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Glyphosate application associated to non-purified residue of biodiesel production: analysis of different prototypes[1]

Aplicação de glyphosate associado a resíduo não-purificado da produção de biodiesel: análise de diferentes protótipos

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Abstract - Glyphosate is an agricultural pesticide of great importance in the world. In this sense, several studies have been developed in order to select adjuvants which elevate the efficacy of the product. Two experiments were performed with the goal to evaluate the efficacy of the glyphosate herbicide when associated to different experimental prototypes of agricultural adjuvants, elaborated from non-purified residue ofbiodiesel production. Gross residue from the biodiesel production was used, as well as its dilution in water, and addition of ammonium sulfate, KOH and/or soybean oil. Control of morning glory (*Ipomoea triloba* L.) was evaluated in experimental plant nursery, as well as the infestation of weeds in the field condition. In general, the presence of adjuvant prototypes in glyphosate herbicide spray did not result in an increase of the control in any experimental condition, as well as, it did not reduce the efficiency of the product.
Keywords: glycerin; burndown; adjuvants; herbicides; efficacy

Resumo - O herbicida glyphosate é um defensivo agrícola de grande importância mundial. Neste sentido, diversos trabalhos têm sido desenvolvidos no intuito de selecionar adjuvantes que elevem a eficácia do produto. Dois experimentos foram realizados com o objetivo de avaliar a eficácia do herbicida glyphosate quando associado a diferentes protótipos experimentais de adjuvantes agrícolas, elaborados a partir de resíduos não-purificados da produção de biodiesel. Foi utilizado resíduo bruto da produção de biodiesel, bem como sua diluição em água, adição de sulfato de amônio, KOH e/ou óleo de soja. Avaliou-se o controle da corda-de-viola (*Ipomoea triloba* L.) em viveiro experimental, bem como a infestação de plantas daninhas em condição de campo. Em geral, a presença de protótipos de adjuvante na calda do herbicida glyphosate não resultou em incrementos de controle em nenhuma condição experimental, porém, também não prejudicou a eficácia do produto.
Palavras-chaves: glicerina; dessecação; adjuvantes; herbicidas; eficácia

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Introduction

Presently, glyphosate (N-(phosphonomethyl) glycine) might be highlighted as one of the most used and available molecules in agriculture. It is a non-selective herbicide with post-emergence application, of systemic action, which is used in the annual control of weeds, perennials and in pre-plantingdesiccation (Timossi et al., 2006; Rodrigues and Almeida, 2011). This product has great importance in the system of production, and currently, it may be considered the herbicide with the greatest world importance (Moreira and Christoffoleti, 2008).

Glyphosate inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), enzyme that participates in the route of synthesis of phenylanine, tyrosine, and tryptophan aromatic amino acids (Cole, 1985). The symptoms resulting from glyphosate application appears from 4 to 20 days after its application, varying according to the species and phenological stage. Initially, leaves become chlorotic, with posterior necrosis and death of the plants (Rodrigues and Almeida, 2011).

Leaf absorption is one of the most critical points, and some formulations need up to six hours for adequate absorption (Rodrigues and Almeida, 2011). Glyphosate foliar absorption is a biphasic process, which involves fast initial penetration by the cuticle, followed by slow symplastic translocation, depending on factors such as the plant's age, environment, adjuvants and concentration of the herbicide in the spray solution (Caseley and Coupland, 1985; Monquero et al., 2004).

In the attempt to elevate glyphosate efficacy, many substances have been added to spray solution. Adjuvants act in different manners, affecting the wetting, adherence, penetration, spreading, foam formation and/or spray dispersion over the target (Mendonça et al., 2007).

Biodiesel is a renewable fuel, originated from non-fossil sources, and its production has been encouraged in the last years, however, a great amount of residue is generated, consisting of glycerin, soaps, catalyzer and water (Suarez et al., 2009). The commercial feasibility of biodiesel implicates in the consumption of the extra volume of glycerin generated, seeking large-scale applications and aggregating value to the production chain (Mota et al., 2009). It is known that approximately 10 m^3 of glycerin are generated at every 90 m^3 of biodiesel is produced by the transesterification process of the vegetal oils (Mota and Pestana, 2011).

One alternative that may be considered for the use of glycerin is its application as agricultural adjuvant, associated to defensives, with potential attention for the glyphosate (Carvalho et al., 2015). Hence, it is considered the hypothesis that the efficacy of the glyphosate may be elevated when applied with residue from the biodiesel production.

