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Full Length Research Paper

Soybean as bioindicador of residual effect of 2,4-D herbicide in an oxisol from the Brazilian cerrado

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Invasive plants, besides competing for space, also compete for water, light, and nutrients against the crop, what may cause drastic production cuts. Chemical control through herbicides is the most widely used method to control these plants. This study aimed to evaluate the persistence of the 2,4-D (dichlorophenoxyacetic acid) herbicide in soybean (*Glycine max* L.) crops in an Oxisol from the Brazilian Cerrado. The experiment was conducted in a greenhouse using a randomized block design, 5x3 factorial, five application periods (0, 3, 5, 7 and 10 days before sowing), and three herbicide doses (0, 750 and 1500 g e. a. ha⁻¹), in four repetitions. Herbicides were sprayed with the assistance of knapsack sprayer at constant pressure (kept through CO₂ compressed). Residual effect was assessed by emergence speed index (ESI), visual plant phytotoxicity, plant height, and shoots and root dry biomass. Persistence of 2,4-D herbicide was short in soybeans planted in an Oxisol. Periods from 0 to 3 days before sowing were the most harmful to soybeans, that is, the closer the spraying was from sowing, higher was its damage to the crop. The 1500 g e. a. ha⁻¹ dose had a higher residual effect.

Key words: Persistence, bioassay, *Glycine max*, dichlorophenoxyacetic acid.

INTRODUCTION

In relation to the increase in grain production, Brazil is estimated to cover an area of 58.5 million hectares, what makes agriculture one of the most important activities in the country, especially in the state of Mato Grosso, the largest national soybean (*Glycine max* L.) producer. In 2015, Brazil's Central-West region accounted for 42% of soybean production, and is also the country's main agricultural region (CONAB, 2015).

Weed occurrence in agricultural areas may reduce crop

yields, resulting in large losses (Fontes, 2003). Weeds are those that directly or indirectly harm human activity (Silva, 2007a), as they compete against the crop for space, water, light and nutrients, may act as pest and disease hosts, exercise allelopathic effects, and reduce biodiversity, among other problems (Vasconcelos et al., 2012)

In order to mitigate weed impacts, it is necessary to adopt control measures, whether mechanical, physical,

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рН	Р	K	Ca	Mg	Н	Al	SB	СТС	V	O.M.	Sand	Silte	Clay
CaCl ₂	mg dm ⁻³		Cmol _c dm ⁻³						%	g dm ⁻³		g kg ⁻¹	
4.8	1.4	23	0.4	0.2	5.4	0.8	0.8	6.8	9.7	27.1	423	133	444

Table 1. Chemical and grain size characterization of an Oxisol in the 0.0 to 0.20 m deep layer.

chemical or biological. Of these, chemical control was and still be the most widely used measure (Pitelli, 1987). On the other hand, the use of chemicals may become a risk, requiring care. According to the National Health Surveillance Agency (ANVISA, 2013), the Brazilian pesticide market expanded 190% in the last decade, more than twice than the global market (93%).

Despite the beneficial effects that pesticides have in that kind of control, excessive or incorrect use may lead to soil, water, and crop contamination, affecting nontarget organisms (Mohiddin et al., 2015; Xiao et al., 2012). When the molecules of a given herbicide are applied in the environment, the final destination is usually the soil. Incorrect chemical use in these soils is a serious threat to the health of humans, animals, and aquatic systems worldwide (Karam et al., 2009; Belo et al., 2007). Concern on this topic has increased, which justifies soil studies aimed at avoiding phytotoxicity of succeeding crops.

Considering the soil as the probable destination of most chemicals, both in pre- or post-emergence application, herbicides are subject to physical and chemical processes that lead their destination in the environment, which may follow different pathways (Law, 2001; Oliveira and Brighenti, 2011). Among processes that may occur with herbicides in the soil, persistence and leaching are the most common.

