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The triangular greenness index indicates the weed control efficacy of stored herbicide spray mixture[1]

Cleyton Batista de Alvarenga[2*], *Dalton Luiz Benz*[2], *Paula Cristina Natalino Rinaldi*[2], *George Deroco Martins*[3], *Edson Aparecido dos Santos*[4], *João Paulo Arantes Rodrigues da Cunha*[4] and *Jair Rocha do Prado*[5]

ABSTRACT

Herbicide spray mixture may need to be stored due to mechanical failures, adverse weather conditions, or the use of concentrated spray mixture. Remote sensing has emerged as a valuable tool for assessing pest control efficacy. This study aimed to assess the effect of prolonged storage of spray solution on weed control efficacy using remote sensing. The experiment adopted a randomized block design with four replicates, using a split-plot arrangement. The following factors were analyzed in the present study: four herbicide spray mixture (glyphosate, dicamba, diquat, and 2,4-D) in the main plot, eight storage periods (14, 12, 10, 8, 6, 4, 2, and 0 days before application) in subplots, and seven time points (1, 3, 5, 7, 9, 14, 21, and 28 days after application) in subsubplots. Data analysis involved ANOVA, assumption testing, and Pearson product-moment correlation between methods. The results indicated that stored spray mixture maintain their efficacy in weed control. In addition, the triangular greenness index effectively estimated the weed control efficacy of all herbicides studied, i.e., glyphosate, dicamba, diquat and 2,4-D.

Keywords: glyphosate, dicamba, diquat, 2,4 D.

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[2] Universidade Federal de Uberlândia, Instituto de Ciências Agrárias (ICIAG), Monte Carmelo, MG, Brazil. cleytonalvarenga@ufu.br, daltonbenz@hotmail.com, renanzampiroli@ufu.br, paularinaldi@ufu.br.

[3] Universidade Federal de Uberlândia, Instituto de Geografia, Geociências e Saúde Coletiva (IGESC), Monte Carmelo, MG, Brazil. deroco@ufu.br.

[4] Universidade Federal de Uberlândia, Instituto de Ciências Agrárias (ICIAG), Uberlândia, MG, Brazil. edsonsantos@ufu.br, jpcunha@ufu.br.

[5] Universidade Federal de Uberlândia, Instituto de Matemática (IME), Uberlândia, MG, Brazil. jairrp@ufu.br.

*Corresponding author: cleytonalvarenga@ufu.br

Editors:
Marihus Baldotto
Marcus Alvarenga Soares

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INTRODUCTION

Old or stored spray mixture of pest control products are often used in field applications. Research on stored mixture has focused on analyzing the products and mixtures within spray tanks and assessing their effects on weed control efficacy. Spray mixture are usually discarded after 24 to 48 h of storage, with high agricultural and environmental costs. The storage period can influence the effectiveness of insecticides, fungicides, and herbicides. Spray solution storage must be followed by a rigorous decontamination of the tank and hydraulic system, especially when using hormonal products, due to the accumulation of residues on tank walls,[1] e.g., as in the case of 2,4-D and dicamba.[2]

The appropriate period between adding pest control products to the tank and completing the application is technically unknown. Manufacturer's instructions recommend preparing spray mixture immediately before applying them. However, various factors can extend the time for which the solution remains in the tank; these include mechanical problems, adverse weather conditions,[3,4] low application rates, in-furrow and preplant incorporated applications, and ready-to-use and concentrated formulations. Scientific studies on herbicide spray solution storage indicate that the time between preparation and application can affect efficacy, especially in the case of growth regulators, such as dicamba and 2,4-D.[5] However, while some studies noted no changes in efficacy,[6,7] others reported decreased efficacy.[8] As a result, the effect of spray solution storage on herbicide efficacy remains controversial in literature.

In Brazil, there is limited understanding of the weed control efficacy of stored herbicide spray mixture. According to Schortgen and Patton,[3] spray solution storage time varies because some farmers prepare spray mixture immediately before application, while others prepare them in advance or store unused leftovers for subsequent use. In this respect, Ramos and Durigan[7] have stated that storage time affects not only product efficacy but also compatibility with other products.

