

INTRODUCTION

The grain yield of the common bean (*Phaseolus vulgaris*) crop is strongly reduced because of the weed interference (Vidal et al., 2010; Lamego et al., 2011; Kalsing and Vidal, 2012, 2013). The control of vegetation with herbicides is one of the main tactics of weed management (Lamego et al., 2011; Kalsing and Vidal, 2012; Cieslik et al., 2014). The plant of *Urochloa plantaginea* (*Brachiaria plantaginea*) is a grass weed with annual life cycle and reproduction by seeds. The plants of this species are among the most competitive with the common bean crop reducing the dry mass by 97% (Cury et al., 2011) and the grain yield by 96% (Kalsing and Vidal, 2013).

Fluazifop-p-butyl (fluazifop) is a herbicide with systemic activity in grass weeds and selectivity for *P. vulgaris* plants. This herbicide belongs to the chemical family aryloxy-phenoxy propionate, which is a strong inhibitor of the enzyme acetyl-coenzyme A carboxylase (ACCase). Inhibition of ACCase by fluazifop precludes the synthesis of malonyl-CoA, the committed step of fatty acid biosynthesis in plants, thus controlling the grass weeds (Cieslik et al., 2013).

Environmental conditions, such as air temperature and relative humidity, as well as irradiation, affect several steps on the herbicide-plant interaction, mainly interception, absorption and the translocation of the chemical within the weed (Cieslik et al., 2013, 2014). The environmental variables during the herbicide spray are affected by the time of the day. Therefore, the time of the day which the herbicide is sprayed may affect the herbicide efficacy (Cieslik et al., 2014). There is enough theoretical knowledge about the impact of the environment on the efficacy of ACCase inhibitors (Cieslik et al., 2013). However, most of available information was developed with experiments conducted in growth-chambers to study the impact of each factor isolated. There is limited information about how fluazifop performance is affected by environmental conditions actually measured under field situations. Experiments evaluating dose-response curves of herbicides sprayed in several times of the day provide a good opportunity to determine the interaction among environmental variables and their effect on herbicide efficacy (Cieslik et al., 2014). The objectives of this work were to identify the time of fluazifop application which gives best performance of *U. plantaginea* control in the common bean crop and to determine the environmental variables most important for the efficacy of this herbicide.

MATERIALS AND METHODS

One experiment was conducted under field conditions at the Agronomy Farm of the Federal Technological University of Paraná, which is located in Pato Branco, Brazil. The soil was an oxisol with a clay content of 60%. The experimental design was a randomized block with a factorial arrangement of the treatments, and four replicates. The first factor consisted of the time at which fomesafen was sprayed (2 a.m., 6 a.m., 11 a.m., 4 p.m. and 9 p.m.), and the second factor consisted of the five fluazifop doses (80, 110, 140, 170 e 200 g ha⁻¹), and with four additional untreated control plots.

The bean cultivar IPR-Tiziu was sowed on October 26, 2010, with rows spaced 45 cm apart and density of 288 thousand seeds ha⁻¹. The fertilizer used at the sowing time was the formula 08-20-20 N-P-K at 370 kg ha⁻¹. The herbicide was sprayed 24 days after crop emergence (DAE) when the common bean plants had three trifoliolates and the plants of the grass weed *U. plantaginea* had up to two tillers (six leaves). The prevailing environmental conditions during the herbicide spray are presented on Table 1. The herbicide was applied with a CO₂ backpack sprayer with nozzle 80.02 and a spray volume of 200 L ha⁻¹. A nonylphenoxy poly(ethyleneoxy) ethanol (Agral®) adjuvant was added to the herbicide spray solution at the concentration of 0.2%.

Leaf angles of *U. plantaginea* were determined in periods (time of the day) and environmental conditions similar to the ones during herbicide application at the field. The leaf angle was measure with a compass and a protractor in greenhouse grown plants to increase the precision of the measurements. The leaf angle was measured in three plants, on the last two expanded leaves on the main stem. Leaves completely on the horizontal were attributed the angle 90°, whereas completely vertical leaves had 0° leaf angle.

