

Glyphosate application associated to different concentrations of non-purified residue of biodiesel production¹

Pulverização de glyphosate associado a diferentes concentrações de resíduo não-purificado da produção de biodiesel

Saul Jorge Pinto de Carvalho²; Edisom Carlos Ribeiro Machado³; Rodrigo Sanini de Oliveira Lima³; Alan Pratezi Penha Silva³; Bruno da Silva Marques³; Marcelo Ferri Gonçalves³

Abstract - Searching for renewable energy sources has motivated the development of biodiesel production units in Brazil. However, for biodiesel being considered fully sustainable, suitable destinations for its production waste should be found, especially for crude glycerin. Therefore, this work was developed to evaluate glyphosate herbicide efficacy on weed control when combined to different concentrations of non-purified residue of biodiesel production. Two experiments were carried out, one in an experimental plant nursery, using sourgrass (*Digitaria insularis*) as bioindicator, and another one in field condition, assessing the natural weed community. Adding biodiesel production waste to the spray mix solution has not increased glyphosate efficacy in controlling sourgrass. Furthermore, there was a reduction in glyphosate effectiveness after addition of waste to the spray mix solution at doses equal to or higher than 0.6% v/v. No results were found justifying the adoption of biodiesel production residue as an agricultural adjuvant. Positive points observed were minimal, with no consistency between species or application times, and glyphosate remains as the best application and recommendation option.

Keywords: glycerin; sustainability; adjuvants; herbicides; biofuels

Resumo - A busca por fontes energéticas renováveis tem estimulado a implantação de unidades de produção de biodiesel no Brasil. Porém, para que o biodiesel seja considerado plenamente sustentável, deve-se encontrar destinos adequados para os resíduos de sua produção, com destaque para a glicerina bruta. Assim sendo, este trabalho foi desenvolvido com o objetivo de avaliar a eficácia do herbicida glyphosate no controle de plantas daninhas quando associado a diferentes concentrações de resíduo não purificados da produção de biodiesel. Dois experimentos foram realizados, um em viveiro experimental, utilizando o capim-amargoso (*Digitaria insularis*) como bioindicador; e outro em campo, avaliando a comunidade natural de plantas daninhas. A adição de resíduo da produção de biodiesel à calda de pulverização não elevou a eficácia do herbicida glyphosate no controle do capim-amargoso. Ainda, foi observada redução na eficácia do glyphosate após adição de resíduo à calda de pulverização em doses iguais ou superiores a 0,6% v/v. Não foram encontrados resultados que justifiquem a adoção de resíduo da produção de biodiesel como adjuvante agrícola. Os pontos positivos observados foram mínimos, sem consistência entre

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² Professor Doutor, IFSULDEMINAS - Campus Machado, Rod. Machado-Paraguaçu, km. 3, Machado, MG, sjpcarvalho@yahoo.com.br.

³ Engenheiro Agrônomo, graduado pelo IFSULDEMINAS - Campus Machado.

espécies ou momentos de aplicação, permanecendo o glyphosate puro como a melhor opção de aplicação e recomendação.

Palavras-chaves: glicerina; sustentabilidade; adjuvantes; herbicidas; biocombustíveis

Introduction

The depletion of traditional energy sources such as petroleum and coal has driven innovative research for fuels derived from renewable sources of energy. As an example, there is biodiesel, a non-fossil, renewable and non-toxic fuel that may totally or partially replace common diesel obtained from petroleum in diesel cycle engines (Gama et al. 2010).

Biodiesel is derived from the transesterification reaction between a triglyceride (vegetable oil or animal fat) and an alcohol. Most of the biodiesel produced today in the world is derived from soybean oil using methanol and an alkaline catalyst. In Brazil, there is the possibility of using anhydrous ethanol, which is advantageous, as this is produced in large volumes to be mixed with gasoline, as well as being a product obtained from biomass and, thus, the process becomes totally independent from petroleum, promoting the production of fuel whose origin is completely agricultural (Ferrari et al. 2005).

