RESEARCH

Unmanned aerial vehicles to determine soybean plant injury caused by pre-emergence herbicides

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ABSTRACT

Images from unmanned aerial vehicles (UAV) can serve as a baseline for studies in weed science, complementing observations obtained in the ground. The objective of this work was to determine soybean (*Glycine max* (L.) Merr.) plant injury caused by pre-emergence herbicides in sandy and clayey soils, using a low-cost UAV. The experiment was conducted in a randomized complete block design, with four replicates and seven treatments consisted of herbicides (diclosulam, chlorimuron, sulfentrazone, flumioxazin, and *S*-metolachlor), hand weeded and untreated treatments. Ground-based evaluations were carried out to assess soybean crop injury, plant stand, leaf chlorophyll content, plant height, canopy distance and grain yield. Images were taken using a UAV equipped with an RGB (red green and blue) camera. Soybean plants sprayed with diclosulam had lower plant reflectance in the R (98.9), G (147.1) and B (74.3) range than the other treatments in sandy soil. In clayey soil, hand weeded treatment had higher plants (30.8 cm) and untreated favored smaller plants (24.9 cm) compared to herbicide treatments. In sandy soil, soybean yield of all treatments was similar, however in clayey soil, soybean yield treated with chlorimuron and flumioxazin was higher than 5000 kg ha^{-1} and better than the others treatments. The nutrient-poor soil (sandy) may have aggravated the plant injury caused by herbicides and explain the lower yield observed compared to clayey soil. It was determined soybean plant injury caused by pre-emergence herbicides with the use of unmanned aerial vehicles, providing complementary results to ground-based measurements, indicating the potential of this technology for low-cost evaluations in weed science.

Key words: Digital weed management, *Glycine max*, low-cost RGB sensor, post-application evaluation, spectral responses.

INTRODUCTION

Application of herbicides is the main method used for control of weeds in commercial fields around the world (Peterson et al., 2018; Moss, 2019). The use of pre-emergence herbicides has increased in the last years, but it requires extra care regarding crop injury (Heap and Duke, 2018; Kumar et al., 2018). Assessing crop injury after herbicide application is important to determine potential yield losses and choose solutions for its mitigation (Huang et al., 2018). Crop injury caused by pre-emergence herbicides is mostly dependent on the product rate and soil type (Jursík et al., 2015). Crops have complex responses to weeds, involving several agronomic interactions. Therefore, the identification of injuries caused by herbicides usually requires field evaluations by trained professionals, which makes the monitoring of large fields difficult for researchers and farmers (Riechers and Green, 2017).

Although ground-based evaluations are the main method to evaluate the effects of herbicides, practical alternatives for the evaluation of crop injury on a large scale can increase the efficiency of injury identification for different crops, soils, environments, and herbicide rates (Arnold et al., 2013). Remote sensing is among these alternatives; it has been recently used to determine crop responses to insecticides (Alves et al., 2017). However, the efficacy of using unmanned aerial vehicles (UAV) to determine crop responses to other agrochemicals in large fields is unknown.

Crop injury can be detected by remote sensing because stressors usually affect plant morpho-physiology characteristics (Prabhakar et al., 2011). Changes in plant reflectance at visible wavelengths may indicate effects on photosynthetic pigments. Vegetation indices are based on mathematical combinations of wavelengths reflected by plants. These equations include spatial and temporal patterns of vegetation photosynthetic activities that are related to canopy properties (Richardson et al., 1992). A combination of wavelengths into vegetation indices may improve the accuracy of predictions when compared to individual wavelengths (Richardson et al., 2002; Alves et al., 2015). Considering sensors that record only visible wavelengths, modified photochemical reflectance index (MPRI), photochemical reflectance index (PRI), and visible atmospherically resistant index (VARI) are the most common vegetation indices used in agriculture (Xue and Su, 2017). Studies have determined herbicide damages to crops, but none have included the use of UAV and pre-emergence herbicides simultaneously (Duddu et al., 2019; Zhang et al., 2019). In general, UAV are easier to use and cheaper for large-scale evaluations than other direct contact systems and ground observations. The UAV images can also be used for crop insurance companies to make legal reports and for quick evaluations of crop quality. Furthermore, UAV can operate in different soil types and rough areas (Saadatseresht et al., 2015).