Table 1 - Environmental conditions and *Urochloa plantaginea* leaf angle at the time of fluazifop-p-butyl application

Time	Air Temperature (°C)	Air relative humidity (%)	PAR ⁽¹⁾ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Leaf angle (°)
2 a.m.	18.0	70	0	62
6 a.m.	22.7	60	670	68
11 a.m.	30.2	32	1853	49
4 p.m.	31.4	41	1305	46
9 p.m.	23.7	56	0	57

⁽¹⁾ Photosynthetically active radiation.

The effect of fluazifop on the grass weed plants of *U. plantaginea* was evaluated 20 days after the herbicide treatment (DAT) through the shoot dry mass and the visual assessment of weed control (in %). To measure the dry mass, the plant shoots were collected on two 0.25 m² sites randomly located inside each experimental unit and dried at 60 °C during three days. The dry shoot mass was transformed in values of dry mass reduction (%) in relation to the untreated controls.

An analysis of variance was carried out on the data. Because an interaction between the time of spraying and the herbicide dose was detected, for each application time, the Sigma-Plot (version 11) software was used to fit a rectangular hyperbole curve between herbicide dose and shoot dry mass, or sigmoid curve between herbicide dose and weed control. Comparisons among application times were performed by analyzing the parameters of the equations obtained from each curve and their respective values of standard error. From the shoot dry mass adjusted equations, it was calculated the herbicide dose that reduces the dry shoot mass by 80% (D_{80}). The 5% confidence interval was used to compare this variable among the different herbicide application times.

The environmental variables (air temperature, relative humidity and photosynthetically active radiation) that were measured at the time of the herbicide spray were correlated with one another and, also, with the *U. plantaginea* leaf angle. Additionally, each of these variables was correlated with the herbicide dose for 50% reduction of shoot dry mass (D_{50}) and a conceptual map with the correlation coefficients was created to identify the variables most important on fluazifop efficacy.