During the production of biodiesel, it is necessary to use a chemical catalyst, where sodium hydroxide has been the most used and can be replaced by potassium hydroxide (Rinaldi et al. 2007). Then, after the catalyst transesterification reaction, there is the presence of biodiesel and by-products in the tank. The main separation method of biodiesel phases is washing, by the addition of water to the system, followed by decantation. Two phases are clearly formed, one consisting in biodiesel, and another one, the glyceridic phase, consisting in glycerin, alcohol waste and a catalyst (NaOH or KOH). Additionally, soap is also formed due to the interaction of the catalyst with the fatty acids (Suarez et al. 2009).

Glycerin is the main byproduct of this reaction and must be purified before sale to increase the economic efficiency of the process

(Silva and Freitas 2008). It is a viscous, colorless, odorless, and hygroscopic liquid, with a sweet taste. According to Mota and Pestana (2011), glycerin chemical characteristic is of high polarity, but with nonpolar components, which are wastes of fatty acids derived from the transesterification process.

From every 90 m³ of biodiesel produced by the transesterification process of vegetable oils, approximately 10 m³ of glycerin are generated (Mota and Pestana 2011). This scenario indicates that biodiesel commercial viability undergoes through the consumption of this extra volume of glycerin, seeking large-scale applications and adding value to the supply chain (Mota et al. 2009).

An alternative that may be considered for using glycerin is its application as an agricultural adjuvant associated to pesticides. Accordingly, among the molecules available and commonly used in agriculture, glyphosate (N- (phosphonomethyl) glycine) may be mentioned. It is an herbicide with application in postemergence (foliar), non-selective, of systemic action, used to control annual and perennial weeds and in the elimination of cover crops (Timossi et al. 2006, Rodrigues and Almeida 2011). This product has great importance in production systems, so that today it can be considered the most important herbicide worldwide (Moreira and Christoffoleti 2008).

Of all the glyphosate characteristics, absorption is one of the most critical points, since some formulations require up to six hours for adequate absorption (Rodrigues and Almeida 2011). The foliar absorption of glyphosate is a two-phase process, which involves rapid initial penetration by the cuticle, followed by slow symplastic absorption, dependent on factors such as the age of the plant, environment, adjuvants and concentration of the

herbicide in the spray mix (Caseley and Coupland 1985, Monquero et al. 2004).

Therefore, the hypothesis that glyphosate efficacy can be high when applied with biodiesel production residue is considered, highlighting the glycerin characteristics, presence of ions in the waste (sodium or potassium) and emulsifiers (soap). Therefore, this work was developed to evaluate glyphosate herbicide efficacy on weed control when combined to different concentrations of non-purified residue of biodiesel production.

Material and Methods

Two experiments were developed at the Federal Institute of Education, Science and Technology of the South of Minas Gerais (IFSULDEMINAS), Machado Campus, Machado - MG (21° 40' S; 45° 55' W; 850 m of altitude). One trial was developed in experimental nursery and other on field condition. Biodiesel residue was collected in the Varginha - MG Biodiesel Plant, managed by Abdiesel.

For biodiesel production, this plant adopts residual frying oil in reaction with methanol and NaOH as a catalyst (approximately 0.5%). The reaction is hot processed, between 45 and 50 °C. The methanol is recovered at the end of the process, leaving crude glycerin as the residue (Table 1).

In an experimental plant nursery, sourgrass (*Digitaria insularis*) was used as a bioindicator once this species is hard to control with glyphosate herbicide (Timossi et al. 2006). The experiment was carried out between October and December 2013. The plots consisted in plastic pots with a capacity of 1 dm³ filled with commercial substrate, properly fertilized. The average density of 10 plants per pot was kept, daily irrigated and with no incidence of water stress. Treatments were the result of a factorial arrangement between two phenological stages of sourgrass (three leaves and tillering) and eight herbicide treatments (Table 2). An experimental design of randomized blocks with six replications was adopted, totaling 96 plots.