The use of UAV in agriculture increased in the last decade and opened new opportunities to make weed management more efficient since it is possible to improve weed monitoring, weed control and crop injury evaluation. Therefore, we hypothesized that the use of UAV could be a useful tool to assess soybean herbicide effects, especially in large areas. This study was undertaken to determine soybean plant injury caused by pre-emergence herbicides in sandy and clayey soils, using a low-cost UAV and ground-based measurements.

MATERIALS AND METHODS

The study was conducted at commercial farms in Rio Verde (17°45'28.7" S, 51°02'06.6" W, 819 m a.s.l.) and Montividiu (17°26'37.2" S, 51°08'35.8" W, 878 m a.s.l.), Goiás State, Brazil, during the rainy season, with non-irrigated crops. One trial was conducted in a sandy soil (82% sand, 10% silt and 8% clay, pH [$CaCl_2$] 5.6, organic matter 1.4 g dm^{-3}, 61.3 mg P dm^{-3}, 23 mg K dm^{-3}, 2.3 $cmol_c$ Ca^{+2} dm^{-3}; 0.7 $cmol_c$ Mg^{+2} dm^{-3} and 2.1 H+Al $cmol_c$ dm^{-3}) located in Rio Verde and another in a clayey soil (20% sand, 12% silt and 68% clay, pH [$CaCl_2$] 5.7, organic matter 3.7 g dm^{-3}, 54 mg P dm^{-3}, 54 mg K dm^{-3}, 5.3 $cmol_c$ Ca^{+2} dm^{-3}; 1.9 $cmol_c$ Mg^{+2} dm^{-3} and 3.9 $cmol_c$ H+Al dm^{-3}) located in Montividiu. According to prior evaluations, the predominant weed species in the study area were Benghal dayflower (*Commelina benghalensis* L.), white eye (*Richardia brasiliensis* Gomes), southern sandbur (*Cenchrus echinatus* L.) and goose grass (*Eleusine indica* (L.) Gaertn.) A broad-spectrum herbicide (paraquat) was applied to the crops in both trials, at the label rate (400 g ha^{-1}), at 5 d before the establishing of the experimental plots.

The experiments were conducted in a randomized complete block design, with seven treatments and four replicates. Each plot had area of 4 × 4 m with soybean (*Glycine max* (L.) Merr.) plants grown from 'P96Y90' seeds (Pioneer Hi-Bred International Inc., Johnston, Iowa, USA), with spacing of 0.5 m between rows and 20 plants m^{-1}. The treatments consisted of applications of pre-emergence herbicides for soybean at the label rates diclosulam at 35.3 g ha^{-1} (*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro-[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide; Corteva Agriscience LLC, Midland, Michigan, USA), chlorimuron at 20 g ha^{-1} (2-[(4-chloro-6-methoxypyrimidin-2-yl)carbamoylsulfamoyl] benzoic acid; FMC Agricultural Caribe Industries Ltd., Manati, Puerto Rico) sulfentrazone at 200 g ha^{-1} (*N*-[2,4-dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-1,2,4-triazol-1-yl]phenyl]methanesulfonamide; FMC Corporation, Baltimore, Maryland, USA), flumioxazin at 50 g ha^{-1} (2-(7-fluoro-3-oxo-4-prop-2-ynyl-1,4-benzoxazin-6-yl)-4,5,6,7-tetrahydroisoindole-1,3-dione; Sumitomo Chemical Co., Ltd., Oita-shi, Oita, Japan) and *S*-metolachlor at 1728 g ha^{-1} (2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-[(2*S*)-1-methoxypropan-2-yl]acetamide; CABB AG, Pratteln, Switzerland); hand weeded and untreated (control treatments). The herbicides were applied under good environmental conditions at the soybean planting time (10 March 2018 for the clayey soil; and 10 August 2018 in the sandy soil), using a

CO_2-pressurized backpack sprayer containing a spray tip model TeeJet AIXR 110.015 (TeeJet Technologies, Glendale Heights, Illinois, USA), operated at a pressure of 275 kPa and calibrated to deliver 100 L ha^{-1} of solution.