Eudicot and monocot herbicides are commonly mixed to broaden the control spectrum. However, contact herbicides can have an antagonistic effect on systemic products, according to Castner et al.[2] For example, glufosinate decreases the efficacy of clethodim in *Eleusine indica* control. Conversely, Harre, Young & Young[9] noted that auxinic herbicides may have an antagonistic effect on Acetyl CoA Carboxylase (ACCase)-inhibiting herbicides.

Assessing herbicide efficacy is crucial in farm management. Field technicians should evaluate the results 24 h after application, depending on the product. Such assessments are subjective and require experienced raters. In addition to rater reliability, other factors should be considered, including the sample size needed to estimate weed control efficacy with reasonable precision, as shown by Voll et al.[10]

Remote sensing increases precision in assessing weed control efficacy, addressing the limitations of subjective evaluations. Using vegetation images or indices is an effective weed management tool. Remotely Piloted Aircraft Systems (RPAS), equipped with low-cost cameras, enable multiple applications by capturing images in the common visible (Red, Green and Blue [RGB]) spectrum bands, in turn providing the Triangular Greenness Index (TGI).[11,12] Hunt Júnior et al.[13] define TGI as a low-cost estimator of chlorophyll levels that can aid and inform technical decisions in the field. Considering the above, the present study aimed to assess the impact of prolonged spray solution storage on the weed control efficacy of herbicides using remote sensing.

MATERIAL AND METHODS

The experiment was conducted in a study area located at 18°43'23.83"S and 47°31'24.48"W, with an average altitude of 980 m. The region has a tropical wet and dry or savanna (Aw) climate, with cold (15/16 ºC) and dry winters, according to the Köppen[14] climate classification. The herbicides were applied in November 2019. Weather conditions during applications were monitored using a Kestrel 4000 weather and environmental meter (São Paulo, São Paulo State, Brazil). The average temperature, relative humidity and wind speed values were 27.5 °C, 57.5% and 8.46 km h^{-1}, respectively.

The experiment was conducted based on a randomized block design, with a 4×7×7 subsubplot scheme. Four herbicide spray mixture were tested in the present study, namely glyphosate (1.400 g ha^{-1}), glyphosate (1.400 g ha^{-1}) + 2,4-D (1.340 g ha^{-1}), glyphosate (1.400 g ha^{-1}) + dicamba (624 g ha^{-1}), and glyphosate (1.400 g ha^{-1}) + diquat (500 g ha^{-1}). The acid equivalent of the glyphosate and 2,4-D doses was the same, and the dicamba and diquat doses contained the same amount of active ingredient. The seven spray mixture were prepared 14, 12, 10, 8, 6, 4, 2, and 0 days before application (DBA), and their efficacy was assessed at seven time points, that is, 1, 3, 5, 7, 9, 14, 21, and 28 days after application (DAA), with four replicates. The plots were 3 x 5 m in size (15 m² total area) and 1.0 m apart, with 2.5 m between blocks and with a 0.5-m border around each plot.

In the field, the first stage of the experiment was the phytosociological survey of weeds, according to the method of Braun-Blanquet.[15] The following species were identified in the present study: *Commelina benghalensis*, *Digitaria sanguinalise*, *Eleusine indica*, *Amaranthus viridis*, *Portulaca oleracea*, *Ipomoea triloba*, *Ipomoea nill*, *Emilia fosbergii*, *Richardia brasiliensis*, *Ricinus communis*, and *Euphorbia heterophylla*. All plants were in the adult stage, close to flowering.

Based on the phytosociological survey, we selected the following herbicides to prepare the pest control mixture: glyphosate (62% Glycine, N-(phosphonomethyl)- potassium salt and 50% acid equivalent), dicamba (48% 3,6-dichloroanisic acid), diquat (20% 9,10-dihydro-8a,10a-diazoniaphenanthrene), and 2,4-D (80.6% 2,4-dichlorophenoxy acetic acid dimethylamine salt and 67% acid equivalent).

