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Assessing the effects of dicamba and 2,4 Dichlorophenoxyacetic acid (2,4D) on soybean through vegetation indices derived from Unmanned Aerial Vehicle (UAV) based RGB imagery

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ABSTRACT

The increase in agricultural production is facing several challenges with future implications for food security and environmental protection. The aim of this study was to evaluate a remote sensing-based low-cost methodology for assessing the effects of dicamba and 2,4 Dichlorophenoxyacetic acid (2,4D) in a non-tolerant soybean crop. Here, we introduced the application of six vegetation indices (VI) derived from Unmanned Aerial Vehicle (UAV) based Red-Green-Blue (RGB) imagery contrasting with a conventional approach of visual injury criteria classification to estimate soybean plant injury and the effect on grain yield. The results demonstrated the feasibility of Modified Green-Red Vegetation Index (MGRVI) and Excess Green (ExG) strongly correlated with the effects of dicamba and 2,4D in soybean. These VIs discriminated plant injury caused by dicamba and 2,4D up to 5% of the recommended dose. The Lethal Dose 50 (LD₅₀) considering the effect on grain yield was around 13% (72.80 g a.e. ha⁻¹), 55% (552.75 g a.e. ha⁻¹) and 48% (482.40 g a.e. ha⁻¹) for dicamba; 2,4D dimethylamine (DMA) and 2,4D choline (CHO) of the recommended dose, respectively. This study revealed noteworthy limitations for the RGB indices to discriminate between the effects of different formulations of the same herbicide, as for 2,4D DMA and 2,4D CHO. With expectations for the introduction of new genetic soybean events and alongside new synthetic auxin compounds, our results pointed out that the proposed methodology can lead to a protocol for identifying and estimating the damage to the off-target movement from these outcoming herbicides on neighbourhood fields.

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1. Introduction

The increase in agricultural production is facing several challenges over the past few years. A new generation of genetically modified crops with resistance to dicamba and 2,4 Dichlorophenoxyacetic acid (2,4D) herbicides, including soybean Xtend (Behrens et al. 2007) and Enlist (Zhou et al. 2016; Wright et al. 2010; Skelton et al. 2017), respectively, has been developed with expectations of improving weed control including glyphosate-resistant and -tolerant weeds. Along with the commercial introduction of these genetically modified crops, synthetic auxin herbicides have been rapidly adopted (Wells, Prostko, and Carter 2019). These compounds present a risk of drift for non-target crops in neighbouring fields (D. R. O. da Silva et al. 2018), which is worrisome for both food security and environmental protection. Many reports have shown that soybean can be highly sensitive to the herbicides dicamba and 2,4D (Egan et al., 2014; Kniss 2018; Weidenhamer, Triplett, and Sobotka 1989). Moreover, it has been demonstrated that grain yield reduction is highly correlated to visual injury ratings from dicamba and 2,4D, with a coefficient of determination (R^2) ranging from 0.95 to 0.99 (Robinson et al. 2013a; Robinson, Simpson, and Johnson 2013b). As reported, a 10% yield decrease was related to 35% visual injury observed at 14 days after treatment (DAT) and by 40%, 19%, and 15% injury observed at 28 DAT when soybean was exposed to 2,4D at V2, V5 and R2 growth stages, respectively (Robinson et al. 2013a). Simulated dicamba drift injured soybean and reduced grain yield by 71% and 90% at 5% and 10% of the recommended label dose, respectively (Huang, Lin Yuan, and Zhang 2016).