Table 1. Physical and chemical characteristics of crude glycerin used in the experiments, collected in the Abdiesel Biodiesel Plant, Varginha - MG, 2013

Component	Analysis*	Component	Analysis*
P ₂ O 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
Moisture (%)	0.72	Cadmium (mg kg ⁻¹)	0.02
Organic carbon (%)	0.22	Chrome (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 ratio ((C:N) or carbon-to-nitrogen ratio)	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

*Means of nine subsamples.

In the field, an experiment was installed in a fallow area whose weed density was estimated at 150 plants per square meter in a general phenological stage of flowering. For the evaluations, the total weed population present in

the area was considered, consisting in: signalgrass (*Brachiaria decumbens* – 65%), beggarticks (among several other names) (*Bidens* spp. – 20%), perennial soybean (*Glycine wightii* – 5%), guinea grass or green

panic grass (*Panicum maximum* – 5%), other species (5%). The experiment was carried out between November and December 2013. Each plot was 2.5 m wide and 5.0 m long, with a total area of 12.5 m² and floor area in the central 6 m². Six treatments were applied (Table 3), with an experimental design of randomized blocks and six replications, totaling 36 plots.

In both experiments, deionized water was used for the preparation of the spray mixes. In the plant nursery, spraying was performed on 10/31/2013, beginning at 09:12 a.m. and finishing at 09:28 a.m. The average air temperature was 22.3 °C, relative humidity of

70.7%, no wind and overcast sky. In the field, spraying was performed on 11/13/2013, beginning at 2:31 p.m. and finishing at 2:57 p.m. The average air temperature was 33.5 °C, relative humidity of 52.5%, 0.9 m/s wind and clear sky. For both applications a CO₂-pressurized knapsack sprayer was used, with XR 110.02-type fan nozzle tips, properly calibrated for a spray mix volume of 200 L ha⁻¹. In a plant nursery a single nozzle tip was used for application. In the field, an application boom was used, with four nozzle tips spaced 0.5 m, 2.0 m wide.

Table 2. Treatments used in the experiment carried out in an experimental plant nursery, including non-purified waste from the biodiesel production, applied to sourgrass in two phenological stages. Machado - MG, 2013

Treatment	Herbicide	Dose of Herbicide ¹	Concentration of waste
1	Check plots without application	Absent	Absent
2	Glyphosate ²	360 g ha ⁻¹	Absent
3	Glyphosate	360 g ha ⁻¹	0.2% v/v
4	Glyphosate	360 g ha ⁻¹	0.4% v/v
5	Glyphosate	360 g ha ⁻¹	0.6% v/v
6	Glyphosate	360 g ha ⁻¹	0.8% v/v
7	Glyphosate	360 g ha ⁻¹	1.0% v/v
8	Glyphosate	720 g ha ⁻¹	Absent

¹Dose in grams of glyphosate acid equivalent per hectare; ²Round up Original®, 360 g L⁻¹.

Table 3. Treatments applied in the experiment carried out in the field using non-purified waste from biodiesel production. Machado - MG, 2013

Treatment	Herbicide	Dose of Herbicide ¹	Concentration of waste
1	Check plots without application	Absent	Absent
2	Glyphosate	360 g ha ⁻¹	Absent
3	Glyphosate	360 g ha ⁻¹	0.25% v/v
4	Glyphosate	360 g ha ⁻¹	0.50% v/v
5	Glyphosate	360 g ha ⁻¹	1.00% v/v
6	Glyphosate	720 g ha ⁻¹	Absent

¹Dose in grams of glyphosate acid equivalent per hectare; ²Round up Original®, 360 g L⁻¹.

Percentage control evaluations were performed at 14, 21 and 28 days after application (DAA). For this purpose, a percentage scale of visual impairment was used, in which zero represented absence of symptoms and 100% represented plant's death (SBCPD 1995). Additionally, just for the experiment developed in a plant nursery, plants dry matter was measured. At this time, any plant material

remaining in the pots was collected and dried in a forced air circulation oven controlled at 70 °C for 72 hours for later weighing.

Data were submitted to application of F-test on the analysis of variance, followed by a means grouping test (Scott and Knott 1974), both with 5% significance.