In the laboratory, the spray mixture were prepared and stored in 2-L black polyethylene terephthalate bottles, labeled by treatment. The bottles were black to mitigate photodegradation and were placed in an environment with an average temperature of 25 ºC until application. The water used to prepare the mixture was collected from an artesian well. The water pH (6.3) was measured using a PHTEK portable pH meter (Curitiba, Paraná, Brazil).

The herbicides were applied using a Herbicat CO_2-pressurized backpack sprayer (Ribeirão Preto, São Paulo State, Brazil), equipped with a 3-m wand with six Magnojet ADIA 11002 flat fan nozzles (Ibaiti, Paraná, Brazil) 0.5 m apart. The operating pressure was 291.3 kPa, producing droplets with diameters ranging from 428 to 622 µm, classified as extremely coarse, according to the American Society of Agricultural and Biological Engineers (ASABE) S572.3 standard,[16] as reported in the spray nozzle catalog. The application rate was set to 200 L ha^{-1} for a good droplet density, target coverage, and compatibility with a 4.56 km h^{-1} spraying speed.

In the visual assessment method, weed control efficacy was determined by averaging the scores of three raters, as established by the Brazilian Society of Weed Science.[17] Weed control efficacy was scored according to the following ranges: < 10% - no control; 11–39% - poor or negligible control; from 40–79% - moderate control, insufficient for infestations; 80–89% - good control, acceptable for infestations in the area; and 90–100% - excellent control, with no effects on crops.

The TGI was determined using images acquired by a DJI Phantom 4 Pro drone (Shenzhen, China) equipped with a camera with a 1-in, 20-MP Complementary Metal-Oxide-Semiconductor (CMOS) sensor and a built-in Global Positioning System (GPS). Flights were conducted 1, 3, 5, 7, and 9 DAA, between 12:00 and 13:00 pm, Brasília Time (BRT), in autonomous mode, at 60- m altitude, with 80% longitudinal coverage and 75% lateral coverage, yielding 1.8-cm pixels. The TGI of each experimental plot was determined before performing Pearson product-moment correlation with the average scores of the evaluators up to 9 DAA.

The data were pre-processed in MAPIR Camera Control (MCC), software [18], for atmospheric correction and digital number transformation into reflectance percentage. The orthomosaic was assembled in Agisoft Photoscan software,[19] using dense point cloud and digital elevation model (DEM) as byproducts. The final processing was implemented using the QGIS software to obtain the indices recorded in the images through zonal statistics for each plot.[20]

Statistical analysis was conducted in the R software environment for statistical computing and graphics.[21] After measuring data normality using the Shapiro-Wilk test and homogeneity using the Oneill and Mathews test, we subjected the data to analysis of variance (ANOVA) using the F test at a 5% probability level. When the results were significant, means were compared using the Scott-Knott test, also at a 5% probability level.

RESULTS AND DISCUSSION

ANOVA revealed a significant interaction effect between herbicide type and spray solution storage duration. For the unstored spray solution, the largest difference in weed control efficacy was 27.8% between glyphosate + 2,4-D and glyphosate + diquat mixtures. However, after eight days of storage, the weed control efficacy of the glyphosate + diquat mixture was 37.8% lower than that of the glyphosate + 2,4-D mixture (Table 1).

The glyphosate + diquat mixture provided poor or negligible control in all periods, except after two days of storage. In turn, spray mixture containing glyphosate, glyphosate + 2,4-D, and glyphosate + dicamba resulted in moderate weed control[17] throughout the storage period. Eure et al.[4] observed no decrease in the *Brachiaria platyphylla*, *Chenopodium album*, *Ipomoea hederacea*, and *Amaranthus palmeri* control efficacy of spray mixture prepared with diclosulam, dimethenamid, flumioxazin, fomesafen, imazethapyr, pendimethalin, and s-metolachlor, following storage for up to nine days.