Early detection of injury from herbicide drift to non-target crops is critical in crop management. The early evaluation of herbicide symptoms is a valuable information for grain yield losses estimation. Although herbicide drifts are recognized worldwide as one of the major risks for crop security, the conventional approach for assessment its damage in crops has several limitations (Suarez, Apan, and Werth 2017). Unmanned Aerial Vehicle (UAV) or Aircraft System (UAS) was introduced in this work for soybean plant injury assessment from dicamba and 2,4D as an alternative to the conventional approach based on visual criteria classification. UAV-based remote sensing has been successfully employed to aid farmers' management (Hunt and Daughtry 2018), analyse dose–response relation to herbicides (Huang, Lin Yuan, and Zhang 2016) and crop yield estimation (Balota and Oakes 2016; Yu et al. 2016). True colour Red-Blue-Green (RGB) channel imageries were introduced for soybean crop monitoring in the last few years. For instance, the RGB-based vegetation index Excess Green (ExG) provided information about how canopy changes over time under different cropping conditions, thus enabling the identification of changes in soybean fields (Yun et al. 2016). Recently, the potential to estimate soybean canopy defoliation using RGB images taken in the field was demonstrated (Liang, Kirk, and Greene 2018). These approaches serve as examples to the potential use of RGB remotely sensed imagery for investigating herbicides damage in a non-target soybean crop and timely intervention with integrated crop management strategies. However, since vegetation indices (VI) and derivative indices used in previous studies were not explicitly designed for dicamba and 2,4D symptoms detection, a more thorough investigation is needed.

Biophysical and biochemical changes in plants can induce reflectance, transmittance, and absorbance spectral variations (Foster and Griffin 2018), which can be evaluated by

means of VIs. The Normalized Difference Vegetation Index (NDVI) is one of the most well-known vegetation indices, as it includes visible and near-infrared reflected electromagnetic radiation (Gracia-Romero et al. 2017; Thelen, Kravchenko, and Lee 2004). Among the numerous VIs, the most usual are those derived from multispectral (Ortiz et al. 2011; Huang et al. 2015; Huang and Thomson 2010) and hyperspectral sensors (Huang, Lin Yuan, and Zhang 2016; Henry et al. 2004). However, a large amount of investment is required for the development and maintenance of satellite-based sensors. Furthermore, the large volume of data generated by hyperspectral sensors makes current crop monitoring expensive, laborious, and time and hardware consuming. At present, the use of information derived from RGB images acquired from UAV cameras represents a time-saving and low-cost alternative (Gracia-Romero et al. 2018).

The need for assessing the effects of dicamba and 2,4D at large-scale crop fields is primarily based on the potential for the increasing use of new herbicide-resistant biotechnology soybean cultivars in the next years. Acknowledging these concerns, we use our data to investigate four questions: (1) could a true colour RGB sensor mounted in a UAV provide reliable information to identify and estimate the damage caused by dicamba and 2,4D in a non-tolerant soybean crop? (2) Is it possible to set up a dose-response relationship in soybean to estimate plant injury caused by these herbicides by applying vegetation indices derived from UAV-based RGB imagery? (3) Is the information content extracted from RGB vegetation indices suitable to predict the impact on soybean grain yield induced by dicamba and 2,4D? (4) Does this method allow discrimination between symptoms caused by dicamba or 2,4D? Seeking to answer these questions, this study aims to assess plant injury and the impact on grain yield caused by dicamba and 2,4D in a non-tolerant soybean crop by using UAV-based RGB vegetation indices.

2. Materials and methods

2.1. Characterization of the experimental area and treatments

A field experiment was conducted in the 2017/2018 growing season at the Agronomic Experimental Station (30°07'17"S; 51°41'12"W) of the Federal University of Rio Grande do Sul (Eldorado do Sul, southern Brazil) (Figure 1), with the glyphosate-resistant genotype BMX Potência RR (Brasmax, GDM Genética do Brasil).