Herbicide persistence is the ability of a compound to show its residual effect, in order to prevent weed development in a certain area. On the other hand, herbicides with long bioactivity may cause subsequent damage to sensitive crops, that is, successor plants in a crop rotation system or crop consortium (Dan et al., 2012a,b). There are many processes that influence herbicide persistence: dissipation, evaporation, leaching, surface runoff, molecule absorption by the plant, biological, physical and chemical degradation (Silva et al., 2007b), and the own initial dose (Blanco and Oliveira, 1987).

The 2,4-D (2,4-dichlorophenylacetic acid) herbicide is a growth regulator that has a similar effect to the auxin hormone (Ashton and Crafts, 1973). Belonging to the phenoxy compound family, it consists of salts or esters with high molecular weight and low volatility, derived from phenoxyacetic acid (Saad, 1978). It is used as herbicide for selecting narrow-leaved plants, causing higher phytotoxicity in broad-leaved species, and is widely used in weed desiccation before soybean sowing, as this herbicide has short to intermediate soil persistence.

An alternative to evaluate herbicide residual effect in the soil is the use of plant species that are highly sensitive to the herbicide of interest (bioindicators). The technique aforementioned is known as bioassay (Inoue et al., 2002; Nunes and Vidal, 2009). Bioassay advantages over other techniques are its simplicity, low cost, and the possibility of detecting the biologically active amount of the herbicide. In addition, it is directly applicable to field conditions (Lima et al., 1999).

The aim of this study was to evaluate 2,4-D (dichlorophenoxyacetic acid) herbicide persistence in an Oxisol from the Brazilian Cerrado through bioassay, using the soybean crop as bioindicator.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse located at 16° 28 'south latitude, 50° 34' west longitude and 284 m altitude. The experiment used a randomized block design, 5x3 factorial, five application periods (0, 3, 5, 7 and 10 days before sowing), and three 2,4-D herbicide doses (0, 750 and 1500 g e.a. ha⁻¹), in four repetitions.

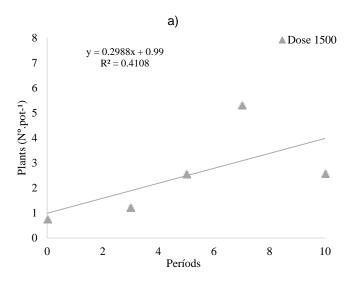
Each experimental unit consisted of pots with 5 dm³ capacity containing eight soybean plants of TMG 132 cv. (seeded at 5 cm depth). The soil used in this study was an Oxisol collected in the Rondonópolis region, MT, in depths from 0 to 0.20 m. After collection, the soil was sifted a 4-mm mesh, and was subsequently characterized by chemical and grain size analysis (according to EMBRAPA's methodology (1997), as shown in Table 1). The soil was kept at 80% of field capacity moisture content, according to the methodology proposed by Bonfim-Silva et al. (2011) throughout the study.

Herbicide were sprayed with the assistance of knapsack sprayer at constant pressure (kept through CO_2 compressed) equipped with spray nozzle XR 11002 and with consumption corresponding to 200 L ha⁻¹. Herbicide persistence in the soil was evaluated by emergence speed index (ESI), visual soybean phytotoxicity (with a score ranging from 1 to 5, where 1 corresponds to no injury and 5 to plant death) (SBCPD, 1995), plant height (cm), and shoot - BDM (g) and root dry matter - BDM (g) at 26 days after sowing, at the end of the study. Statistical analysis was conducted in accordance with the polynomial regression model.

RESULTS AND DISCUSSION

In the Emergence Speed Index - ESI parameter, only the 1500 g e.a. ha⁻¹ dose (the highest dose in the experiment) was significant, linearly reducing emergence speed as herbicide and sowing application approached (Figure 1a).