The glyphosate + 2,4-D mixture exhibited the best weed control efficiency, thanks to the synergistic effect of the products, particularly on weeds found in the area, and to the development stage of those weeds. Both products are systemic: 2,4-D is effective against eudicots, whereas glyphosate is often more effective against monocots. The relatively slow action of the herbicides enables them to reach the sites of action, achieving the necessary damage to the weeds. However, the mixture of glyphosate and diquat can have an antagonistic effect because diquat acts quickly, causing cell membrane oxidation and tissue death. The rapid effect hinders glyphosate translocation, compromising its effectiveness.[22]

In the herbicide storage analysis, only the glyphosate-containing mixture exhibited a significant effect (Figure 1). Intentionally storing the spray solution did not lead to significant benefits to weed control, with efficacy scores in the 40.0%–79.0% range.[17] Nevertheless, stored spray mixture delayed weed development, which could provide a competitive advantage if there was a crop in the area. According to Schortgen and Patton[3], spray solution storage time does not affect weed control efficacy.

Based on the assessment data, spray solution composition impacted weed control efficacy. The weed control efficacy of spray mixture composed of glyphosate, glyphosate + 2,4-D, and glyphosate + dicamba increased up to 28 DAA. By contrast, the efficacy of the glyphosate + diquat spray solution decreased over time. The glyphosate

Table 1. Weed control scores based on herbicide and spray solution storage time

Storage (days)	Herbicides			
	Glyphosate	**Glyphosate + 2,4 D**	**Glyphosate + Dicamba**	**Glyphosate + Diquat**
0	45.5b	54.0a	42.0c	39.0d
2	47.8b	53.4a	45.0b	41.3c
4	46.4b	57.2a	41.2c	36.8d
6	49.8b	54.5a	46.8c	39.0d
8	41.6c	57.0a	46.1b	35.5d
10	41.6c	56.2a	48.1b	35.7d
12	43.5c	54.2a	46.5b	38.2d
14	44.5c	53.1a	48.0b	39.5d
CV (%)	20.7			
p-value (herbicide x storage)	0.0100*			
p-value (homogeneity)	0.99*			
p-value (normality)	1.94×10^{-11ns}			

* Significant at 5% probability. ^{ns} Non-significant.
Means with the same letters in rows do not significantly differ from each other according to the Scott-Knott test at 5% probability.

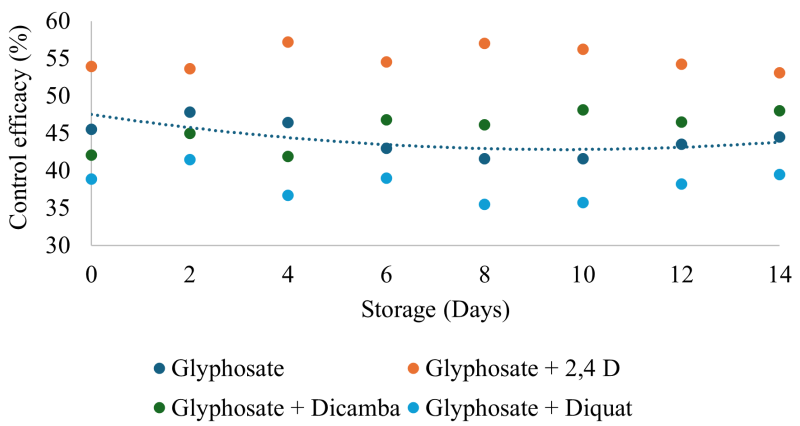


Figure 1. Spray solution storage time effect on weed control efficacy for different herbicide spray solutions.

+ diquat spray solution immediately affected weed control efficacy due to its contact action. At 1 DAA, the weed control efficacy of this spray solution was 88.5, 75.9, and 83.0% higher than that of glyphosate, glyphosate + 2,4-D, and glyphosate + dicamba spray mixture, respectively (Table 2).

