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Glyphosate and Diuron Selectivity in Jatropha Plants When Applied with Directed Spray Technology

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Abstract: There is little information about the selectivity of herbicides in jatropha crops (*Jatropha curcas* L.) in Brazil. The objective was to evaluate the selectivity of different rates and mixtures of glyphosate and diuron in applications with directed spray on jatropha plants under green-house conditions. A randomized block design with five replicates was used. Treatments evaluated included: Glyphosate applied alone at 720 and 1440 g of active ingredient per hectare (g ai ha^{-1}), diuron applied alone at 1000 and 2000 g ai ha^{-1}, glyphosate+diuron (720+1000 g ai ha^{-1}), glyphosate+diuron (720+2000 g ai ha^{-1}), glyphosate+diuron (1440+1000 g ai ha^{-1}), glyphosate+diuron (1440+2000 g ai ha^{-1}) and an untreated control. The application was performed at 70 days after sowing, directed spray in the lower middle third of the plants. It was concluded that diuron applied alone at 1000 and 2000 g ai ha^{-1} the mixes with glyphosate (720+2000, 1440+1000 and 1440+2000 g ai ha^{-1}) impaired the development and photosynthetic activity of jatropha plants in applications with directed spray. Glyphosate at 720 and 1440 g ai ha^{-1} and the mix glyphosate+diuron 720+1000 g ai ha^{-1} showed potential good selectivity on jatropha plants in directed spray applications.

Key words: *Jatropha curcas* L., herbicides, biodiesel, weed

INTRODUCTION

The search for renewable energy sources has increased in recent decades, and among the alternative sources it is highlighted the use of vegetable oils as feedstock for biofuel production, like the oil extracted from jatropha seeds (*Jatropha curcas* L.).

Jatropha belongs to the family Euphorbiaceae. It is a perennial, rough plant and adapted to the various soil and climatic conditions. It is characterized by being a shrub that can reach up to 5 m in height, deciduous leaf, large and green petiole, smooth and cylindrical stem, trilocular capsule type fleshy fruit, having three seeds. By presenting quite prolonged reproductive cycle, the plant can reach 40 years, with average yield of 2.0 t ha^{-1} of seeds (Arruda *et al*., 2004).

With the possibility of using jatropha oil for biodiesel production, broad prospects are opened up for growth of planted areas; however, there is currently little information about the technical recommendations for management, especially those relating to the control of weeds.

Therefore, similar to other perennial crops, the young plants of jatropha may suffer negative interference of the weed community and have their growth and vegetative development compromised, as well as seed and oil production (Erasmo *et al*., 2009).

However, among the methods used weed management in perennial crops, the use of selective herbicides applied in isolation or combined, in pre-or post-emergence of weed plants, can be a great alternative, especially considering the management of these species in large planting areas.

Although, there being no herbicides registered for jatropha crop in Brazil, glyphosate has being used in areas of commercial cultivation in the State of Tocantins, with jatropha plants showing tolerance to simulated drift of glyphosate (Costa *et al*., 2009), highlighting the potential for use in management of weed plants in applications with direct spray technology.

The use of broad spectrum herbicides and distinct mechanisms of actions mixes can assist on weed control at areas with high species variability. Add to that, the use of only one herbicide can promote inefficient control and increase the selection pressure of resistant species to herbicide used (Christoffoleti and Lopez-Ovejero, 2003; Erasmo *et al*., 2009).

The use of glyphosate and diuron, individually or combined, is an excellent alternative for weed control, similar to what is used in the citrus crops, forest species and other perennial fruit species (Caetano *et al*., 2001; Santos *et al*., 2006; Yamashita *et al*., 2009; Gravena *et al*., 2009).

Moreira *et al*. (2010) found *Conyza bonariensis* and *C. canadensis* control (81.6%) in plants at ten leaves stage using the mix of glyphosate plus diuron at 1440+1500 g of active ingredient per hectare (g ai ha^{-1})

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in crop citrus area. However, an unsatisfactory control of 28.3% was obtained when glyphosate was used alone at 1440 g ai ha^{-1}.

Particularly for jatropha cultivation, there is little information regarding the phytotoxic effect of formulations based on glyphosate and diuron available in the market, as well the tolerance levels of crops to herbicides.

According to Galon *et al*. (2010), herbicides can influence directly or indirectly the photosynthetic activity by having different mechanisms of action. The damage due to phytotoxicity of plants can be more efficiently measured from the photosynthetic rate and variables associated with this. These data can assist in the development of herbicide selectivity studies on plants of economic interest.

The hypotheses of this study were based on the fact of mixes containing different mechanisms of action herbicides, such as glyphosate and diuron, can contribute to reduce the selection pressure of resistant biotypes and promote greater efficiency in the management of weeds.

As well, the selectivity for jatropha crop could be promoted by herbicide directed spray application technology only over de weeds. Thus, the present study aimed to evaluate the selectivity of different rates and mixes of glyphosate and diuron on jatropha plants (*J. curcas* L.) using direct spray application technology.

MATERIALS AND METHODS

Experimental design: The study was conducted in a nursery cloth (shade cloth with 50% brightness) at Center for Agricultural Sciences, State University of West Paraná, Marechal Cândido Rondon-PR.