The injuries (chlorosis + necrosis) caused by the herbicides were determined at 7, 14, 21, 28, and 35 d after planting (DAP) by rating soybean plants on a scale from 0 (no injury) to 100 (dead plant). Leaf chlorophyll contents were indirectly measured at 35 DAP, using a portable radiometer (GreenSeeker, Falcon Inc., Sunnyvale, California, USA). Plant stand (14 DAP), plant height, and canopy distance (35 DAP) were also evaluated. All measurements were carried out using the two central rows of each plot.

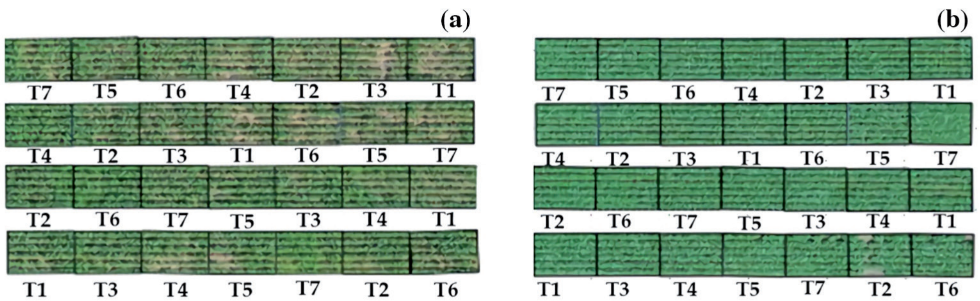
Trial flights were carried out at 35 DAP using a quadcopter UAV (Phantom 4 Advanced, DJI, Shenzhen, China) equipped with an RGB (red green and blue) camera (20 MP CMOS, DJI) with lateral and frontal overlaps of 80% (pixel of 1.0 cm). The flights were conducted at 30 m height between 10:00 and 11:00 h under cloudiness < 30%. The images were orthorectified using the Pix4D 3.2.23 program (Pix4D SA, Lausanne, Switzerland), and analyzed using an imaging program (QGIS Development Team, Boston, Massachusetts, USA). Common vegetation indices were calculated using the RGB bands (Table 1). Spectral reflectance from the sampling points was obtained by averaging the values of the pixels in six areas (0.05 × 0.05 m each) within the two central rows of each plot.

The data of injury, ground-based data, individual wavelengths and vegetation indices were subjected to ANOVA by the F-test ($\alpha = 0.05$), considering the blocks as a fixed effect. When the F-value was significant, the means were subjected to the Tukey's pairwise comparison test using the software Minitab 18 (Minitab Statistical Software).

RESULTS AND DISCUSSION

The reflectance of soybean plants in the different treatments was similar, regardless of the soil type (Figure 1), except for those subjected to application of the herbicide diclosulam, which had lower plant reflectance in the Red (98.9), Green (147.1) and Blue (74.3) range than the other treatments in the sandy soil (Figure 1, Table 2). Despite the effects on chlorophyll content (Table 3), changes at these visible wavelengths may have been affected by other photosynthetic pigments, such as carotenoids or anthocyanins (Carter and Knapp, 2001). Chlorimuron, sulfentrazone, flumioxazin and *S*-metolachlor did not affect soybean reflectance at individual wavelengths and vegetation indices, in the sandy soil (Table 2). Considering the researched literature, this study is the first report of potential effects of pre-emergence herbicides, based on remote sensing data.

Figure 1. Orthomosaic map with images of soybean plants to evaluate injuries caused by application of pre-emergence herbicides on sandy (a) and clayey (b) soils. Images taken 35 d after planting, in both trials.



T1: Diclosulam at 35.3 g ai ha^{-1}; T2: chlorimuron at 20 g ai ha^{-1}; T3: sulfentrazone at 200 g ai ha^{-1}; T4: flumioxazin at 50 g ai ha^{-1}; T5: *S*-metolachlor at 1728 g ai ha^{-1}; T6: hand weeded; T7: untreated.

Table 1. Vegetation index equations used to evaluate crop response in soybean experiments.