The glyphosate + diquat mixture induced more visible symptoms in plants up to 5 DAA. The glyphosate + 2,4-D mixture was effective in controlling weeds from 7 to 28 DAA. After this period, the plots in which the glyphosate + diquat mixture was applied showed no difference in phytotoxicity symptoms, and weed emergence started at 21 DAA.

The study herbicides have no pre-emergence effects. In other words, they only affect sprayed plants once they have emerged, so that new plants can quickly emerge after defoliation. For example, diquat can be used in areas with seed banks or specific environmental conditions,[23] such as water availability and sunlight exposure. For this reason, herbicides, particularly herbicide mixtures, are recommended, considering the cultivation system, whether no-till farming or conventional tillage. Thus, scoring weed control efficacy scores is essential for agricultural management.

Based on SBCPD,[17] the weed control effects of glyphosate, glyphosate + 2,4-D, glyphosate + dicamba and glyphosate + diquat spray mixture were poor up to 5, 3, and 3 DAA, and from 21 DAA, respectively. Between 7 and 28 DAA, the glyphosate and glyphosate + dicamba spray mixture achieved moderate control, that is, they failed to control all species identified in the phytosociological survey. Adding 2,4-D to glyphosate provided good weed control efficacy at 21 DAA. When examining herbicide antagonism and synergism, Kalina et al[24] noted that dicamba's activity may be greater or less than that of a contact herbicide, depending on the duration and timing of exposure, due to the physiological effects of systemic and contact herbicides.

The addition of 2,4-D to glyphosate increased weed control efficacy across all time points. Up to 7 DAA, both dicamba and diquat also enhanced efficacy when combined with glyphosate. The findings suggest that herbicide mixtures should be prepared according to the needs for immediate/fast or slower/prolonged weed control. When spraying coincide with the critical period of the crops, farmers should select the most appropriate mixture to minimize competition.

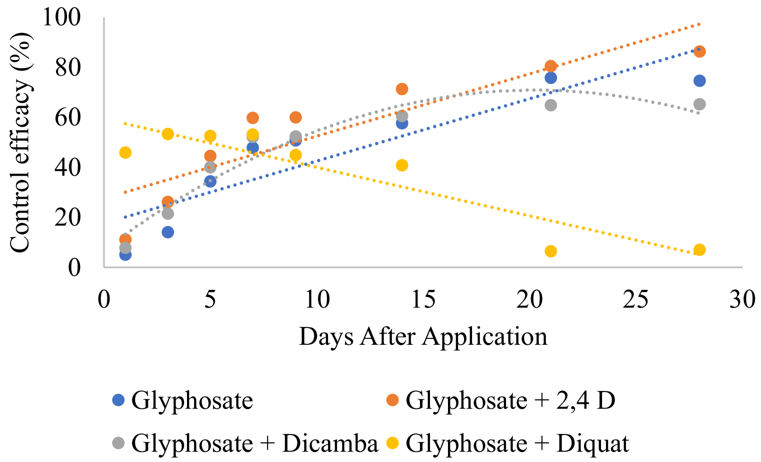
The impact of each herbicide or mixture on weed control efficacy varies over time. The weed control efficacy of glyphosate and glyphosate + 2,4-D spray mixture increased linearly from 1 to 28 DAA. In contrast, the glyphosate + dicamba spray solution exhibited a polynomial effect, with its weed control efficacy increasing up to 15 DAA and plateauing thereafter until the emergence of new weeds in the plot. Conversely, the weed control efficacy of the glyphosate + diquat spray solution decreased linearly due to the diquat contact effect and to the weak residual glyphosate effect (Figure 2).