Regarding periods in each dose applied, only the periods of 0, 3 and 5 days before sowing were significant. The period of 0 days before sowing caused higher



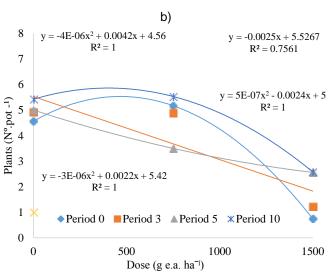
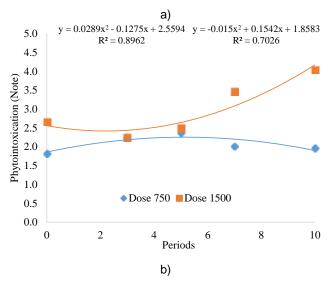


Figure 1. Emergence speed index of soybean seeds in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

emergence speed reductions. The intermediate dose (750 g e.a. ha⁻¹) provided the highest emergence speed, even at 0 days before sowing (Figure 1b). Schäfer et al. (1999) found that 2,4-D (2,4-Dichlorophenoxyacetic acid) exerted hormonal action when applied in small doses. According to Mortensen et al. (2012), 2,4-D acts as a herbicide that controls dicotyledonous weed species. However, 2,4-D also has hormonal action, acting as a synthetic auxin which can be used as plant growth regulator.

For the other periods (3 and 5 days before sowing), increasing doses caused linear emergence speed reduction, which was mitigated as spraying distanced sowing. A lower emergence speed index was observed when the highest 2,4-D dose was applied at sowing (0



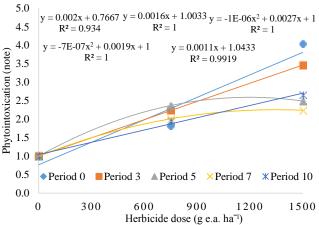


Figure 2. Visual phytointoxication of soybean plants in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

days before sowing), result also found by Silva et al. (2011) and Peres-Oliveira et al. (2016).

According to Procópio et al. (2008), herbicides that have residual activity in the soil are important inputs to ensure commercial crop yields, especially for crops with long weed interference periods. However, after that period is over, which often coincides with crop canopy closing, herbicide presence in the soil may become undesirable, and may result in carryover (Belo et al., 2007). The 2,4-D herbicide has short to intermediate persistence in soils. In normal doses, 2,4-D residual activity does not exceed four weeks in clay soils and hot weather (Silva et al., 2007a). In this parameter, it was observed that the herbicide did not exercise residual action in periods distant from soybean sowing.

Soybean phytotoxicity (Figure 2) was observed in all

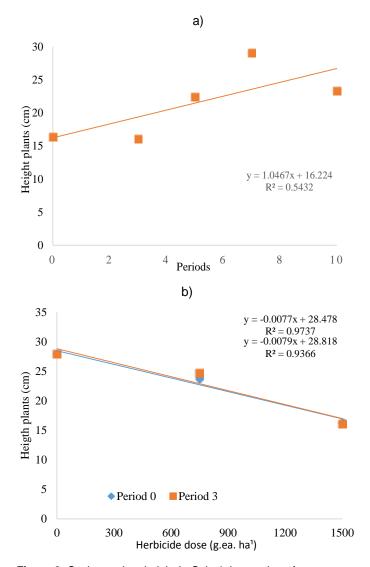


Figure 3. Soybean plant height in Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant a 0.1, 1 and 5% respectively.

application periods for both doses. The first symptoms, which occurred 10 days after sowing, were mild injuries, insufficient to cause yield reductions. Phytotoxicity visual symptoms observed were leaf shriveling, petiole epinasty and rib bleaching. One of the most common techniques to identify and quantify herbicides in the soil or water is to use bioassays through indicator plants with easy cultivation, fast development, and high sensitivity to the herbicide evaluated (Nyffeler et al., 1982; Souza et al., 1999). According to Thill (2003), intoxication symptoms produced in the leaves of various dicotyledonous plants by auxinic herbicides are easily characterized. Therefore, they are widely used to detect residues of these herbicides in the soil.