Index	Description	Equation	Reference
MPRI	Modified photochemical reflectance index	(G - R)/(G + R)	Yang et al. (2009)
PRI	Photochemical reflectance index	(B - G)/(B + G)	Gamon et al. (1997)
VARI	Visible atmospherically resistant index	(G - R)/(G + R - B)	Gitelson et al. (2003)

G: Green; R: red; B: blue.

The results found using images obtained from the quadcopter UAV (Table 2) were complementary with those of ground-truthing measurements (Tables 3 and 4). The spectral responses of soybean plants grown in the sandy soil showed some variability, which was unnoticed by the ground measurements. In the clayey soil, the hand weeded treatment had higher plants (30.8 cm) than the other treatments (Table 3). Additionally, with the application of herbicides plant height was greater than or equal to the untreated treatment (24.9 cm). The results obtained with the UAV could be used for weed evaluations as a baseline for detailed studies and analyses, revealing particularities that extend visual, ground-based observations. However, well-trained professionals are important to complement the image information by locally inspecting the area (Maes and Steppe, 2019; Marston et al., 2019). Moreover, the low injury rate observed during the evaluations carried out by a trained researcher may be unnoticed during inspections of large commercial fields.

Table 2. Reflectance of soybean plants, at visible wavelengths and derived vegetation indices (mean ± standard deviation), to application of pre-emergence herbicides on sandy and clayey soils, evaluated at 35 d after soybean planting.

Treatments	Red	Green	Blue	MPRI	VARI	PRI
			Sandy soil			
Diclosulam	98.9 ± 9.3b	147.1 ± 0.5b	74.3 ± 0.01b	0.201 ± 0.1a	0.286 ± 0.01ab	-0.340 ± 0.01a
Chlorimuron	107.1 ± 8ab	159.0 ± 9.2ab	82.5 ± 0.01ab	0.200 ± 0.01a	0.288 ± 0.01ab	-0.324 ± 0.04a
Sulfentrazone	111.8 ± 12.1ab	162.4 ± 7.7ab	84.0 ± 0.01ab	0.188 ± 0.1a	0.270 ± 0.01ab	-0.324 ± 0.02a
Flumioxazin	109.1 ± 4.8ab	162.6 ± 7.1ab	88.5 ± 0.01ab	0.205 ± 0.1a	0.303 ± 0.01a	-0.304 ± 0.05a
S-metolachlor	119.6 ± 8.3a	170.5 ± 9.0a	96.0 ± 0.01a	0.178 ± 0.1a	0.265 ± 0.01ab	-0.287 ± 0.03a
Untreated	121.8 ± 6.5a	174.0 ± 1.6a	98.0 ± 0.01ab	0.181 ± 0.01a	0.268 ± 0.01b	-0.292 ± 0.04a
Hand Weeded	115.9 ± 7.2a	166.5 ± 4.9a	81.2 ± 0.01a	0.183 ± 0.01a	0.254 ± 0.01ab	-0.357 ± 0.08a
			Clayey soil			
Diclosulam	88.4 ± 10.5a	156.6 ± 9.3a	81.0 ± 0.5a	0.284 ± 0.01a	0.426 ± 0.1a	-0.322 ± 0.03a
Chlorimuron	88.3 ± 8.6a	157.1 ± 8.0a	83.1 ± 9.2a	0.288 ± 0.01a	0.432 ± 0.01a	-0.316 ± 0.03a
Sulfentrazone	85.6 ± 13.6a	155.5 ± 12.1a	80.0 ± 7.7a	0.295 ± 0.01a	0.444 ± 0.1a	-0.325 ± 0.02a
Flumioxazin	86.5 ± 6.6a	155.6 ± 4.8a	79.4 ± 7.1a	0.289 ± 0.01a	0.430 ± 0.01a	-0.329 ± 0.03a
S-metolachlor	83.8 ± 9.2a	152.1 ± 8.3a	75.4 ± 9.0a	0.296 ± 0.01a	0.435 ± 0.1a	-0.344 ± 0.03a
Untreated	87.0 ± 5.4a	154.3 ± 6.5a	75.1 ± 1.6a	0.282 ± 0.01a	0.410 ± 0.01a	-0.349 ± 0.03a
Hand Weeded	79.5 ± 3.7a	145.9 ± 7.2a	73.6 ± 4.9a	0.304 ± 0.01a	0.445 ± 0.01a	-0.345 ± 0.02a

Means followed by the same letter in the columns within each soil type are not different by the Tukey's test ($p > 0.05$).
MPRI: Modified photochemical reflectance index; VARI: Visible atmospherically resistant index; PRI: Photochemical reflectance index.