The experimental design was a randomized block with five replications. The treatments consisted of different herbicides rates and mixes, as following: glyphosate applied alone at 720 and 1440 g ai ha^{-1}, diuron applied alone at 1000 and 2000 g ai ha^{-1}, glyphosate+diuron at 720+1000 g ai ha^{-1}, glyphosate+diuron at 720+2000 g ai ha^{-1}, glyphosate+diuron at 1440+1000 g ai ha^{-1}, glyphosate+diuron at 1440+2000 g ai ha^{-1} and a non-applied control.

The sowing of Jatropha (*J. curcas* L.) was performed on 05/01/2011 using five seeds in plastic pots with 17.81 dm^3 capacity. It was performed the thinning at 15 Days After Seeding (DAS), leaving only one plant per pot.

The soil had the following physical characteristics: 143 g kg^{-1} of clay, 35.95 g kg^{-1} of silt and 821.05 g kg^{-1} of total sand. The chemical analysis showed pH = 4.85 at $CaCl_2$; MO = 6.15 g dm^{-3}, P = 29.93 g dm^{-3}; K = 0.10 $cmol_c$ dm^{-3}, Ca = 0.95 $cmol_c$ dm^{-3}; Mg = 0.29 $cmol_c$ dm^{-3}; Al_3+H = 0.20 $cmol_c$ dm^{-3}; CTC = 4.48 $cmol_c$ dm^{-3} and V = 29.91%. The soil was adjusted to 60% of bases saturation remaining in plastic bags for an incubation period of approximately 15 days before sowing. The nitrogen fertilization was performed at 15 and 30 DAS, using urea at 11.6 g pot^{-1}.

Herbicides application: It was performed at 70 DAS through directed spray application technology in the lower middle third of plants. The plants were arranged linearly one-meter spaced and the application was performed by walking on both sides of the plants.

It was used a CO_2 pressured backpack sprayer equipped with nozzle Jacto F110/0-8/3 (110/LD/02) with pressure adjusted to 22 Lb-$inch^2$ and calibrated to provide a spray volume of 200 L ha^{-1}. The environmental conditions were suitable for the application, with temperature of 25°C, 70% of air relative humidity and wind speed varying between 5 and 6 km h^{-1}.

Evaluations: The plant height (cm) from the ground surface to apex was measured with the aid of ruler and the stem diameter (cm) using a digital caliper (0.01 mm precision) based on the height of 10 cm from the soil. The measurements were performed at 0, 7, 14, 21, 28 and 35 days after herbicide application (DAA).

Herbicide plant phytotoxicity visual evaluations were done at 7, 14, 21, 28 and 35 DAA according to the percentage scale notes, where 0 corresponded to no intoxication and 100% to death of plants. The visual evaluation was performed according to the growth inhibition, quantity and uniformity of injuries, leaf abscission and plant re-growth capacity.

It was also evaluated the dry mass (g) of leaves, stem, root and total plant mass at 35 DAA, where each collected material was packaged in paper bags and taken to the oven at 65°C until constant weight. The leaf area (cm^2) was measured with the equipment LI-3100C Portable Leaf Area Meter, being analyzed all leaves with more than 3 cm length in the main vein.

Gas exchanges was performed just to determine the net photosynthesis rate (A), stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci) and water efficiency use (WEU = A/E). The gas exchanges were obtained measuring the third leaf fully expanded from the plant apex and using the equipment IRGA LI-6400XT (liquor Inc. Lincoln, NE),. Measurements were performed on sunny days, between 8 and 10 h in the morning at 8, 16, 23 and 31 DAA. The flux of photosynthetically active photons during gas exchange measurements was 1800 ± 0.75 mol m^{-2} sec^{-1}.

The same leaf used in the evaluations of gas exchange was collected at 35 DAA to determine the levels (mg g^{-1}) of chlorophyll a, chlorophyll b and total chlorophyll, carotenoids and anthocyamins and chlorophyll a/chlorophyll b ratio, according to the methodology described by Sims and Gamon (2002).

Statistical analysis: The results were subjected to analysis of variance by the F test and means comparison was performed by the LSD test at 5% probability. The percentage of phytotoxicity data were transformed into arcsine v $\sqrt{x/100}$.

RESULTS AND DISCUSSION

Results of the visual phytotoxicity assessment in jatropha plants are presented at Table 1. All the treatments showed slight phytotoxicity symptoms at 7 days after application (DAA), with progressive increases of symptoms occurring until 28 DAA. It was found that diuron applied alone at 2000 g ai ha^{-1} and all mixes involving the herbicides glyphosate and diuron provided higher phytotoxicity at 28 DAA, with means ranging from 23.5-41.5%.

The phytotoxicity symptoms on jatropha decreased at 35 DAA, especially when the herbicides where applied alone. Considered phytotoxicity symptom reduction was also observed when glyphosate was applied in tank mix with diuron at 720+1000 g ai ha^{-1} (<4.4%), being statistically similar to herbicides and rates applied alone. However, slight reduction on phytotoxicity symptoms were observed at same evaluation date on all herbicide mixes and rates.

The symptoms observed after glyphosate and diuron directed spray application corresponded to yellowing of immature leaves in the stem apex and some leaves showed some reddish purple coloration. The most severe phytotoxicity symptoms provided by herbicides mixes consisted of leaf yellowing observed in the whole plant, followed by browning and necrosis, especially of immature leaves from the stem apex.

