Hammock, 1992). However, these techniques often require expensive equipment plus technical expertise (Oyeniyi & Akinyemiju, 1990). Bioassays, on the other hand, are a simple and inexpensive way to detect not only atrazine but also its main phytotoxic metabolites. Moreover, bioassays have been considered the only suitable methods for recording biological activity of herbicides (Santelmann, 1977).

Plant sensitivity to a herbicide has been expressed in bioassays in terms corresponding to the LD_{50} values of animal toxicity studies. In that sense, the amount of herbicide in the soil which reduced the yield of a specific species by 50 % has been expressed as DE_{50} or GR_{50} (Santelmann, 1977). We obtained GR_{50} values of 0.64, 0.90, and 0.30 $\mu g.g^{-1}$ DSW in soils from Balcarce, A. G. Chaves and San Cayetano, respectively. These values could be speculatively compared to 1.10, 1.55, and 0.50 $kg.ha^{-1}$ (a.i.) doses of atrazine applied in the field, respectively. Thus, atrazine phytotoxicity expressed in terms of GR_{50} followed the order: San Cayetano > Balcarce > A. G. Chaves. Even though our results are insufficient to assess a relation among soil parameters and GR_{50} values, San Cayetano's soil had both the lowest OM and CEC, and the highest pH of all the soils under study (Table 1). All these parameters have been shown to enhance atrazine bioavailability in soils (Weber, 1991).

TABLE 1. Physicochemical properties of soils.

SITE	TEXTURE	SAND (%)	SILT (%)	CLAY (%)	OM (%)	pH	CEC
Balcarce	Loam	33,2	39,0	27,8	5,70	6,9	32,7
A. G. Chaves	Clay Loam	26,7	44,4	28,9	5,15	6,1	31,4
San Cayetano	Clay Loam	38,8	32,8	28,4	3,84	7,3	27,6

OM: organic matter; CEC: cation exchange capacity (meq.100 g^{-1}).

Using a bioassay method, Crafts & Dreyer (1960) calculated persistence of herbicides in soils by determining the number of runs required to reduce acti-vity of each chemical to the 50 % growth level. Later on, the same investigators (Sheets *et al.,* 1992) compared the residual activities of s-triazines through fresh weight changes of eight successive crops of oats at three concentrations of each herbicide. Similarly, we determined atrazine persistence in three different soils, by studying shoot relative dry weight (RDW) hanger of four consecutive oat crops, at three herbicide concentrations. Table 2 shows the RDW evolution in shoots from four consecutive crops of *A. Sativa* cv. Millauquén sown in soils from the SE of Buenos Aires Province previously treated with

TABLE 2. Shoot RDW* vs. days after treatment (DAT) in oat crops obtained from soils treated with atrazine.

SOIL SITE	DOSE	DAYS AFTER TREATMENT			
	$\mu g. g^{-1}$	42	105	160	223
Balcarce	0	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$
	0,58	$53,3^{c}$	$81,2^{bc}$	$84,2^{bc}$	$95,2^{ab}$
	1,16	$22,3^{ef}$	$75,0^{c}$	$82,3^{bcd}$	$94,0^{ab}$
	2,32	$20,8^{ef}$	$50,3^{d}$	$74,0^{cd}$	$94,9^{ab}$
A. G. Chaves	0	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$
	0,58	$71,5^{b}$	$86,0^{b}$	$94,3^{ab}$	$96,1^{ab}$
	1,16	$24,3^{de}$	$81,3^{bc}$	$88,4^{abc}$	$97,9^{ab}$
	2,32	$18,0^{f}$	$57,2^{d}$	$83,0^{bcd}$	$97,8^{ab}$
San Cayetano	0	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$	$100,0^{a}$
	0,58	$28,0^{d}$	$55,9^{d}$	$69,0^{d}$	$90,9^{bc}$
	1,16	$20,8^{ef}$	$35,6^{e}$	$44,6^{e}$	$84,9^{cd}$
	2,32	$19,8^{ef}$	$26,7^{e}$	$22,8^{f}$	$78,1^{d}$
Variation coefficient		8,4	11,2	14,9	7,0
Standard error of the mean		1,6	3,2	4,8	2,7

a-f Means followed by the same letter in columns are not significantly different (P=0.05). *As percent of control.

single doses of atrazine applied at three different concentrations.

At 42 and 104 DAT, shoot RDWs were significantly lower than controls for all the atrazine doses applied to the soils under study. After 160 days herbicide treatment, however, RDWs were not significantly different to controls in A. G. Chaves soils at 0.58 and 1.16 µg.g^{-1} DSW doses (Table 2). This trend was also evident after 223 days for Balcarce and A. G. Chaves soils, at each herbicide concentration assayed. San Cayetano soil on the other hand, showed herbicide effects on oat shoot RDWs after 223 DAT at 0.58, 1.16, or 2.32 µg.g^{-1} DSW doses (Table 2). Additional comparisons among soils could be made at each sampling period (Table 2). First, shoot RDW obtained 42 DAT was not significantly different ($P = 0.05$) among soils when the herbicide was applied at 1.16 and 2.32 µg.g^{-1} DSW doses. On the contrary, RDW followed the order A. G. Chaves > Balcarce > San Cayetano at 0.58 µg.g^{-1} DSW atrazine dose. Second, RDWs were significantly lower in San Cayetano soil than in the other two soils after 104 DAT at 1.16 and 2.32 µg.g^{-1} DSW doses. Third, at 223 DAT oat shoot RDW obtained from San Cayetano's soil treated with 0.58 µg.g^{-1} DSW atrazine dose did not differ significantly to that obtained from Balcarce at 1.16 µg.g^{-1} DSW dose, or to those obtained from Balcarce or A. G. Chaves soils treated with the highest atrazine dose.