The experiment was carried out in a randomized block design in a factorial scheme. Factor A consisted of the herbicides Dicamba (diglycolamine salt of dicamba; 3,6-dichloro-*o*-anisic acid, *XtendiMax*[®] *VaporGrip*[®], 350 g L⁻¹ acid equivalent, Monsanto Company, St. Louis, MO, USA); 2,4D choline salt (2-hydroxy-N,N,N-trimethylethanaminium (2,4-Dichlorophenoxy) acetate, *Enlist*TM *Colex-D*[®]; 456 g L⁻¹ acid equivalent, Dow AgroSciences Industrial Ltda., Barueri, SP, Brazil) (CHO) and 2,4D dimethylamine salt (dimethylammonium (2,4-Dichlorophenoxy) acetate, *DMA*[®] 806 BR, 806 g L⁻¹, Dow AgroSciences Industrial Ltda., São Paulo, SP, Brazil) (DMA). Factor B consisted of different doses applied (zero, 1%, 5%, 10%, 20%, 40%, 60%, 80%, and 100% of the maximum-recommended label rate of each herbicide, being 560 and 1005 g a.e. ha⁻¹ for dicamba and 2,4D, respectively). Each plot comprised five rows of 10 m in length, with a row spacing of 0.45 m (Figure 1). Herbicide applications were made at V6 growth stage (six nodes at the main stem with fully developed leaves) from 8:30 a.m. to 9:45 a.m. with



Figure 1. Characterization of the study area: (a) overview of Brazil country and part of South America; (b) representative area of the experimental plots; (c) a high-resolution UAV-based orthophoto mosaic of the experimental field at 8 days after herbicide treatments (8 DAT); (d) plot under treatment with 5% 2,4D choline; (e) untreated control; (f) plot under treatment with 5% dicamba; (g) plot under treatment with 10% 2,4D dimethylamine. (page 7).

temperature variation between 23°C and 27°C, relative humidity of 60% to 75%, and wind speed below 1 m s^{-1} . Applications were made using a CO_2 -pressurized backpack sprayer with AFXR 100–02 nozzles at 200 kPa, delivering 200 L ha^{-1} of herbicide solution. The choice on the V6 stage was conditioned to most critical time regarding crop stage, climatic conditions, and the application on surrounding fields that could impact herbicide drift. Thus, we anticipated for the coexistence among the UAV flights and the summery season, that occurred earlier and mid-January 2018.

Before sowing, seed treatment was performed with triadimenol fungicide (0.0027 L kg^{-1}), imidacloprid insecticide (0.001 L kg^{-1}), and inoculation with *Bradyrhizobium Japonicum* inoculant (0.003 L kg^{-1} – SEMIA 5079 and 5080 strains (Nitragin Cell Tech HC)). Insect pest control was performed with the biological insecticide *Bacillus thuringiensis var. kurstaki* HD-I strain at 0.5 L ha^{-1} (Dipel) and Karate Zeon 50 insecticide. Disease control was performed with trifloxystrobin + prothioconazole (Fox, $150 + 175 \text{ g L}^{-1}$, Bayer S. A. São Paulo, SP, Brazil) at $60 + 70 \text{ g ha}^{-1}$ and the adjuvant-methylated seed oil (Aureo, 720 g L^{-1} , Bayer S. A. São Paulo, SP, Brazil) at 0.25% volume per volume (v v^{-1}) at the beginning of flowering and 15 days later. Weed control was provided by the application of glyphosate herbicide (isopropylamine salt, N-(phosphonomethyl) glycine, Glifosato Nortox, 480 g L^{-1} , Nortox S.A., Arapongas, PR, Brazil) at 1080 g ha^{-1} plus hand weeding. Sprinkler irrigation was used when necessary to supplement natural rainfall.

2.2. Data acquisition

Evaluation was performed on spectral data (digital images) acquired from a UAV-based RGB sensor, plant injury analysis, and grain yield. The UAV system comprised a DJI™ Zenmuse X3 RGB sensor (12.4 megapixel digital camera) onboard on a DJI™ Matrice 100 quadcopter, equipped with Inertial Navigation System (IMU) and Global Satellite Navigation System (GNSS). The UAV was configured for automated measurements at 50 m above the ground with an average speed of 6 m s⁻¹ and 80% lateral and frontal overlaps. Flights were carried out concurrently to the plant injury analysis between 10:00 a.m. and 10:30 a.m. Plant injury of soybean associated with the effect of herbicides was assigned based on visual estimative from zero being no injury to 100% standing for complete necrosis and plant death. All evaluations were performed at 8, 15, and 56 DAT. Grain yield was determined in an area of 3.60 m² in each plot, and the grains weight was expressed at 130 g kg⁻¹ of moisture.