Some basal leaves suffered abscission after two or three days of the onset of severe symptoms. Costa *et al.* (2009) found similar symptoms after simulation of glyphosate drift on jatropha plants.

Table 2 shows no statistical differences for the variables plant height, leaf dry mass and root dry mass at 35 DAA. Diuron at 2000 g ai ha^{-1} and the mixes of

Table 1: Phytotoxicity in jatropha plants after herbicide application.

Treatments	Rate (g ai ha^{-1})	Days after application (DAA) (%) 7		14		21		28		35	
Control	-	0.00	$(0.00)^{b}$	0.00	$(0.00)^{d}$	0.00	$(0.00)^{e}$	0.00	$(0.00)^{c}$	0.00	$(0.00)^{b}$
Glyphosate	720	1.13	$(4.65)^{ab}$	1.13	$(3.87)^{cd}$	1.00	(2.58)de	1.50	$(3.18)^{c}$	1.40	$(4.21)^{b}$
Glyphosate	1440	1.33	$(5.87)^{ab}$	2.07	$(5.78)^{bcd}$	2.00	$(5.17)^{bcde}$	9.00	$(14.86)^{bc}$	1.60	$(4.58)^{b}$
Diuron	1000	1.27	$(5.49)^{ab}$	3.73	$(9.48)^{abc}$	1.67	$(2.99)^{bcd}$	9.50	$(13.65)^{bc}$	3.40	$(6.76)^{b}$
Diuron	2000	1.60	$(6.41)^{a}$	1.87	$(4.83)^{bcd}$	11.67	$(15.74)^{abc}$	32.00	$(32.76)^{a}$	27.00	$(29.71)^{a}$
Glyphosate+diuron	720+1000	3.27	$(10.31)^{a}$	4.60	$(10.87)^{abc}$	4.07	$(10.34)^{abcde}$	23.50	$(28.19)^{ab}$	4.40	$(10.48)^{b}$
Glyphosate+diuron	720+2000	3.20	$(10.13)^{a}$	6.33	$(12.76)^{ab}$	7.80	$(13.44)^{abcd}$	37.50	$(37.03)^{a}$	30.00	$(32.58)^{a}$
Glyphosate+diuron	1440+1000	3.20	$(7.68)^{a}$	8.33	$(15.01)^{a}$	11.67	$(17.42)^{ab}$	34.00	$(34.96)^{a}$	27.00	$(31.14)^{a}$
Glyphosate+diuron	1440+2000	2.93	$(7.17)^{a}$	4.20	$(10.64)^{abc}$	14.67	$(19.35)^{a}$	41.50	$(39.01)^{a}$	30.70	$(31.03)^{a}$
$F_{(Herbicides)}$		2.261*		2.937*		2.486*		6.786**		8.581**	
$F_{(Blocks)}$		0.943^{ns}		1.614^{ns}		0.363^{ns}		0.379^{ns}		0.122^{ns}	
d.m.s		5.925		8.093		13.141		16.5541		13.709	
CV(%)		71.720	`	75.4		105.49		56.79		63.64	

Percentage of original data were transformed into arcsine v $\sqrt{x/100}$ and they are highlighted in parentheses. Means followed by the same letter in the column do not differ significantly by the LSD test at 5% probability. **Significant at 1%, *Significant at 5%, ns: Not significant by the F test

Table 2: Height (H), diameter (D), dry mass of leaf+petiole (DMLP), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM) and leaf area (La) of jatropha plants at 35 days after herbicide application

Treatments	Rate (g ai ha^{-1})	H (cm)	D (cm)	DMLP (g)	SDM (g)	RDM (g)	TDM (g)	La (cm^2)
Control	-	51.2	2.87^{a}	31.85	59.30^{ab}	29.99	118.04^{a}	3605.53^{a}
Glyphosate	720	53.8	2.84^{a}	28.08	62.66^{a}	32.46	130.46^{a}	3617.57^{a}
Glyphosate	1440	52.1	2.72^{abc}	24.57	52.92^{ab}	24.33	104.91^{b}	2357.40^{abc}
Diuron	1000	56.3	2.69^{abc}	24.75	48.70^{bc}	25.78	99.28^{b}	3054.80^{ab}
Diuron	2000	56.7	2.49^{bc}	16.28	37.34^{d}	21.82	78.26^{c}	2392.56^{abc}
Glyphosate+diuron	720+1000	49.4	2.79^{ab}	15.89	57.57^{ab}	24.55	100.50^{b}	1686.86^{c}
Glyphosate+diuron	720+2000	53.0	2.48^{c}	20.01	38.48^{cd}	22.59	76.59^{c}	1368.41^{c}
Glyphosate+diuron	1440+1000	53.8	2.65^{abc}	22.60	36.15^{d}	22.69	77.37^{c}	1704.48^{c}
Glyphosate+diuron	1440+2000	55.8	2.41^{c}	20.63	38.08^{cd}	22.14	82.00^{c}	1894.61^{bc}
$F_{(Herbicides)}$		0.76^{ns}	2.24*	2.20^{ns}	7.29*	1.99^{ns}	5.71**	3.59**
$F_{(Blocks)}$		0.40^{ns}	2.11^{ns}	0.99^{ns}	0.44^{ns}	0.46^{ns}	0.96^{ns}	0.951^{ns}
d.m.s		8.10	0.32	10.16	11.32	7.60	8.12	1280.76
CV(%)		11.73	9.28	34.67	18.34	23.46	18.35	41.27