Herbicide persistence in soils was represented as relative plant growth vs. number of crops in bioassays (Sheets *et al.,* 1962). In order to obtain a value for atrazine persistence at any chosen growth level, we preferred to plot RDW vs. log DAT. In this way, a line traced parallel to the x axis at 80 % RDW intercepts the curves at values that represent log DAT for each atrazine dose initially applied to the soil. Figure 1 shows the evolution of oat shoot RDW when atrazine was applied at 0.58, 1.16, or 2.32 µg.g^{-1} DSW doses, to the different soils under study. The reasons for preferring this level of growth instead of the more common 50 % were: a) to avoid confusing with GR_{50} values; and b), to provide data more closely related to agronomic requirements. Figure 1 shows that 70, 100 and 191 DAT were required to reach the 80 % RDW limit when 0.58 µg.g^{-1} DSW was applied to soils from A. G. Chaves (B), Balcarce (A) and San Cayetano (C), respectively. When the atrazine dose was duplicated, the same order of herbicide persistence in relation to soil was followed, and the periods of time extrapolated from the curves were of 100, 143 and 221 DAT, respectively. At 2.32 µg.g^{-1} DSW atrazine dose, the 80 % RDW limit was reached at 160 and 176 DAT in A. G. Chaves and Balcarce soils, respectively, while the maximum 223 DAT period of time considered here was insufficient to allow oat plants reach the 80 % RDW limit in San Cayetano's soil.

Atrazine doses used in this work were equivalent to: a) zero (control), b) 1 kg.ha^{-1}, c) 2 kg.ha^{-1} (recommended at field trials), and d) 4 kg.ha^{-1}. The recommended dose is slightly higher than the one used in Europe after July, 1990, when a law fixing the treatment of maize crops with atrazine at 1.5 kg.ha^{-1} (a.i.), was passed (Mirgain *et al.,* 1993). Considering the results presented in Figure 1, a minimum of 100, 143 and 221 days waiting should be taken into consideration prior to sowing atrazine-sensitive crops in A. G. Chaves, Balcarce and San Cayetano after a recommended dose of atrazine is applied to soils. Nevertheless, the herbicide has a biocide persistence of more than 223 days in soils with a lower content of organic matter (San Cayetano), increasing the potential danger of contaminating sub-superficial waters (Table 2). Therefore, in order to minimize the danger of environmental contamination, the rational use of atrazine at some southeastern soil of Buenos Aires implies the use of the herbicide within the recommended doses and taking into account the characteristics of the soils of each region.

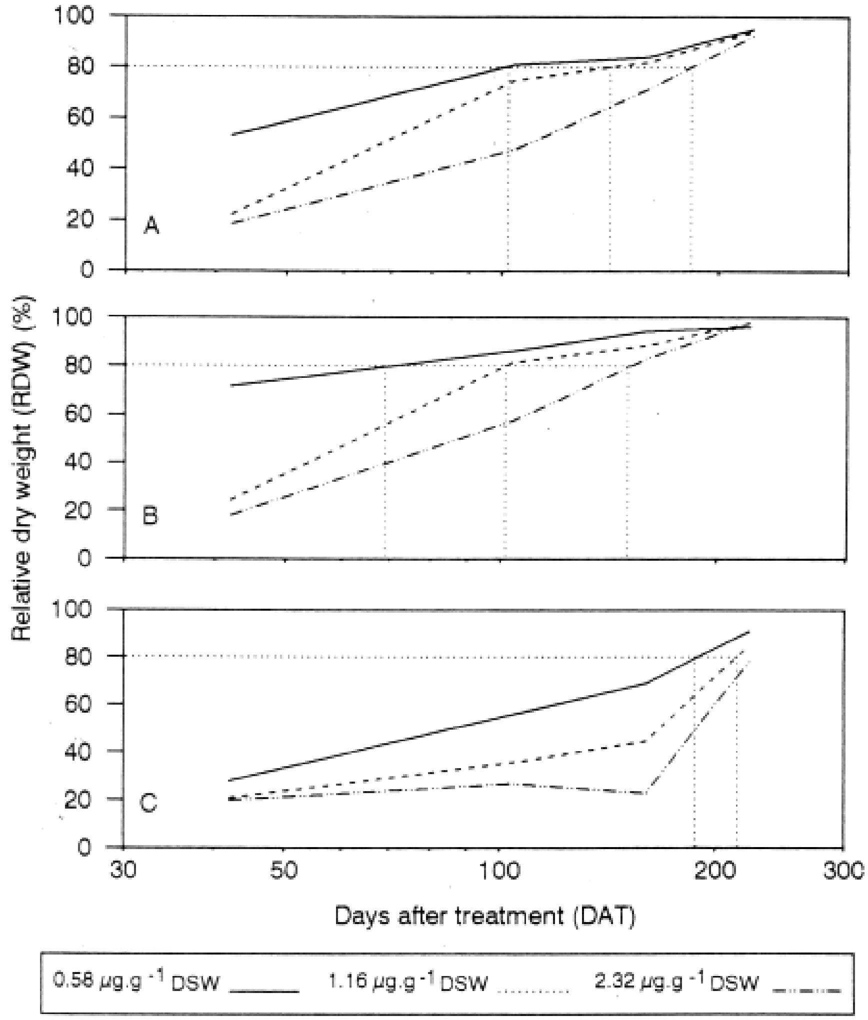

FIGURE 1. Shoot RDW vs. DAT obtained from consecutive oat crops grown up to two leaves, in soils from Balcarce (A), A. G. Chaves (B) and San Cayetano (C), treated with different doses of atrazine. DSW: dry soil weight.

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