The present work was developed with the goal to assess the efficacy of the glyphosate herbicide in the control of weeds, when associated to different experimental prototypes of agricultural adjuvants, elaborated from non-purified residues of biodiesel production.

Material and Methods

Two experiments, one in an experimental plant nursery and the other in the field, were developed at the Federal Institute of Education, Science and Technology of South Minas Gerais - IFSULDEMINAS, Machado Campus (21º 40' S; 45º 55' W; 850 m of altitude). Biodiesel residue used in the experiments was collect at the Varginha Biodiesel Plant, in the State of Minas Gerais, administered by Abdiesel. For the biodiesel production, this plant uses frying residual oil in reaction with methanol and NaOH as catalyzer (approximately 0.5%). The reaction is processed hot, between 45°C and 50°C. The methanol is recovered by the end of the process, and the crude glycerin remains as a residue (Table 1). Five adjuvants prototypes were produced from such crude residue, which were later used in the experiments (Table 2).

Table 1. Physical and chemical characteristics of crude glycerin* used in the experiments, collected in Varginha's Biodiesel Plant - Abdiesel.

Component	Analysis*	Component	Analysis*
P_2O_5/AC (%)	0.03	Iron (mg kg^{-1})	33.25
Total nitrogen (%)	0.11	Manganese (mg kg^{-1})	1.92
pH	8.71	Zinc (mg kg^{-1})	1.60
Humidity (%)	0.72	Cadmium (mg kg^{-1})	0.02
Organic carbon (%)	0.22	Chromium (mg kg^{-1})	0.51
Calcium (g kg^{-1})	0.46	Lead (mg kg^{-1})	0.10
Magnesium (g kg^{-1})	0.10	Molybdenum (mg kg^{-1})	0.14
Potassium (g kg^{-1})	1.64	Nickel (mg kg^{-1})	0.33
Phosphorus (g kg^{-1})	0.04	Density (g mL^{-1})	1.01
Sodium (g kg^{-1})	10.70	C/N Relation	214.35
Sulfur (g kg^{-1})	0.02	Organic Matter (%)	41.03
Boron (mg kg^{-1})	1.78	Methyl ester (%)	35.5
Copper (mg kg^{-1})	0.78	Sodium adsorption ratio (SAR)	37.21

*Averages of nine subsamples.

Table 2. Adjuvant prototypes used in the experiments developed in plant nursery and in the field. Machado, 2013.

Prototype	Description
P1	Gross original residue (GOW)*, collected in the plant
P2	GOW, dilution 50% v/v in water
P3	GOW (100 mL) + 5 g of ammonium sulfate
P4	GOW (50 mL) + Water (50 mL) + 5g of ammonium sulfate
P5	GOW (50 mL) + Water (25 mL) + soy oil (25 mL) + 0.5 g of KOH

*Gross original residue collected in the ABDiesel Plant in Varginha, in the State of Minas Gerais, arising from the catalytic reaction between dry residual oil, methanol and NaOH.

In the experimental plant nursery, morning glory (*Ipomoea triloba* L.) was used as bioindicator, due to its control difficulty with glyphosate herbicide (Carvalho et al., 2011). The experiment was carried out from March to April, 2013. Plots consisted of plastic vases with 1,000 mL capacity, filled with commercial substrate, duly fertilized. The average density of four plants per vase was maintained, daily irrigated, without the occurrence of water deficiency. Applications were performed over plants with phenological stage of six definitive leaves. Due to the influence of the irrigation line, the experimental design of randomized blocks was adopted, with eight treatments (Table 3) and eight repetitions, totalizing 64 plots.

In the field, one experiment was installed in a fallow area, whose average density of weeds was estimated in 100 weeds m^{-2}, in general phenological stage of flowering. The total population of weeds present in the area was considered for the assessments, which was composed of: hairy beggarticks (*Bidens* spp. - 25%), guinea grass (*Panicum maximum* - 25%), signal grass (*Brachiaria decumbens* - 30%) and other species (20%). The experiment was carried out from October to November 2013. Each plot had 2.5 m of width and 5.0 m of length, with total area of 12.5 m^2 and useful area in the central 6 m^2. Six treatments were applied (Table 4) with experimental design of randomized blocks and six repetitions, totalizing 36 plots.