Means followed by the same letter in the column do not differ significantly by the LSD test (p<0.05) **Significant at 1%, *Significant at 5%, ns: Not significant by the F test, 1: Percentage of diameter stem reduction

Table 3: Chlorophyll a, chlorophyll b, total chlorophyll, a/b Chlorophyll ratio, carotenoids and anthocyanins contents of jatropha plants after 35 days of herbicides application

Treatments	Rate (g ai ha^{-1})	Chlorophyll a	Chlorophyll b	Total chlorophyll	a/b Chlorophyll ratio	Carotenoids	Anthocyanins
		($\mu g\ g^{-1}$)					
Testemunha	-	33.34	48.61	3.36	1.02	39.91	166.280
Glyphosate	720	27.66	45.40	3.15	0.99	32.01	116.800
Glyphosate	1440	21.61	39.10	2.66	0.91	28.45	91.820
Diuron	1000	29.03	45.58	3.32	0.99	36.54	161.000
Diuron	2000	21.97	26.86	2.13	1.13	26.00	102.990
Glyphosate+diuron	720+1000	31.79	46.00	3.35	1.03	34.64	150.470
Glyphosate+diuron	720+2000	27.76	40.29	3.05	1.00	32.61	142.280
Glyphosate+diuron	1440+1000	29.67	39.70	2.97	1.06	36.79	155.180
Glyphosate+diuron	1440+2000	38.86	53.56	3.31	1.10	33.08	134.410
$F_{(Herbicides)}$		1.371^{ns}	0.981^{ns}	1.36^{ns}	1.80^{ns}	0.748^{ns}	0.766^{ns}
$F_{(Blocks)}$		1.127^{ns}	1.031^{ns}	0.83^{ns}	0.68^{ns}	0.020^{ns}	0.101^{ns}
d.m.s		13.23	21.82	1.01	0.05	14.29	86.850
CV(%)		35.33	39.80	25.78	10.79	33.26	49.680

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). ns: Not significant by the F-test

glyphosate+diuron at 720+2000 g ai ha^{-1} and at 1440+2000 g ai ha^{-1} provided the lowers stem diameters of 2.49, 2.48 and 2.41 cm, respectively, being statistically inferior when compared with the untreated control. Likewise, these treatments also significantly reduced the stem dry weight, as well as the mix of glyphosate+diuron at 1440+1000 g ai ha^{-1}, where the values obtained were up to 38.5 g.

Only glyphosate applied alone at 720 g ai ha^{-1} did not reduce the total plant dry mass compared with control. However, applications of glyphosate+diuron significantly reduced the leaf area of jatropha plants, independently of the rate used in the mix.

These results indicate that the directed spray application in the third lower middle of jatropha plants can result in herbicide absorption by the stem and basal leaves and consequently provide moderate and severe injuries in plants (Table 1).

Peixoto *et al.* (2010) found that jatropha plants had impaired growth when applied increasing rates of diuron at pre-emergence, but this effect was more intense when the rate was increased from 600-2000 g ai ha^{-1}.

The chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, carotenoids and anthocyanins values observed in all herbicide treatments were statiscally equal to the values obtained on untreated plants at 35 DAA (Table 3). These results indicate that the phytotoxicity caused by herbicides did not affect the synthesis and maintenance of the contents of photosynthetic pigments in the jatropha leaves. However, the action of these pigments in the photosynthetic process was hindered mainly by the diuron as observed in the results of gas exchange evaluations.

Regarding the net photosynthesis (A), it was found that diuron applied alone or in tank mix with glyphosate reduced the plant's A when compared to control, except for the mixture of glyphosate+diuron at 720+1000 g ai ha^{-1} from 16-31 DAA (Table 4). Glyphosate applied alone did not damage the plant's A in all periods. This indicates that despite causing mild symptoms of phytotoxicity, glyphosate did not affect the ability of plants to absorb atmospheric CO_2 and produce biomass, corroborating the results in Table 2.

Table 4: Net photosynthesis (a)in jatropha plants after herbicides application

Treatments	Rate (g ai ha^{-1})	μmol (CO_2 m^{-2} sec^{-1}) (DAA) 8	16	23	31
Control	-	7.58	11.28^{b}	15.01^{a}	9.21^{b}
Glyphosate	720	8.32	13.84^{ab}	15.01^{a}	10.75^{b}
Glyphosate	1440	5.97	16.14^{a}	14.57^{a}	14.89^{a}
Diuron	1000	9.35	7.32^{c}	6.30^{c}	2.64^{c}
Diuron	2000	3.66	0.25^{d}	-0.78^{d}	-1.18^{d}
Glyphosate+diuron	720+1000	11.21	16.52^{a}	11.20^{b}	12.28^{ab}
Glyphosate+diuron	720+2000	6.57	0.64^{d}	-0.30^{d}	-0.57^{cd}
Glyphosate+diuron	1440+1000	6.84	1.73^{d}	0.15^{d}	-0.14^{cd}
Glyphosate+diuron	1440+2000	7.39	2.73^{d}	1.30^{d}	-0.08^{cd}
$F_{(Herbicides)}$		1.46^{ns}	24.77**	51.23**	28.11**
$F_{(Blocks)}$		1.43^{ns}	2.65^{ns}	1.8^{ns}	0.64^{ns}
d.m.s		5.06	3.91	2.84	3.47
CV(%)		7.43	7.82	3.73	50.82

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). **Significant at 1%; ns: Not significant by the F-test. Days After Application (DAA)

However, Zobiole *et al.* (2010) found significantly impaired on photosynthetic rate and chlorophyll content in soybean glyphosate resistant plants submitted to increasing herbicide rates application.