Results and Discussion

Effectiveness on sourgrass

In the experimental plant nursery, for all assessments of percent control, the interaction of herbicide treatments with different phenological stages of sourgrass was identified, which justified the factorial decomposition

(Table 4). At 14 DAA, the presence of biodiesel production waste in the spray mix reduced glyphosate effectiveness on sourgrass plants. For younger plants, negative effect was observed at 0.6% v/v concentrations of the adjuvant in the spray mix. For plants in tillering, lower control was evident in all treatments which received the waste (Table 4).

Table 4. Sourgrass (*Digitaria insularis*) percent control in two phenological stages after glyphosate application associated to biodiesel production waste collected in the Varginha Biodiesel plant. Machado - MG, 2013

Treatments	Phenology	
	Three leaves	Tillering
Evaluation performed at 14 DAA		
Check plots without application	0.0 D a	0.0 C a
Glyphosate 360 g ha ⁻¹	52.5 B a	33.2 A b
Glyphosate (360 g ha ⁻¹) + waste (0.2% v/v)	52.8 B a	28.0 B b
Glyphosate (360 g ha ⁻¹) + waste (0.4% v/v)	43.8 B a	26.2 B b
Glyphosate (360 g ha ⁻¹) + waste (0.6% v/v)	31.3 C a	21.3 B a
Glyphosate (360 g ha ⁻¹) + waste (0.8% v/v)	22.8 C a	19.2 B a
Glyphosate (360 g ha ⁻¹) + waste (1.0% v/v)	26.0 C a	23.8 B a
Glyphosate 720 g ha ⁻¹	81.0 A a	40.0 A b
F _(treat) = 34.151* F _(phenol) = 47.098* F _(int) = 5.128* CV (%) = 33.75		
Evaluation performed at 21 DAA		
Check plots without application	0.0 a	0.0 D a
Glyphosate 360 g ha ⁻¹	74.2 B a	42.8 B b
Glyphosate (360 g ha ⁻¹) + waste (0.2% v/v)	89.5 A a	33.3 C b
Glyphosate (360 g ha ⁻¹) + waste (0.4% v/v)	58.3 C a	31.2 C b
Glyphosate (360 g ha ⁻¹) + waste (0.6% v/v)	47.5 D a	23.3 C b
Glyphosate (360 g ha ⁻¹) + waste (0.8% v/v)	32.5 D a	23.7 C a
Glyphosate (360 g ha ⁻¹) + waste (1.0% v/v)	39.2 D a	26.3 C b
Glyphosate 720 g ha ⁻¹	97.3 A a	56.2 A b
F _(treat) = 66.402* F _(phenol) = 149.531* F _(int) = 9.716* CV (%) = 23.93		
Evaluation performed at 28 DAA		
Check plots without application	0.0 D a	0.0 D b
Glyphosate 360 g ha ⁻¹	70.8 B a	50.8 B b
Glyphosate (360 g ha ⁻¹) + waste (0.2% v/v)	89.8 A a	39.2 C b
Glyphosate (360 g ha ⁻¹) + waste (0.4% v/v)	59.5 B a	40.0 C b
Glyphosate (360 g ha ⁻¹) + waste (0.6% v/v)	52.5 C a	27.5 C b
Glyphosate (360 g ha ⁻¹) + waste (0.8% v/v)	35.8 C a	28.8 C a
Glyphosate (360 g ha ⁻¹) + waste (1.0% v/v)	41.7 C a	34.2 C a
Glyphosate 720 g ha ⁻¹	99.5 A a	78.7 A b
F _(treat) = 54.294* F _(phenol) = 55.679* F _(int) = 4.721* CV (%) = 26.39		

*F-test significant at 1%; ¹Means followed by the same uppercase letter in the column and a lowercase letter in the row do not differ according to the Scott-Knott test means grouping with 5% probability.

The potential benefits arising from the addition of glyphosate waste on the spray mix were minimal. At the experimental plant nursery, positive points were observed only at 21 and 28 DAA, when spray mix with 0.2% v/v

of residue was applied on young plants (three leaves). In this case, control was increased of up to 19.2%, but without consistency between stages, once for tillering plants the same

treatment had lower control than the application of pure glyphosate at 360 g ha⁻¹ (Table 4).