In both experiments, deionized water was used for the spray preparation. In the experimental plant nursery, pulverization was performed on March 25, 2013, beginning at 2:16 pm and finishing at 2:36 pm. The air average temperature was 29ºC, relative humidity of 66%, and winds of 1 m s^{-1}. In the field, the pulverization was performed on October 9, 2013, beginning at 3 pm and finishing at 3:18 pm. The average air temperature was 31.1°C, relative humidity of 49.9%, and winds of 0.4 m s^{-1}. A CO_2-pressurized backpack sprayer, with flat fan nozzles XR 110.02, duly calibrated for spray volume of 200 L ha^{-1} was used for both applications. A single nozzle was used for the application in the plant nursery. In the field, an application bar with four nozzles was used, spaced in 0.5 m and useful width estimated in 2 m.

Table 3. Treatments used in the experiment developed in the experimental plant nursery, including adjuvant prototypes obtained from non-purified residue of biodiesel production, applied over morning glory. Machado - MG, 2013.

Treatments	Herbicide		Prototype	
	Active	Dose[1]	Code	Concentration
1	Check plots without application	Absent	Absent	Absent
2	Glyphosate[2]	720 g ha^{-1}	Absent	Absent
3	Glyphosate	720 g ha^{-1}	P1	1.0% v/v
4	Glyphosate	720 g ha^{-1}	P2	1.0% v/v
5	Glyphosate	720 g ha^{-1}	P3	1.0% v/v
6	Glyphosate	720 g ha^{-1}	P4	1.0% v/v
7	Glyphosate	720g ha^{-1}	P5	1.0% v/v
8	Glyphosate	1440 g ha^{-1}	Absent	Absent

[1]Dose in grams of glyphosate acid equivalent by hectare; [2]Round up Original®, 360 g L^{-1}.

Table 4. Treatments used in the experiment developed in the field, including adjuvant prototypes obtained from non-purified residue of biodiesel production. Machado - MG, 2013.

Treatments	Herbicide		Prototype	
	Active	Dose[1]	Code	Concentration
1	Check plots without application	Absent	Absent	Absent
2	Glyphosate[2]	180 g ha^{-1}	Absent	Absent
3	Glyphosate	180 g ha^{-1}	P2	1,0% v/v
4	Glyphosate	180 g ha^{-1}	P4	1,0% v/v
5	Glyphosate	180g ha^{-1}	P5	1,0% v/v
6	Glyphosate	360 g ha^{-1}	Absent	Absent

[1]Dose in grams of glyphosate acid equivalent by hectare; [2]Round up Original®, 360 g L^{-1}.

Percentage control assessments were evaluated within 7, 14, 21 and 28 days after application (DAA), using the visual damage percentage scale, in which zero represented the absence of symptoms and 100% represented the death of the plants. For the experiment developed in the plant nursery, the mass of the plants' dry matter was measured. The vegetal material remaining in the vases was collected and dried in a forced air circulation oven, regulated at 70°C, for 72 hours, for posterior weighing. All data obtained was submitted to the application of the F test on the variance analysis, followed by Scott Knott test (Scott and Knott, 1974), both with 5% of significance.

Results and Discussion

In the experiment developed in the experimental plant nursery, significance for the effect of treatments in the control of morning glory was detected in all of the assessment dates; however, differences among the treatments with glyphosate were only observed at the 7^{th} and the 28^{th} DAA (Table 5). In the first assessment, performed within 7 DAA, treatments 5 and 6 promoted superior control than the isolated application of glyphosate, in the same dose (Treatment 2), equaling to the biggest dose of the product (Treatment 8). The differential of treatments 5 and 6 was the addition of ammonium sulfate to the prototypes (P3 and P4; Table 2).

It is believed that the use of water with high concentration of salts reduces glyphosate efficacy, due to the formation of salt of low foliar absorption. Ammonium sulfate presented in the prototypes promotes the formation of ammonium-glyphosate salts, which have higher absorption easiness (Nalewaja et al., 1992; Thelen et al., 1995) and may improve the efficacy in the control of weeds. In addition, ammonium sulfate may alter the drop morphology, delaying or preventing the glyphosate crystallization in the foliar surface (MacIsaac et al., 1991). Cellular absorption of weak acid herbicides may be facilitated by the acidification of the foliar apoplast (Gronwald et al., 1993; Ruiter and Meinen, 1996; Young et al., 2003), so that the addition of the ammonium sulfate may promote this apoplastic acidification, increasing the efficacy on plant control.

Table 5. Control (%) and dry matter mass of the morning glory (*Ipomoea triloba*) after the application of the glyphosate herbicide with adjuvant prototypes produced from non-purified residue of biodiesel production, arising from the Varginha in Minas Gerais State. Machado, 2013.