Studies with cotton (Constantin et al., 2007) and soybean (Silva et al., 2011) crops showed symptoms such as leaf shriveling and petiole epinasty. In both studies, 2,4-D herbicide intoxication caused bleaching of leaf blade ribs, symptom also found in this study. In general, the main 2,4-D effects in the plant are leaf shriveling, leaf bending to the underside, stem bending to the soil and rigidity (with cracks sometimes) or swelling in almost its entire length. In addition, barks are parted, branches and roots appear within these cracks, developing shoots stop growing, poorly-constituted organs appear, and plants lose their green color, yellowing or even dying (Saad, 1978).

Doses of 750 and 1500 g e.a.ha⁻¹ were significant. The 1500 g. e.a.ha⁻¹ dose provided higher crop damage and higher phytotoxicity scores. As spraying and sowing came closer, phytointoxication scores increased, causing from light injuries to plant death (Figure 2a).

Similar results were observed by Silva et al. (2011), when they used the 1005 g e.a. ha⁻¹ dose in soybean. Farinelli et al. (2005) used the same dose in a millet crop and did not find phytotoxicity effects. The 750 g. e.a.ha⁻¹ dose caused slight injuries, which were insufficient to affect plant growth and/or yield.

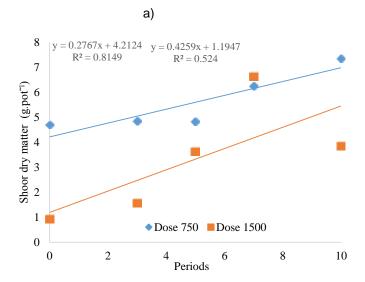
All periods (0, 3, 5, 7 and 10 days before sowing) were significant (Figure 2b), where periods of 0 and 3 days before sowing had the highest phytotoxicity scores. Symptoms such as severe injuries and reduced growth were observed, which may drastically reduce yield. The farther the spraying was conducted from sowing, lower was the plant damage.

When some herbicides reach the soil, their redistribution and degradation processes begin, which may be extremely short, as for some simple and non-persistent molecules, or may last for months or years, for highly persistent compounds (Filizola et al., 2002).

Auxinic or auxin mimic herbicides were the first selective organic herbicides for weed control. They are still extensively used in rice, corn, wheat, sugarcane, and pastures due to their characteristics (Thill, 2003). They are latifolicide products, and 2,4-D has short to intermediate soil persistence, which, according to Silva et al. (2007a), may cause intoxication in sensitive species, such as soybean, bean, cotton, and other dicotyledonous, when these are grown in areas where the herbicide was applied.

In the plant height variable (Figure 3), only the 1500 g e.a.ha⁻¹ dose was significant, causing plant height reduction. Plant height reduction was increased as spraying approached sowing date (Figure 3a). Pacheco et al. (2007) observed a reduction from 7 to 27% in millet (*Pennisetum americanum* L.) plant height using the 1005 g e. a. ha⁻¹ dose.

Regarding the outcome of doses over periods, only the periods of 0 and 3 days before sowing were significant. As the period between spraying and sowing decreased along dose application, plant height was gradually reduced. Silva



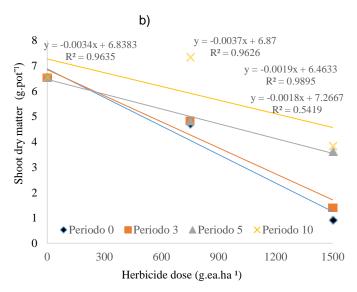


Figure 4. Soybean shoots dry matter in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant at 0.1, 1 and 5% respectively.

et al. (2011) observed lower height at 0 days before sowing, and herbicide dose increase caused significant soybean height reduction.