Glyphosate and glyphosate + 2,4-D mixtures performed similarly, with weed control efficacy increasing linearly. When added to glyphosate, 2,4-D helped to increase weed

Table 2. Spray solution composition affects weed control efficacy over time

Assessment (DAA)	Herbicides			
	Glyphosate	Glyphosate + 2,4 D	Glyphosate + Dicamba	Glyphosate + Diquat
1	5.3d	11.1b	7.8c	46.0a
3	14.1d	26.2b	21.6c	53.3a
5	34.4d	44.5b	40.0b	52.7a
7	47.9c	59.8a	52.2b	53.1b
9	50.9b	59.9a	52.3b	45.0c
14	57.6c	71.3a	60.6b	40.9d
21	75.8b	80.4a	64.8c	6.6d
28	74.7b	86.3a	65.2c	7.1d
CV (%)	12.7			
p-value (herbicide x storage)	0.00*			
p-value homogeneity	0.99*			
p-value normality	1.94×10^{-11ns}			

* Significant at 5% probability. ns Non-significant.
Means with the same letters in rows do not significantly differ from each other according to the Scott-Knott test at 5% probability.



Glyphosate – $y = 2.485149x + 17.725156$, $R^2=0.8156$

Glyphosate + 2,4 D – $y = 2.485919x + 27.597974$, $R^2=0.7973$

Glyphosate + Dicamba – $y = -0.154904x^2 + 6.280276x + 7.180993$, $R^2=0.9324$

Glyphosate + Diquat – $y = -1.932078x + 59.336918$, $R^2=0.8437$

Figure 2. Variation of the herbicide effect on weed control efficacy over time.

control efficacy from 1 DAA. From 3 to 20 DAA, dicamba also had a positive effect on weed control efficacy. However, after 20 DAA, dicamba shortened the period of action of glyphosate because glyphosate alone increased weed control efficacy up to 28 DAA.

The diquat spray solution allowed rapid control of the weeds described in the phytosociological survey. However, new plants were observed quickly emerging from under crop residues. Glyphosate had no effect on emerging plants, providing a limited contribution to this treatment.

The weather conditions during herbicide applications did not constrain droplet size. However, high temperature and low relative humidity conditions may increase volatility, as observed by Kalina et al.[24] Davkota and Johnson[25] noted that these conditions affect dicamba and glyphosate absorption and efficacy. In addition, herbicide performance might have been affected by spray solution pH during the application. When analyzing the weed control efficacy of glyphosate and dicamba at pH 4.0, 6.5, and 9.0, Marques et al[26] found that efficacy peaked at the lowest pH value.

Determined using TGI 1 DAA, the weed control efficacy of glyphosate, glyphosate + 2,4-D, and glyphosate + dicamba spray mixture were 86.6, 85.7, and 96.6%, respectively. At 3 DAA, the TGI values were similar. From 5 to 9 DAA, the glyphosate + 2,4-D mixture had the highest TGI (Table 3).

In the TGI assessment, the glyphosate + diquat spray solution ceased to induce visible symptoms between 3 and 5 DAA, in contrast to the glyphosate + 2,4-D spray solution. However, the difference was observed between 5 and 7 DAA in the visual assessment. This discrepancy between TGI and visual assessment indicates that TGI enables us to visualize the effects and weed control efficacy of the glyphosate + 2,4-D spray solution more rapidly.

TGI correlates with herbicide absorption and activity at the site of action, as indicated by weed control efficacy. Depending on the herbicide, TGI values may either increase, as seen with glyphosate, glyphosate + 2,4-D, and glyphosate + dicamba, or decrease, as observed with glyphosate + diquat. These changes are reflected in leaf chlorophyll content (Figure 3).

Glyphosate, 2,4-D, and dicamba are systemic, slow-acting herbicides, which damage weeds, primarily causing homogeneous chloroses. These chloroses intensify over time. In contrast, diquat acts quickly, and its effect stops within a few hours after causing cell damage.[23] Therefore, TGI is a promising tool for managing herbicide applications, particularly when they are followed by rainfall or plagued by mixture incompatibility and water quality problems and equipment failures.