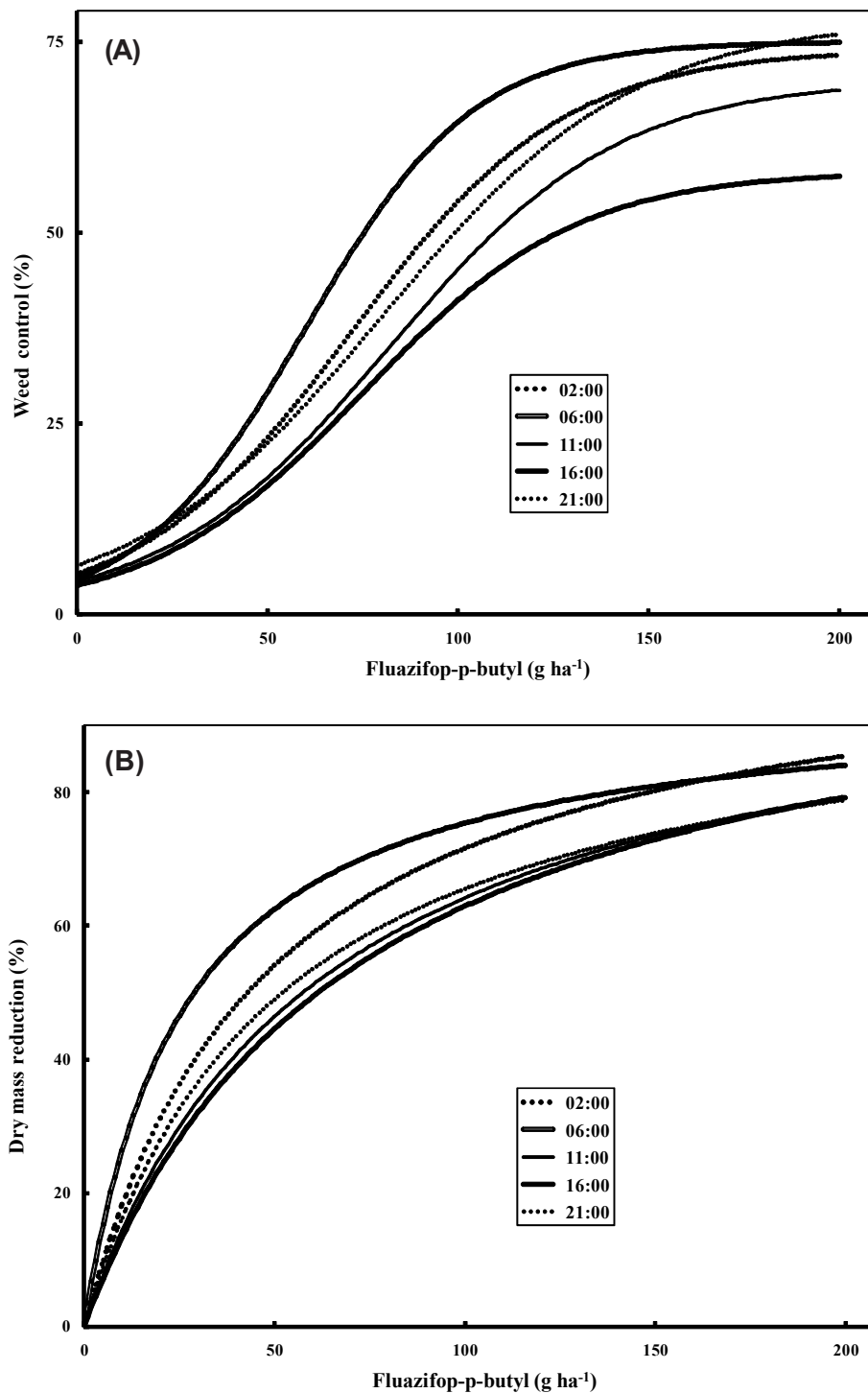
RESULTS AND DISCUSSION

The grass weed control also was dependent ($p < 0.01$) of the factors fluazifop dose and time of the day that the herbicide was sprayed. At each spray time, a sigmoid equation gave the best fit between fluazifop dose and the weed control. The best weed control was observed when the herbicide was sprayed at 6:00h, which contrasted with the results observed when fluazifop was sprayed at 6 p.m. (Figure 1A and Table 2).

The reduction of shoot dry mass of *U. plantaginea* was dependent ($p < 0.05$) of the factors fluazifop dose and time of the herbicide application. At each time of the day that the herbicide was sprayed, the impact of fluazifop rate on the weed shoot dry mass reduction followed the rectangular hyperbole equation (Figure 1A and Table 2). The maximum asymptote estimated by the equation did not differ from 100%, thus the comparisons among equations could be performed using the D_{50} (and its standard error). The variation of this parameter ranged between 26 (± 12) and 70 (± 22) g ha⁻¹. When fluazifop was applied at 6 p.m., the D_{50} was numerically the lowest of the experiment, indicating this was the best spraying time to optimize the efficacy of this herbicide. Contrarily, when fluazifop was sprayed at 4 p.m., the D_{50} was numerically the highest, indicating lowest herbicide performance when applied in the mid-afternoon (Table 2).

The value of D_{80} (determined on the dry mass reduction data) may be used to establish the herbicide dose for field conditions (Lamego et al., 2011). When fluazifop was sprayed at 2 a.m. and at 6 a.m., the values of D_{80} was 25% lower than the D_{80} values estimated for the herbicide application at 11 a.m. and 4 p.m. Indeed, for fluazifop application at 11 a.m. and 4 p.m., only the maximum fluazifop dose tested (200 g ha⁻¹) on the experiment was able to reach D_{80} (Figure 2).

The environmental variables measured in this experiment (Table 1) were consistent with typical field spring conditions during common bean crop in Southern Brazil. The lowest air temperature and highest air relative humidity were registered early in the day (2 a.m. and 6 a.m.). In contrast, the maximum air temperature and minimal relative humidity were recorded at 11 a.m. and 4 pm.



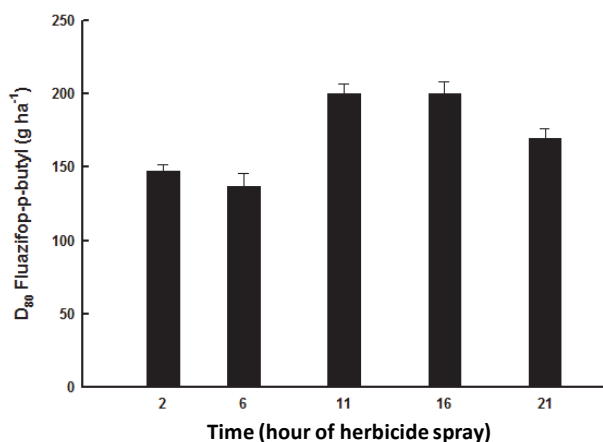
Equations on Table 2.