In this case, an explanation for lower glyphosate efficacy observed in the treatments with the addition of waste would be the possible change in the spray mix pH. It is known that the catalyst used in the transesterification reaction is of alkaline nature, affecting even the final pH of the waste, rated at 8.7 (Table 1). Consequently, by adding the waste to the spray mix, the pH balance point of the glyphosate spray mix, typically around 4.5 (Carvalho et al. 2009), may have been changed to more alkaline levels, undermining the product effectiveness.

In all visual evaluations, better control obtained with glyphosate application on

younger plants is clear, so that after tillering sourgrass control becomes difficult (Table 4). In this regard, Christoffoleti et al. (2005), Dias et al. (2013) and Gonçalves Netto et al. (2015) have also observed interference of plants' phenological stage in glyphosate efficacy, observing less control for more developed plants of ryegrass (*Lolium multiflorum*), Bengal dayflower (*Commelina benghalensis*), and sourgrass itself, respectively.

As for the residual dry matter, there was no interaction between treatment factors (Table 5), not even an isolated effect of sourgrass phenology. In short, all herbicide treatments reduced the species dry matter, with no difference between them.

Table 5. Sourgrass (*Digitaria insularis*) residual dry matter in two phenological stages after glyphosate application associated to biodiesel production waste collected in the Varginha Biodiesel plant, evaluated 28 days after application. Machado - MG, 2013

Treatments	Dry matter(g pot ⁻¹)
Check plots without application	15.5 B
Glyphosate 360 g ha ⁻¹	4.3 A
Glyphosate (360 g ha ⁻¹) + waste (0.2% v/v)	4.4 A
Glyphosate (360 g ha ⁻¹) + waste (0.4% v/v)	4.6 A
Glyphosate (360 g ha ⁻¹) + waste (0.6% v/v)	5.0 A
Glyphosate (360 g ha ⁻¹) + waste (0.8% v/v)	6.2 A
Glyphosate (360 g ha ⁻¹) + waste (1.0% v/v)	4.9 A
Glyphosate 720 g ha ⁻¹	4.1 A
F _(treat) = 63.909* F _(phenol) = 3.180 ^{NS} F _(int) = 0.279 ^{NS} CV (%) = 27.36	

*F-test significant at 1%; ¹Means followed by the same uppercase letter in the column do not differ according to the Scott-Knott test means grouping with 5% probability.

Effectiveness in the field

In accordance with the first experiment, for any evaluation under field conditions, control increments resulting from adding waste to the glyphosate spray mix were not observed (Table 6). Clearly, it is possible to highlight the treatment with glyphosate at 720 g ha⁻¹, which obtained the highest control levels of the weed population, but also without a satisfactory agronomic efficacy, i.e., control levels always below 80%. The other treatments, with lower dose of glyphosate (360 g ha⁻¹), were equal to each other, except for the evaluation at 28 DAA (Table 6). In this latest assessment (28 DAA),

the addition of waste at any concentration has impaired glyphosate agronomic efficacy.

Therefore, it is clear that the correct choice of glyphosate dose is more important than the presence of adjuvants in the spray mix, which imposes responsibility on the agricultural recommendations of herbicides under field conditions. Notably, greater glyphosate dose is necessary for an adequate control of the species, highlighting the difficulty in controlling sourgrass and signalgrass, which may require doses around 1440 g ha⁻¹ (Timossi et al. 2006, Rodrigues and Almeida 2011, Gonçalves Netto et al. 2015).

In plant cells, glyphosate inhibits EPSPS (5-enolpyruvylshikimate 3-phosphate synthase), an enzyme that participates in the synthesis pathway of the aromatic amino acids phenylalanine, tyrosine and tryptophan (Cole 1985). Symptoms resulting from glyphosate

application are slow, starting with the progressive yellowing of leaves, wilting with subsequent necrosis and death of plants, which may take 4-20 days, depending on the species and phenological stage (Rodrigues and Almeida 2011).