Glyphosate has action in the shikimic acid pathway by inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate Synthase (EPSPS), which catalyzes the synthesis of aromatic amino acids (tyrosine, phenylalanine and tryptophan), while stopping the synthesis of these products can cause plant death (Peterson *et al.*, 1996; Velini *et al.*, 2009; Priestman *et al.*, 2005). According to the review by Kruse *et al.* (2000) about 35% of plants dry mass and 20% of carbon fixed by photosynthesis are products derived from the shikimic acid pathway.

It is noteworthy that some researchers have found that partial inhibition of EPSPs promoted stimulatory effect on the growth of Eucalyptus plants after sub-rates application of glyphosate (Santos *et al.*, 2006; Cedergreen, 2008; Velini *et al.*, 2008). Likewise, Costa *et al.* (2009) mentioned that jatropha plants showed good tolerance to simulated glyphosate drift applied up to 22.5 g ai ha^{-1}. Probably, jatropha plants have the ability to metabolize glyphosate at low concentrations or immobilize it in the vacuole. Thus, the directed spraying can contribute to increasing the tolerance and consequently this herbicide selectivity to the jatropha plants.

The herbicide diuron inhibits the photosystem II (PS II) electrons transport and bind to the D1 protein of PS II reaction center, thereby blocking the transfer of electrons to plastoquinone. This blockage prevents the conversion of absorbed light energy into electrochemical energy and results in the production of singlet oxygen and triplet chlorophyll which induce lipid peroxidation of the membrane, destroy the membrane integrity, leading to cellular disorganization (Fuerst and Norman, 1991; Breitenbach *et al.*, 2001; Hiraki *et al.*, 2003).

Thus, the increasing rate of diuron individually and mixed with glyphosate increased the level of intoxication of jatropha plants and reduced leaf area useful for realization of the photosynthetic process, leading to lower and later negative values of net photosynthesis (a) until the 31 DAA as showed at Table 4.

This indicates that plants may require more time to recover from injuries caused by diuron in directed spray application early in the development of jatropha plants. However, there was a possible synergistic effect for the tank mix of glyphosate+diuron applied at 720+1000 g ai ha^{-1}, suggesting a possible protective action of glyphosate on the oxidative stress caused by diuron when it was used at the lowest rate of both herbicides, probably due to the inhibition of herbicides translocation in the plant (Steele *et al.*, 2008).

This mixture can be an excellent alternative for weed management in jatropha crops by presenting distinct mechanisms of action and thus avoiding the selection of resistant biotypes to herbicides, besides exploring the residual effect of diuron on soil's seed bank.

The results of stomatal conductance (gs), transpiration (E), internal CO_2 concentration (Ci) and water efficiency use (WEU) of jatropha plants described in Table 5-8 were similar to the data obtained for A (Table 4), mainly because these photosynthetic parameters are correlated. The stomatal conductance (gs) provided by glyphosate+diuron at 720+1000 g ia ha^{-1} was statistically superior when compared to untreated control at 16 and 31 DAA. However, the other glyphosate+diuron tank mixes clearly diminish along the evaluation period, being statistically inferior to the values obtained on untreated jatropha plants (Table 5). The worst stomatal conductance was observed at 21 DAA, where diuron at 2000 g ai ha^{-1} applied alone and the tank mixes glyphosate+diuron applied at 720+2000, 1440+1000 and 1440+2000 g ai ha^{-1} with values up to 0.02 mol m^{-2} sec^{-1} and statistically inferior to the others treatements (Table 5).

High plant transpiration also was observed on glyphosate+diuron applied at 720+1000 g ia ha^{-1}, where the values between 2.66 and 3.95 mol H_2O m^{-2} sec^{-1}. Again, the lower values were provided by glyphosate+diuron applied at 720+2000, 1440+1000 and 1440+2000 g ai ha^{-1} (Table 6).

The internal CO_2 concentration practically were the same on evaluation done at 8 DAA, where the values varying between 130.86 and 253.07 µmol CO_2 mol ar^{-1} with no statistical differences. However, The internal CO_2

Table 5: Stomatal conductance (gs) in jatropha plants after herbicide application