Herbicide action is initially observed through a reduction in leaf chlorophyll content, which corresponds to an increase in the Triangular Greenness Index (TGI). This response mirrors the herbicide modes of action. Systemic herbicides, which affect amino acid synthesis or have hormonal effects, generally lead to an increase in TGI over time. In contrast, contact herbicides, which target the photosystem, cause an increase in TGI from the earliest time point. TGI can also be used to identify the characteristic symptom of each herbicide in plants. For example, TGI holds much potential for detecting dicamba symptoms in soybean crops, according to Marques et al.[26]

Using TGI, we were able to objectively identify plant symptoms in each plot. A time series of flights performed after herbicide applications made it possible to visualize the effects of each treatment on plants growing in the study area (Figure 4).

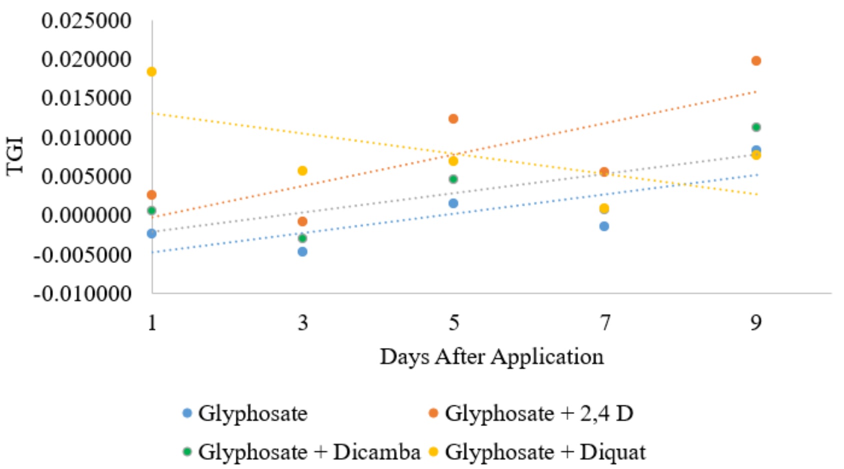
Weed control efficacy assessment based on TGI was more strongly correlated with visual assessment at 1 and 3 DAA and less strongly correlated at 5, 7, and 9 DAA (Table 4).

The correlation between efficacy assessment methods allows us to evaluate the reliability of the TGI method, especially in the absence of raters with visual assessment skills. TGI is derived from images captured by RGB cameras, which are less expensive than multispectral cameras. However, the main limitation of TGI is the need for technical knowledge to extract data in the field. Although

Table 3. Post-application TGI by spray solution and time point

Assessment (DAA)	Herbicides			
	Glyphosate	Glyphosate + 2,4 D	Glyphosate + Dicamba	Glyphosate + Diquat
1	-0.002458d	0.002626b	0.000628c	0.018329a
3	-0.004765d	-0.000862b	-0.002935c	0.005634a
5	0.001472d	0.012376a	0.004581c	0.006859b
7	-0.001476c	0.005474a	0.000684b	0.000897b
9	0.008335c	0.019666a	0.011292b	0.007666c
CV (%)	84.8			
p-value (herbicide x DAA)	0.00*			
p-value homogeneity	0.96*			
p-value normality	6.03×10^{-19ns}			

* Significant at 5% probability. ns Non-significant.
Means with the same letters on rows did not differ significantly from each other according to the Scott-Knott test at 5% probability.



Glyphosate – y = 0.001244x - 0.005997, R^2=0.6047

Glyphosate + 2,4 D – y = 0.002021x - 0.002248, R^2=0.6073

Glyphosate + Dicamba – y = 0.001247x - 0.003387, R^2=0.5003

Glyphosate + Diquat – y = -0.001303x + 0.014393, R^2=0.4140

Figure 3. Variation of TGI as a function of herbicide spray solution over time.