Table 3. Plant stand, plant height, canopy distance, and chlorophyll content (mean ± standard deviation) of soybean crops at 35 d after application of pre-emergence herbicides on grown in sandy and clayey soils.

Treatments	Plant stand	Plant height	Canopy distance	Chlorophyll content
	Plants per plot	cm		Dimensionless
Sandy soil				
Diclosulam	20.5 ± 1.3a	19.3 ± 3.0a	10.6 ± 2.6a	72.8 ± 11.0a
Chlorimuron	19.0 ± 1.4a	20.7 ± 1.7a	8.9 ± 2.8a	75.0 ± 5.7a
Sulfentrazone	20.5 ± 0.6a	19.7 ± 1.3a	10.4 ± 2.1a	74.8 ± 6.5a
Flumioxazin	20.3 ± 0.5a	19.7 ± 1.9a	7.8 ± 3.7a	79.0 ± 2.8a
S-metolachlor	20.5 ± 1.9a	20.0 ± 1.7a	10.0 ± 2.3a	74.7 ± 4.3a
Untreated	20.0 ± 0.8a	20.1 ± 0.6a	8.1 ± 2.6a	72.35 ± 6.3a
Hand weeded	20.3 ± 1.0a	18.8 ± 1.7a	13.4 ± 3.7a	77.5 ± 4.4a
		Clayey soil		
Diclosulam	20.0 ± 0.1a	25.0 ± 1.5b	5.6 ± 1.9ab	80.9 ± 1.2a
Chlorimuron	19.0 ± 1.4a	24.9 ± 1.8b	6.3 ± 1.8ab	81.6 ± 0.8a
Sulfentrazone	19.3 ± 0.5a	26.3 ± 1.5b	4.4 ± 2.0b	81.9 ± 3.2a
Flumioxazin	19.8 ± 1.0a	26.9 ± 2.2b	3.2 ± 3.5b	82.3 ± 2.5a
S-metolachlor	19.5 ± 0.6a	26.7 ± 0.8b	4.0 ± 1.3b	83.7 ± 0.5a
Untreated	20.3 ± 1.0a	24.9 ± 2.1b	4.2 ± 0.9b	81.8 ± 1.9a
Hand weeded	21.0 ± 0.8a	30.8 ± 1.7a	9.5 ± 2.1a	84.7 ± 1.4a

Means followed by the same letter in the columns within each soil type are not different by the Tukey's test ($p > 0.05$).

Table 4. Soybean plant injury (mean ± standard deviation) caused by application of pre-emergence herbicides on sandy and clayey soils. Injury scale is between 0 (no injury) and 100 (dead plant).

Treatments	7 DAP	14 DAP	21 DAP	28 DAP	35 DAP
			Sandy soil		
Diclosulam	0	0.3 ± 0.5a	0.0b	0.8 ± 1.0a	0
Chlorimuron	0	0.5 ± 1.0a	1.5 ± 1.0a	1.3 ± 1.0a	0
Sulfentrazone	0	0.5 ± 1.0a	0.3 ± 0.5b	0.5 ± 1.0a	0
Flumioxazin	0	0.8 ± 1.0a	0.0b	0.3 ± 0.5a	0
S-metolachlor	0	1.0 ± 0.8a	0.8 ± 0.5ab	0.8 ± 1.0a	0
Untreated	0	0.0a	0.0b	0.0a	0
Hand weeded	0	0.0a	0.0b	0.0a	0
			Clayey soil		
Diclosulam	0	1.5 ± 1.0a	1.0 ± 1.2ab	0.3 ± 0.5ab	0
Chlorimuron	0	1.0 ± 1.2a	1.3 ± 1.5ab	0.3 ± 0.5ab	0
Sulfentrazone	0	0.5 ± 1.0a	2.5 ± 0.6a	1.0 ± 0.1a	0
Flumioxazin	0	0.5 ± 1.0a	0.5 ± 1.0ab	0.3 ± 0.5ab	0
S-metolachlor	0	1.0 ± 1.2a	1.5 ± 1.0ab	0.5 ± 0.6ab	0
Untreated	0	0.0a	0.0b	0.0b	0
Hand weeded	0	0.0a	0.0b	0.0b	0