Treatments	Rate (g ai ha^{-1})	mol (m^{-2} sec^{-1}) (DAA) 8	16	23	31
Control	-	0.10	0.11^{cde}	0.13^{a}	0.09^{b}
Glyphosate	720	0.11	0.13^{c}	0.12^{a}	0.10^{b}
Glyphosate	1440	0.05	0.21^{b}	0.13^{a}	0.23^{a}
Diuron	1000	0.10	0.11^{cd}	0.08^{b}	0.05^{b}
Diuron	2000	0.05	0.05^{de}	0.02^{c}	0.03^{b}
Glyphosate+diuron	720+1000	0.12	0.28^{a}	0.11^{ab}	0.19^{a}
Glyphosate+diuron	720+2000	0.10	0.06^{de}	0.01^{c}	0.04^{b}
Glyphosate+diuron	1440+1000	0.10	0.07^{cde}	0.02^{c}	0.06^{b}
Glyphosate+diuron	1440+2000	0.08	0.05e	0.02^{c}	0.03^{b}
$F_{(Herbicides)}$		0.65^{ns}	11.56**	19.30**	6.48**
$F_{(Blocks)}$		3.25*	2.97*	5.39*	0.18^{ns}
d.m.s		0.09	0.07	0.03	0.08
CV(%)		74.20	43.92	38.13	71.77

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). **Significant at 1%; *Significant at 5%; ns: Not significant by the F-test. Days After Application (Daa)

Table 6: Transpiration (E) in jatropha plants after herbicide application

Treatments	Rate (g ai ha^{-1})	mol (H_2O m^{-2} sec^{-1}) (DAA) 8	16	23	31
Control		3.17	1.91^{cde}	3.03^{a}	1.72^{bc}
Glyphosate	720	3.21	2.31^{bc}	2.95^{a}	2.02^{c}
Glyphosate	1440	2.02	3.10^{b}	3.24^{a}	3.50^{a}
Diuron	1000	3.30	2.15^{cd}	2.08^{b}	1.16^{bcd}
Diuron	2000	2.04	1.14^{e}	0.51^{c}	0.58^{d}
Glyphosate+diuron	720+1000	3.94	3.95^{a}	2.66^{ab}	3.18^{a}
Glyphosate+diuron	720+2000	3.33	1.18^{e}	0.35^{c}	0.92^{cd}
Glyphosate+diuron	1440+1000	3.38	1.44^{de}	0.76^{c}	1.29^{bcd}
Glyphosate+diuron	1440+2000	2.74	1.11^{e}	0.64^{c}	0.55^{d}
$F_{(Herbicides)}$		0.716^{ns}	11.22**	19.75**	9.34**
$F_{(Blocks)}$		2.758*	4.06**	6.96**	0.47^{ns}
d.m.s		2.16	0.84	0.79	1.00
CV(%)		55.74	32.11	34.08	47.23

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). **Significant at 1%; *Significant at 5%; ns: Not significant by the F-test. Days After Application (DAA)

Table 7: Internal CO_2 concentration (Ci) in jatropha plants after herbicide application

	Rate	µmol (CO_2 mol ar^{-1}) (DAA)			
Treatments	(g ai ha^{-1})	8	16	23	31
Control		160.07	163.04^{e}	148.620^{d}	168.54^{d}
Glyphosate	720	166.93	167.50^{e}	122.310^{d}	166.05^{d}
Glyphosate	1440	130.86	180.54^{de}	157.140^{d}	230.25^{cd}
Diuron	1000	157.21	243.22^{cd}	213.000^{cd}	275.28^{c}
Giuron	2000	253.07	375.19^{a}	571.060^{a}	477.48^{a}
Glyphosate+diuron	720+1000	174.69	245.20^{cd}	156.020^{d}	230.03^{cd}
Glyphosate+diuron	720+2000	212.12	356.51^{a}	514.410^{ab}	409.13^{ab}
Glyphosate+diuron	1440+1000	237.95	339.72^{ab}	364.37b^{c}	391.33^{c}
Glyphosate+diuron	1440+2000	161.15	288.07^{bc}	229.26^{cd}	380.73^{c}
$F_{(Herbicides)}$		1.29^{ns}	12.42**	7.77**	20.24**
$F_{(Blocks)}$		1.52^{ns}	0.40^{ns}	1.73^{ns}	0.64 ^{ns}
D.m.s		103.8	67.44	173.82	72.92
CV(%)		43.84	19.97	49.04	18.67

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). **Significant at 1%; ns: Not significant by the F-test. Days After Application (DAA)

Table 8: Water efficiency use (WEU) in jatropha plants after herbicide application

	Rate	mol (CO_2 mol H_2O^{-1}) (DAA)			
Treatments	(g ai ha^{-1})	8	16	23	31
Control		2.92	6.05^{ab}	4.97^{a}	5.43^{a}
Glyphosate	720	2.89	6.16^{a}	5.52^{a}	5.48^{a}
Glyphosate	1440	2.84	5.50^{abc}	4.66^{a}	4.28^{a}
Diuron	1000	3.05	3.77^{cd}	3.28^{ab}	2.52^{b}
Diuron	2000	1.49	-0.06^{f}	-2.74^{c}	-2.59^{d}
Glyphosate+diuron	720+1000	2.86	4.34^{bc}	4.77^{a}	4.09^{ab}
Glyphosate+diuron	720+2000	2.20	0.60^{ef}	-2.85^{c}	-0.67^{c}
Glyphosate+diuron	1440+1000	1.81	1.10^{ef}	0.08^{bc}	-0.36^{c}
Glyphosate+diuron	1440+2000	2.99	2.31^{de}	2.16^{ab}	-0.12^{c}
$F_{(Herbicides)}$		1.16^{ns}	15.16**	7.46**	26.51**
$F_{(Blocks)}$		0.06^{ns}	1.22^{ns}	1.53^{ns}	1.38^{ns}
D.m.s		1.55	1.75	3.48	1.68
C.V. (%)		46.75	41.10	122.44	64.96