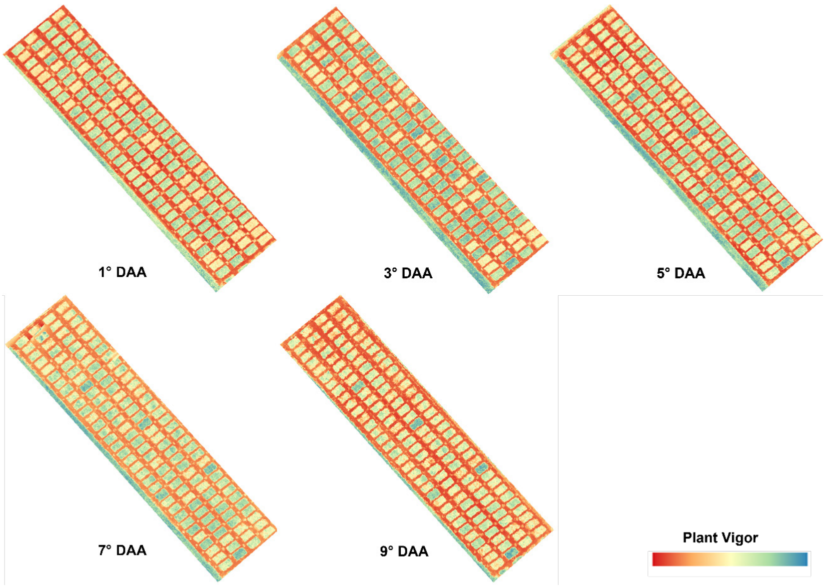


Figure 4. Plant vigor in plots according to TGI at 1, 3, 5, 7, and 9 DAA.

Table 4. Correlation between weed control efficacy assessment methods (visual assessment and TGI)

Analysis	Days after application				
	1	3	5	7	9
Pearson product-moment correlation	0.8977	0.9409	0.3858	0.6003	0.4694

TGI is based on a straightforward mathematical equation, specific image processing software must be used to interact with the data.

CONCLUSIONS

Stored spray mixture may retain their weed control efficacy over different periods.

In particular, the stored spray mixture of glyphosate and glyphosate + 2,4 D mixture maintained their efficacy up to 28 days after application (DAA).

Tank mixing increases efficacy regardless of herbicide storage.

The glyphosate + 2,4 D mixture exhibited the highest weed control efficacy at all time points, except for the initial phase of diquat application.

Storying a spray solution with a contact herbicide decreases the weed control efficacy.

The Triangular Greenness Index (TGI) demonstrates potential as an efficient estimator of herbicide weed control efficacy.

DATA AVAILABILITY

All data supporting the results of this study are contained within this article.

AUTHOR CONTRIBUTIONS

Conceptualization: Cleyton Batista de Alvarenga, João Paulo Arantes Rodrigues da Cunha, Paula Cristina Natalino Rinaldi.

Data curation: Cleyton Batista de Alvarenga, Dalton Luiz Benz.

Formal analysis: Cleyton Batista de Alvarenga, Edson Aparecido dos Santos, George Deroco Martins, Jair Rocha do Prado.

Methodology: Cleyton Batista de Alvarenga, Dalton Luiz Benz, Edson Aparecido dos Santos, George Deroco Martins, Jair Rocha do Prado, João Paulo Arantes Rodrigues da Cunha, Paula Cristina Natalino Rinaldi.

Project administration: Cleyton Batista de Alvarenga.

Software: Dalton Luiz Benz, George Deroco Martins, Jair Rocha do Prado.

Validation: Cleyton Batista de Alvarenga, Dalton Luiz Benz, Edson Aparecido dos Santos, George Deroco Martins, Jair Rocha do Prado.

Visualization: Cleyton Batista de Alvarenga.

Writing – original draft: Cleyton Batista de Alvarenga, Dalton Luiz Benz, Edson Aparecido dos Santos

, George Deroco Martins , Jair Rocha do Prado , João Paulo Arantes Rodrigues da Cunha , Paula Cristina Natalino Rinaldi .

Writing – review & editing: Cleyton Batista de Alvarenga , João Paulo Arantes Rodrigues da Cunha , Paula Cristina Natalino Rinaldi .

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