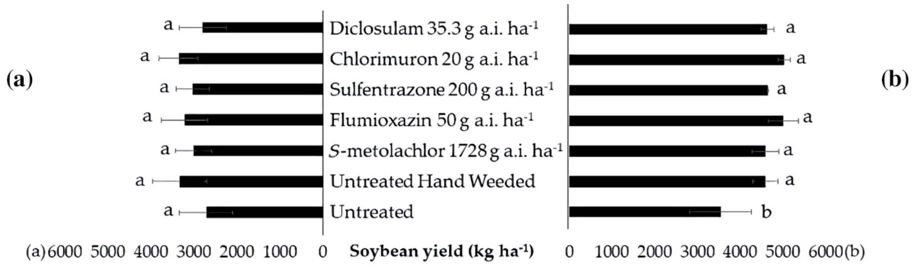
Means followed by the same letter in the columns within each soil type are not different by the Tukey's test ($p > 0.05$).
DAP: Days after application.

The pre-emergence herbicides caused no crop injuries at 7, 14, and 35 DAP, regardless of the soil type, presenting similar results to those of the control treatments (Table 4). In the sandy soil, chlorimuron and *S*-metolachlor caused slightly more plant injury than the other treatments at 21 DAP. In the clayey soil, sulfentrazone caused slightly more plant injury than the control treatments at 21 and 28 DAP (Table 4). Considering the evaluation times within each treatment, diclosulam, chlorimuron, and *S*-metolachlor caused minor injuries (≤ 1.0) in plants at 14 DAP in the sandy soil, which presented few chlorotic symptoms that disappeared at 35 DAP (Table 4). Diclosulam, chlorimuron, sulfentrazone, and *S*-metolachlor caused few injuries at the first evaluations in the clayey soil, and the few chlorotic symptoms also disappeared at 35 DAP. Similarly, flumioxazin caused nonsignificant soybean plant injury (≤ 0.5) in the clayey soil, in any evaluation time.

In the sandy soil, the soybean yield of the seven treatments was similar. In the clayey soil, the yield of soybean plants treated with pre-emergence herbicides was better compared to the untreated treatment or no weed control (3000 kg ha^{-1}) (Figure 2). The weed competition in that treatment caused a lower nutrient uptake by reducing water and nutrient availability to soybean plants (Thevathasan et al., 2000; Nadeem et al., 2018). The limited nutritional resources in the sandy soil may have aggravated the plant injury caused by pre-emergence herbicides and explain the lower yield observed with application of herbicides compared to clayey soil (Figure 2).

The evaluation of soybean plant injury caused by applications of pre-emergence herbicides can be carried out using UAV. Remote sensing can be used to facilitate weed management and provide timely identification of injuries caused by herbicides and information for other crop management practices (Robles et al., 2010). Further studies should evaluate carotenoids and anthocyanins to better understand the correlation of photosystems and modes of action of herbicides that may not be associated with chlorophyll contents. Furthermore, the results of the present study showed that pre-emergence herbicides applied at the label rates did not affect soybean plants, regardless of the soil type (Table 4). These pesticides could promote higher crop injury when applied at higher rates than those recommended for the crop and production environment (Jursík et al., 2015). Considering the soil types when deciding on the use of pre-emergence herbicides is important to prevent injuries caused by herbicides and to have an efficient weed control (Yamaji et al., 2016). Therefore, adjusting herbicide rates according to field environmental conditions and applying the products at the label rates is recommended.

Figure 2. Yield of soybean plants after application of pre-emergence herbicides on sandy (a) and clayey (b) soils.



Means separated by the same letter did not differ by the Tukey's test ($p > 0.05$).

CONCLUSIONS

It was determined soybean plant injury caused by pre-emergence herbicides with the use of unmanned aerial vehicles, providing complementary results to ground-based measurements, indicating the potential of this technology for low-cost evaluations in weed science.

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