Means followed by the same letter in the column do not differ significantly by the LSD test ($p<0.05$). **Significant at 1%; ns: Not significant by the F-test. Days After Application (DAA)

concentration in jatropha plants were statistically superior on treatments where diuron was applied alone at 1000 (275.28) 2000 g ai ha^{-1} (477.48 µmol CO_2 mol ar^{-1}) and he mixes of glyphosate plus diuron at 720+2000 (409.13 µmol CO_2 mol ar^{-1}), 1440+1000 (391.33 µmol CO_2 mol ar^{-1}) and 1440+2000 g ai ha^{-1} (380.73 µmol CO_2 mol ar^{-1}), when compared to the untreated plants on final evaluation done at 35 DAA (Table 7).

The performance of stomatal conductance and transpiration directly impacted on the water efficiency use (WEU), were values of WEU provided by glyphosate applied alone at 720 or 1440 g ai ha^{-1} were similar to values obtained on untreated plants among all evaluation period. The worst WEU performance was provided by diuron applied alone at 2000 g ai ha^{-1} and the tank mixes of glyphosate+diuron at 1440+1000, 720+2000 and 1440+2000 g ai ha^{-1} (Table 8).

Diuron applied alone at 1000 g ai ha^{-1} showed intermediated WEU of 2.52 mol CO_2 mol H_2O^{-1} and its performance was clearly improved to 4.09 mol CO_2 mol H_2O^{-1} when the same rate of diuron was mixed with glyphosate at 720 g ai ha^{-1} (Table 8).

According to Galon *et al.* (2010), photosynthetic parameters g_s, E and WEU are efficient to identify herbicide damage to crops, especially those with effect on the photosynthetic rate, which indirectly influences the parameters associated with water use. Diuron promoted negative effects on the net photosynthetic rate due to damage caused in leaf tissue after peroxide substances production. These peroxide substances also promote an induced abscisic acid (ABA) production and stomatal closuring, both responsible to a progressively stomatal conductance and CO_2 internal contents alterations (Zhang *et al*., 2001).

The reduction in leaf transpiration can also be a result of oxidative stress promoted by the diuron action. Consequently, lower transpiration can be attributed to lower stomatal conductance due to stomatal closure, being more pronounced at 23 DAA (Table 5, 6).

Overall, the treatment with diuron showed higher Ci and lower WEU, indicating less plant efficiency to convert the atmospheric CO_2 into photoassimilates (Table 7, 8), as well as water use to produce biomass, confirming data in Table 2 and 4.

Galon *et al.* (2009) also observed an increased Ci and reduced A in sugarcane genotypes provided by ametryn (inhibitor of PS II) at 2000 g ai ha^{-1} 85 days after planting. Machado *et al.* (2010) found that glyphosate applied in eucalyptus plants on rates up to 86.4 g ai ha^{-1} promoted an increased dry mass production per unit of water transpired. However, rates above 86.4 g ai ha^{-1} reduced the WEU on eucalyptus plants. According to the authors, the water efficiency use reduction may be correlated to leaves necrosis and plant photosynthesis reduction, both responsible to biomass accumulation reduction.

CONCLUSION

Thus, it can be concluded that the diuron (1000 and 2000 g ai ha^{-1}) or in tank mixes with glyphosate (720+2000 g ai ha^{-1}, 1440+1000 and 1440+2000 g ai ha^{-1}) impaired the development and photosynthetic activity of jatropha plants when used as a directed spraying technology. Glyphosate applied alone at 720 and 1440 g ai ha^{-1} or in tank mix with diuron at 720+1000 g ai ha^{-1} showed potential selectivity for jatropha plants.

However, it is emphasized the importance of further studies to verify the actual selectivity of herbicides considering the effects on the production and seed oil content of jatropha plants. Also, it is necessary to improve the technology of herbicides application to prevent the damage on the initial development of plants. Since it is a perennial crop, it must still be evaluated the consequences of consecutive applications for a long period in the same area and the possible beneficial effects that glyphosate and other herbicides can promote in the growth and development of jatropha plants.

REFERENCES

Arruda, F.P., N.E.M. Beltrao, A.P. Andrade, W.E. Pereira and L.S. Severino, 2004. Physic nut (*Jatropha curca* L.) cultivation as an alternative crop for the northeast semi-arid of Brazil. Revista Brasileira de Oleaginosas e Fibrosas, 8: 789-799.

Breitenbach, J., C. Zhu and G. Sandman, 2001. Bleaching herbicide norflurazon inhibits phytoene desaturase by competition with the cofactors. J. Agric. Food Chem., 49: 5270-5272.

Caetano, R.S.X., P.J. Christoffoleti and R.V. Filho, 2001. Weed seed bank of a pera citrus orchard. Sci. Agricola, 58: 509-517.

Cedergreen, N., 2008. Herbicides can stimulate plant growth. Weed Res., 48: 429-438.

Christoffoleti, P.J. and R. Lopez-Ovejero, 2003. Main aspects on weed herbicide resistance to glyphosate. Planta Daninha, 21: 507-515.