Figure 1 - Impact of fluazifop-p-butyl doses and five periods of herbicide spray on: a) weed control (%), and b) reduction (% in relation to untreated) of *Urochloa plantaginea* dry shoot mass; evaluated 20 days after treatment.

Table 2 - Parameters of the equations that describe the reduction of *Urochloa plantaginea* dry shoot mass as a function of fluazifop-p-butyl doses and the time of herbicide spray

Spray Time	Equation parameters for weed control ⁽¹⁾			R ²	P
	A (SE ⁽²⁾)	D ₅₀ (SE)	b (SE)		
2 a.m.	75 (5)	72 (10)	28 (10)	0.97	<0.01
6 a.m.	75 (4)	60 (11)	28 (10)	0.97	<0.01
11 a.m.	70 (5)	82 (08)	30 (09)	0.98	<0.01
4 p.m.	58 (4)	75 (08)	28 (09)	0.98	<0.01
9 p.m.	78 (6)	80 (10)	33 (10)	0.97	<0.01
	Equation parameters for shoot dry mass ⁽³⁾			R ²	P
	A (SE ⁽²⁾)	D ₅₀ (SE)			
2 a.m.	106 (05)	48 (09)		0.99	<0.01
6 a.m.	95 (07)	26 (12)		0.99	<0.01
11 a.m.	104 (08)	62 (16)		0.99	<0.01
4 p.m.	107 (11)	70 (22)		0.99	<0.01
9 p.m.	99 (06)	51 (10)		0.99	<0.01

⁽¹⁾ Sigmoid equation with three parameters: $y = A/(1+\exp(-(d-D_{A50})/b))$, where A = maximum asymptote; d = dose of fluazifop-p-butyl; D₅₀ = dose to decrease 50% the value of the asymptote, d = curve declivity at the inflection point. P is the probability of significance of the equation by the F test. ⁽²⁾ SE= standard error of the estimative of the parameter in parentheses. ⁽³⁾ Rectangular hyperbole with two parameters: $y = A*d/(D_{50}+d)$, where A, D₅₀, d and P = as above;



Bars represent the confidence interval of the value at $p < 0.05$.

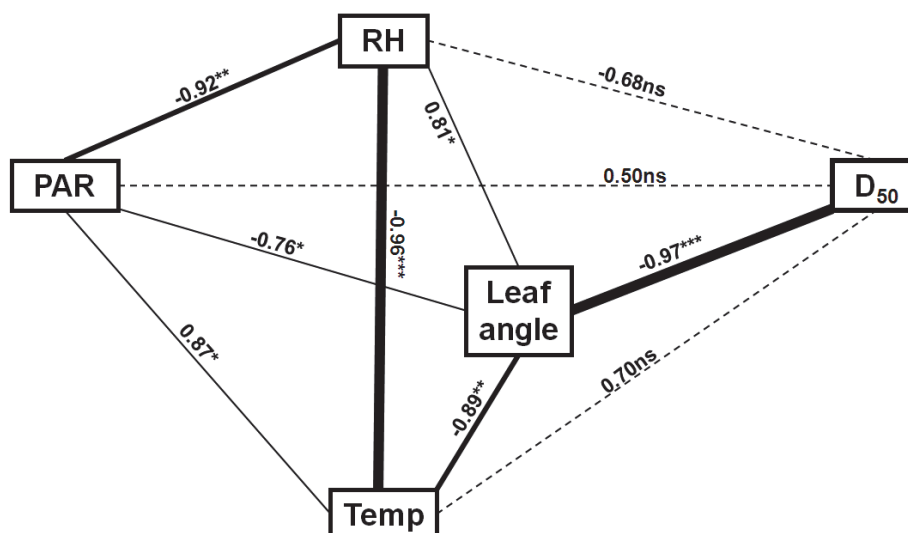
Figure 2 - Dose of fluazifop-p-butyl to reduce 80% the dry shoot mass (D₈₀) of *Urochloa plantaginea* in several times of application, when evaluated 20 days after treatment.