Treatments	Variables[1]				
	Control (%)				Dry mass
	7 DAA	14 DAA	21 DAA	28 DAA	(g/plot)
1	0.0 c	0.0 b	0.0 b	0.0 c	7.07 b
2	33.8 b	96.5 a	99.8 a	100.0 a	0.46 a
3	31.3 b	97.1 a	99.5 a	100.0 a	0.69 a
4	31.9 b	97.8 a	99.9 a	100.0 a	0.38 a
5	38.1 a	97.9 a	99.5 a	99.8 b	0.64 a
6	38.8 a	97.6 a	99.3 a	99.5 b	0.46 a
7	35.6 b	98.9 a	99.9 a	100.0 a	0.66 a
8	43.7 a	99.4 a	100.0 a	100.0 a	0.48 a
$F_{(treatment)}$	28.13*	1516.37*	28535*	84122.7*	60.33*
CV (%)	22.62	2.94	0.68	0.39	62.15

* Significant in F test with 1%; [1] Means followed by equal letters in the column do not differ among themselves according to the Scott-Knott test with 5% probability.

Although there are explanations for efficacy improvements observed within 7 DAA, such improvement was not identified at 28 DAA. In said date, treatments with the addition of ammonium sulfate had reduced control in relation to the other treatments, without practical expression however, since all treatments resulted in control superior to 99%. In addition, no differences between herbicide treatments were identified for the dry matter mass of the morning glory (Table 5).

The use of glycerin as vehicle for the application of herbicides over *Acanthococos emensis* was assessed. The initial manifestation of symptoms after the application of triclopyr and triclopyr + fluroxypyr was slower in treatments with glycerin, maintaining statistically different and inferior up to 30 DAA for the triclopyr herbicide and up to 90 DAA for triclopyr + fluroxypyr. The control level obtained by all treatments was equal within 250 DAA (Pereira et al., 2012).

In a similar work, Carvalho et al. (2014a,b) added non-purified residues from biodiesel production as adjuvant to the glyphosate spray. However, in these works, it was used residues obtained in controlled reaction, in laboratory, using NaOH or KOH as catalyzer. In addition, in these cases, adjuvant prototypes did not increase glyphosate efficacy on the control of signal grass (*Brachiaria decumbens*), common sandbur (*Cenchrus echinatus*), morning glory (*I. triloba*) and sourgrass (*Digitaris insularis*).

On the other hand, by comparing the results of several adjuvants added to the glyphosate spray, Balah et al. (2006) reported that the presence of glycerin reduced shoot fresh and dry matter of purple nutsedge (*Cyperus rotundus*), dry matter mass of roots and SPAD units, surpassing the efficiency of the pure product and with addition of several others adjuvants.

In the second experiment, performed in the field, no weed satisfactory control was obtained, that is, control superior than 80% (Frans et al., 1986), for all treatments, due to the reduced doses that were used. The addition of adjuvant prototypes did not interfere on glyphosate efficiency, whose treatments were equal to the isolated applied product. The highest dose of the herbicide, applied in isolation, had control superior to the other treatments, without reaching, however, the agronomic efficacy levels (Table 6).

Table 6. Control (%) of weeds in the field after the application of the glyphosate herbicide with adjuvant prototypes produced from non-purified residue of biodiesel production, arising from the Varginha in Minas Gerais State. Machado, 2013.

Treatments	Control (%)			
	7 DAA	14 DAA	21 DAA	28 DAA
1	0.0 c	0.0 c	0.0 c	0.0 c
2	24.5 b	36.3 b	28.3 b	22.3 b
3	27.5 b	39.8 b	31.7 b	25.8 b
4	24.7 b	33.7 b	27.5 b	22.8 b
5	22.3 b	34.2 b	26.8 b	24.5 b
6	39.8 a	59.2 a	54.2 a	55.0 a
$F_{(treatment)}$	31.79*	35.22*	42.01*	83.18*
CV (%)	24.3	23.31	23.18	18.78

* Significant in F test with 1%; [1] Means followed by equal letters in the column do not differ among themselves according to the Scott-Knott test with 5% probability.

In this point, it is clear that the correct choice of the glyphosate dose was more important than the presence of adjuvants in the spray solution. It was detected the need for a higher dose of glyphosate for the adequate control of the species, with special attention to the difficulty in controlling signal grass (*Brachiaria decumbens*), which may demand doses of 1440 g ha^{-1} (Rodrigues and Almeida, 2011).

Conclusions

The presence of adjuvant prototypes in the glyphosate herbicide spray did not result in control increments, however, said presence did not reduced the efficacy of the product.

The application of reduced doses of glyphosate associated to the addition of adjuvant prototypes, in the field, did not increase the efficacy on the weed control.

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