Table 6. Weeds percent control in the field after glyphosate application associated to biodiesel production waste collected in the Varginha Biodiesel plant. Machado - MG, 2013

Treatments	Percent control		
	14 DAA	21 DAA	28 DAA
Check plots without application	0.0 C	0.0 C	0.0 D
Glyphosate 360 g ha ⁻¹	39.0 B	40.0 B	44.0 B
Glyphosate (360 g ha ⁻¹) + waste (0.25% v/v)	30.0 B	36.7 B	32.5 C
Glyphosate (360 g ha ⁻¹) + waste (0.50% v/v)	30.0 B	32.8 B	31.7 C
Glyphosate (360 g ha ⁻¹) + waste (1.00% v/v)	29.2 B	28.3 B	27.5 C
Glyphosate 720 g ha ⁻¹	55.0 A	64.3 A	65.0 A
F _(treat)	28.29*	19.18*	35.62*
CV (%)	27.03	34.44	26.12

*F-test significant at 1%; ¹Means followed by the same letter in the column do not differ according to the Scott-Knott test means grouping with 5% probability.

In an attempt to increase glyphosate efficacy on plant cover, speeding senescence, as well as cuticle penetration and cellular absorption of the molecule, several substances have been added to the spray mix solution. According to Mendonça et al. (2007), adjuvants act differently from each other, affecting wetting, sticking, spreading, foaming and/or dispersion of the spray mix on the target.

At this point, an aspect of interest in using non-purified waste from biodiesel production for glyphosate application is emphasized, which is the presence of a monovalent ion in waste from the catalyst, which may be sodium or potassium. With respect to the addition of salts to increase glyphosate efficacy, Wills and McWhorter (1985) have observed that salts of monovalent cations (NH₄⁺, K⁺ and Na⁺), usually result in higher toxicity of the product, while salts of divalent or trivalent cations (Zn²⁺ and Fe³⁺) provide lower herbicide activity. Working with ammonium ion, from the ammonium sulfate fertilizer, Carvalho et al. (2009, 2010) have found greater efficacy of glyphosate herbicide on some weed species.

Still another product commonly added to glyphosate spray mix is urea, whose main control increment mechanism relates to the contribution in cuticle penetration due to the facilitated diffusion of this molecule and the rupture of ester, ether and di-ether bonds of cutin (Durigan 1992, Witte et al. 2002). In this study, this effect of penetration could be achieved due to the incidence of soap in biodiesel waste, derived from the saponification reaction between the catalyst and the vegetable oil used, with the potential effect of detergent on the leaf cuticle.

Additionally, the glycerin viscosity could stimulate the spray mix adhesion to leaves, improving the absorption time and reducing the drop extinction rate. Besides these aspects, the addition of vegetable oil to agricultural spray mixes is also a routine with recognized efficacy and less potential for phytotoxicity when compared to mineral oils (Mendonça et al. 2007, Andrade et al. 2010).

Considering both experiments, significant results that justify the adoption of biodiesel production waste as an agricultural adjuvant were not found. The positive points

observed were minimal, with no consistency between species or application times, then glyphosate remains as the best application and recommendation option.

Other research pathways should be evaluated in order to allocate crude glycerin derived from biodiesel production. In the literature, among the possible allocations for glycerin, chemical, cosmetics and pharmaceutical destinations, resins, food and beverage, tobacco, pulp and paper, etc. (Mota et al. 2009) and composting (Lima et al. 2009) can be included. Also, increase in research evaluating the use of glycerin in animal feed, including pigs, has been observed (Berenchtein et al. 2010), birds (Cerrate et al. 2006) and ruminants (Lage et al. 2010).

Conclusions

Adding biodiesel production waste to the spray mix solution has not increased glyphosate efficacy in controlling sourgrass.

There may be reduced glyphosate effectiveness after addition of waste to the spray mix solution at doses equal to or higher than 0.6% v/v.

No results were found justifying the adoption of biodiesel production waste as an agricultural adjuvant.

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