among these variables (Cieslik et al., 2014). Indeed, in this conceptual map, the paths with highest significance (thickest lines) may be the causal explanations for the performance of fluazifop in *U. plantaginea*. According to this map, the D₅₀ was directly affected by the leaf angle ($p < 0.01$), whereas the environmental variables affected the leaf angle. Actually, when compared to other times of fluazifop application, the lowest leaf angle measured on *U. plantaginea* plants during the warmest parts of the day (11 a.m. and 4 p.m.) (Table 1) may be consequence of slightly rolled leaves, which acquired a more vertical position (low leaf angle). The leaves of grass plants contain bulliform cells on their epidermis, which promote their rolling and protect them against dehydration because reduce the surface area exposed to the atmosphere (Alvarez et al., 2005).

The results of this paper support the hypothesis that fluazifop performance is superior when applied early morning (6 a.m.) in relation to the results obtained when sprayed at the warmest parts of the day (11 a.m. and 4 p.m.) (Table 2; Figures 1 and 2). At 6 a.m., mild air temperatures

Each of the environmental variables (air temperature, air relative humidity and PAR) was correlated with each of the other variables and also with the leaf angle (Figure 3). PAR was negatively correlated ($p < 0.05$) with relative humidity and positively correlated ($p < 0.10$) with air temperature. Relative humidity was negatively correlated ($p < 0.01$) with air temperature (Figure 3). The correlation coefficient between each of these three environmental variables with D₅₀ (determined on the dry mass reduction data) was not significant ($p > 0.10$). The leaf angle had higher correlation coefficient with air temperature ($p < 0.05$) than with PAR ($p < 0.10$) or with the relative humidity ($p < 0.10$). The correlation coefficient between the leaf angle and D₅₀ was highly significant ($p < 0.01$).

The conceptual map organizing the correlation coefficients among all the dependent variables determined in this work (Figure 3) may suggest a causal relationship



Dashed lines a lack of significance (ns, $p > 0.10$) and thicker lines represent correlation coefficient with a higher degree of significance by the t test as follows: * ($p < 0.10$); ** ($p < 0.05$) and *** ($p < 0.01$), with five pairs of data in each correlation. The dry shoot mass was determined at 20 days after herbicide spray and the other variables were measured at the time of the fluazifop-p-butyl spray.

Figure 3 - Conceptual map of the correlation coefficients among the environmental variables (photosynthetically active radiation (PAR), relative humidity of the air (RH) and air temperature (Temp)), the leaf angle and the fluazifop-p-butyl dose for 50% reduction of *Urochloa plantaginea* dry shoot mass (D_{50}).

associated to high relative humidity would be favorable to a more horizontally positioned leaf angle (Table 1). Other researchers (Sellers et al., 2003; Mohr et al., 2007) also found evidence about the relationship between horizontal leaf angle and increased herbicide efficacy. In fact, mild temperature and high relative humidity are related to elevated leaf cuticle hydration (Ramsey et al., 2005; Xu et al., 2010), high herbicide deposition on the leaf surface (Sellers et al., 2003), decreased droplet evaporation (Xu et al., 2010) and increased herbicide absorption and efficacy (Hatterman-Valenti et al., 2006).

Another hypothesis of this work was that night application (2 a.m.) of fluazifop would mitigate the detrimental impact of afternoon environmental variables on herbicide efficacy. This is supported by the D_{80} attained when the herbicide was sprayed at 2 a.m., which is lower than the one determined at 11 a.m. or 4 p.m. (Figure 2). Other researchers (De Villiers et al., 2001) also found evidence of high efficacy of tralkoxydim (an ACCase inhibitor) on evening applications of the herbicide.

Fluazifop-p-butyl efficacy on *Urochloa plantaginea* depends on the herbicide dose and on the hour of the day in which the product is sprayed. The best performance of fluazifop-p-butyl occurred at 6 a.m. and at 2 a.m. and the worst one occurred with applications during the warmest hours of the day (11 a.m. and 4 p.m.). Air temperature, relative humidity and photosynthetically active radiation affect the leaf angle, which is highly correlated to fluazifop-p-butyl efficacy on *Urochloa plantaginea* control.

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