Costa, N.V., E.A.L. Erasmo, P.A. Queiroz, D.F. Dornelas and B.F. Dornelas, 2009. Effect of simulated glyphosate drift on the initial growth of physic nut plants. Planta Daninha, 27: 105-110.

Erasmo, E.A.L., N.V. Costa, M.A. Terra and R.R. Fidelis, 2009. Initial tolerance of physic nut plants to pre and post-emergence herbicide application. Planta Daninha, 27: 571-580.

Fuerst, E.P. and M.A. Norman, 1991. Interactions of herbicides with photosynthetic electron transport. Weed Sci., 39: 458-464.

Galon, L., F.A. Ferreira, A.A. Silva, G. Concenco and E.A. Ferreira *et al*., 2009. Influence of herbicides on the photosynthetic activity of sugarcane genotypes. Planta Daninha, 27: 591-597.

Galon, L., G. Concenco, E.A. Ferreira, I. Aspiazu and A.F. Silva *et al*., 2010. Water use efficiency in sugarcane genotypes submitted to herbicide application. Planta Daninha, 28: 777-784.

Gravena, R., R.V. Filho, P.L. Alves, P. Mazzafera and A.R. Gravena, 2009. Low glyphosate rates do not affect *Citrus limonia* (L.) Osbeck seedlings. Pest Manage. Sci., 65: 420-425.

Hiraki, M., J.J.S Van Rensen, W.J. Vredenberg and K. Wakabayashi, 2003. Characterization of the alterations of the chlorophyll a fluorescence induction curve after addition of Photosystem II inhibiting herbicides Photosynth Res., 78: 35-46.

Kruse, N.D., M.T. Michelangelo and R.A. Vidal, 2000. Herbicides EPSPs inhibitors: Literature review. Revista Brasileira de Herbicida, 1: 139-146.

Machado, A.F.L., L.R. Ferreira, L.D.T. Santos, F.A. Ferreira, R.G.Viana, M.S. Machado and F.C.L. Freitas, 2010. Photosynthetic efficiency and water use in eucalyptus plants sprayed with glyphosate. Planta Daninha, 28: 319-327.

Moreira, M.S., M.S.C. Melo, S.J.P. Carvalho, M. Nicolai and P.J. Crhistoffoleti, 2010. Alternative herbicides to control glyphosate-resistant biotypes of *Conyza bonariensis* and *C. canadensis*. Planta Daninha, 28: 167-175.

Peixoto, M.F.S.P., A.C. dos Santos, A.N. Santiago, C.P. Peixoto and J.C.C. Moraes, 2010. Action of diuron on the growth of jatropha (*Jatropha curcas* L.) under greenhouse. Proceedings of the IV Brazilian Congress of Castor and I International Symposium on Energy Oilseeds, June 8-11, 2010, Joao Pessoa, Brazil, pp: 1146-1150.

Peterson, M.L., S.D. Corey, J.L. Font, M.C. Walker and J.A. Sikorski, 1996. New simplified inhibitors of EPSP synthase: The importance of ring size for recognition at the shikimate 3-phosphate site. Bioorg. Med. Chem. Lett., 6: 2853-2858.

Priestman, M.A., M.L. Healy, T. Funke, A. Becker and E. Schonbrunn, 2005. Molecular basis for the glyphosate-insensitivity of the reaction of 5-enolpyruvylshikimate 3-phosphate synthase with shikimate. FEBS Lett., 579: 5773-5780.

Santos, L.D.T., F.A. Ferreira, L.R. Ferreira, W.M. Duarte, R.A.S. Tiburcio and M.V. Santos, 2006. Intoxication of eucalypt species under glyphosate drift. Planta Daninha, 24: 359-364.

Sims, D.A. and J.A. Gamon, 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structure and developmental stages. Remote Sens. Environ., 81: 337-354.

Steele, G.L., S.A. Senseman, A.S. Sciumbato and J.M. Chandler, 2008. Diuron reduces absorption and translocation of glyphosate in sharppod morningglory (*Ipomoea cordatotriloba*). Weed Technol., 22: 414-419.

Velini, E.D., E. Alves, M.G. Godoy, D.K. Meschede, R.T. Souza and S.O. Duke, 2008. Glyphosate applied at low doses can stimulate plant growth. Pest Manage. Sci., 64: 489-496.

Velini, E.D., S.O. Duke, M.L.B. Trindade, D.K. Meschede and C.A. Carbonari, 2009. Mode of Action of Glyphosate. In: Glyphosate, Velini, E.D., D.K. Meschede, C.A. Carbonari and M.L.B. Trindade (Eds.). FEPAF, Botucatu, pp: 113-134.

Yamashita, O.M., J.R. Betoni, S.C. Guimaraes and M.M. Espinosa, 2009. Influence of glyphosate and 2,4-D in initial development of forest species. Sci. Forestalis, 37: 359-366.

Zhang, X., L. Zhang, F. Dong, J. Gao, D.W. Galbraith and C.P. Song, 2001. Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol., 126: 1438-1448.

Zobiole, L.H.S., R.S. de Oliveira Jr., R.J. Kremer, J. Constantin, C.M. Bonato and A.S. Muniz, 2010. Water use efficiency and photosynthesis of glyphosate-resistant soybean as affected by glyphosate. Pestic. Biochem. Physiol., 97: 182-193.