Santos et al. (2013) observed plant height decrease in plants that are sensitive to auxinic herbicides, and this variable was inversely proportional to 2,4-D dose increase in a short period between herbicide application and sowing. The 2,4-D herbicide has short soil persistence, allowing for sowing susceptible crops two weeks after application (Rodrigues and Almeida, 2011).

For shoot dry matter (Figure 4), only 750 and 1500 g e.a.ha⁻¹ doses were significant. Dry matter had better increases in more distant periods between spraying and

sowing, that is, both doses linearly reduced shoot dry matter as spraying approached sowing (Figure 4a). The 1500 g e.a.ha⁻¹ dose was the most severe and reduced shoot dry matter.

Regarding the assessment of days according to each dose applied (Figure 4b), except for period 7, all other periods were significant (0, 3, 5 and 10 days before sowing). The period of 0 days before sowing drastically reduced shoot plant development, leading to lower dry matter content, i.e., the short time period damaged dry matter production. Root dry matter reduction was due to the fact that herbicides such as 2,4-D induce intense cell proliferation in tissues, causing leaf and stem epinasty, besides phloem interruption, preventing photoassimilate movement from leaves to the root system (Silva et al., 2007b).

Decrease was evidenced as the dose increased, a tendency that continued in all other periods (0, 3, 5 and 10 days before sowing) and causing shoot dry matter reduction (Figure 4b). Increasing the dose may significantly reduce dry matter biomass during application periods of 3 and 14 days before sowing (Silva et al., 2011). Peres-Oliveira et al. (2016) studied a mixture of 2,4-D and glyphosate, and found the lowest shoot dry matter index at 0 days before sowing, in contrast with the evaluation at 14 days before sowing, which had the highest shoot dry matter increase. The farther the spraying was from sowing, higher was crop development.

In the root dry matter variable (Figure 5), only the 1500 g e.a.ha⁻¹ dose was significant, where it reduced dry matter as spraying approached sowing (Figure 5a). As spraying distanced from sowing, root yield increased.

Regarding the assessment of days according to each dose applied (Figure 5b), only the periods of 0 and 3 days before sowing were significant. Root dry matter was influenced by the distance between sowing and herbicide spraying in both applied doses. The period of 0 days before sowing drastically reduced root development, resulting in a lower dry matter content. As the dose increased, reduction became more evident. This behavior could also be observed in the period of 3 days before sowing.

Similar results were found by Silva et al. (2011), where dry matter was reduced in all treatments, with the lowest values observed at 0 days before sowing. In this study there was no statistical difference for 2250 and 3000 doses in relation to application periods.

Conclusion

Persistence of 2,4-D herbicide was short in an Oxisol for soybean; Periods of 0 and 3 days before sowing were the most harmful to soybeans, that is, the closer the spraying was from sowing, higher was the crop damage. The 1500 g e. a. ha⁻¹ dose had a higher residual effect in the soil for soybean.

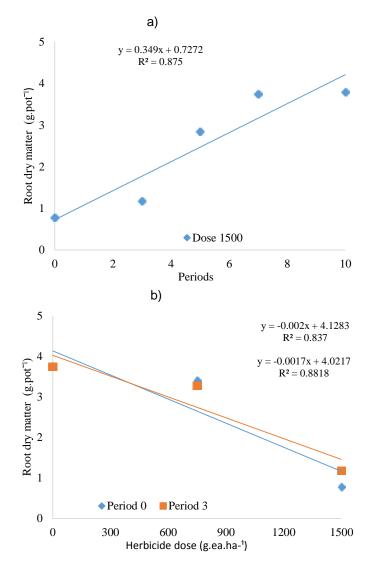


Figure 5. Soybean root dry matter in an Oxisol. Interaction of treatments in the analysis of doses over periods (A) and interaction of treatments in the analysis of periods between sowing and spraying as a function of increasing doses (B). ***, **, *: significant at 0.1, 1 and 5% respectively.

Conflicts of Interests

The authors have not declared any conflict of interests.

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