2.3. Digital image processing

Digital images pre-processing was performed with PhotoScan Professional Edition v.1.4.0 (Agisoft LLC, St. Petersburg, Russia) software according to the following workflow: i) Loading Images: Images were imported with their corresponding altitude data determined by the IMU, plus the ellipsoidal height and geographic coordinates (WGS84 datum) of its central point measured by the GNSS receiver; ii) Aligning Images: Once the images were loaded, PhotoScan found the camera position and orientation for each image (Geotags); then, a sparse point cloud model was built. This model carries with it the digital numbers (DN) of the respective spectral s (Red, Green, and Blue) and elevation related to the flight surface in its dataset; iii) Alignment Optimization: As poor input can influence the alignment result negatively, this step was performed to improve the camera position estimates. Thus, it was possible to get the georeferenced precision from this image block optimization, and was settled a coordinate reference system to the orthomosaic; iv) Building Dense Point Cloud: New depth points were set based on the estimated image positions and sparse points model, then combined into a single dense point cloud; v) Building Mesh: A polygonal model (mesh) was rebuilt based on the dense point cloud; vi) Building Orthomosaic: As a result, the orthomosaic building allowed the generation of a 3 cm pixel high-resolution imagery based on the source images and reconstructed mesh model. The orthomosaic of the study area was exported in Geotiff archive format, with Universal Transverse Mercator (UTM) coordinate system, Zone 22S, in the WGS84 reference system.

After the initial processing described above, six vegetation indices were derived by applying band arithmetic on software QGIS Las Palmas v.2.18: Carotenoid Reflectance Index 1 (CRI₁; Equation (1)) (Gitelson, Yoav Zur, and Merzlyak 2002), Excess Green (ExG; Equation (2)) (Meyer and Neto 2008; Zheng et al. 2017), Excess Green minus Excess Red (ExGR; Equation (3)) (Ballesteros et al. 2018; Meyer and Neto 2008; Zheng et al. 2017), Modified Green-Red Vegetation Index (MGRVI; Equation (4)) (Bendig et al. 2015), Modified Photochemical Reflectance Index (MPRI; Equation (5)) (Li, Li, and Sun 2014) and Red-Green-Blue Vegetation Index (RGBVI; Equation (6)) (Bareth et al. 2016; Bendig et al. 2015). The vegetation indices were calculated as follows:

$$CRI_1 = \frac{1}{\rho_{Blue}} - \frac{1}{\rho_{Green}} \quad (1)$$

$$ExG = 2\rho_{Green} - \rho_{Red} - \rho_{Blue} \quad (2)$$

$$ExGR = (ExG) - 1.4\rho_{Red} - \rho_{Green} \quad (3)$$

$$MGRVI = \frac{\rho_{Green}^2 - \rho_{Red}^2}{\rho_{Green}^2 + \rho_{Red}^2} \quad (4)$$

$$MPRI = \frac{\rho_{Green} - \rho_{Red}}{\rho_{Green} + \rho_{Red}} \quad (5)$$

$$RGBVI = \frac{\rho_{Green}^2 - \rho_{Blue} \times \rho_{Red}}{\rho_{Green}^2 + \rho_{Blue} \times \rho_{Red}} \quad (6)$$

where ρ_{Red} , ρ_{Green} and ρ_{Blue} are the reflectance values of the Red, Green, and Blue bands. The VIs were all normalized between 1 and -1 in order to easily compare among them.

2.4. Statistical analysis

Initially, data were tested for Shapiro-Wilk normality and Hartley homoscedasticity. Grain yield data were subjected to a two-way ANOVA ($p \leq 0.05$) to test for effects of dose, dicamba and 2,4D treatments, and the interaction between dose and herbicides related to plant injury at 8, 15, and 56 DAT. Herbicide doses were analysed by a three or four parameters log-logistic model:

$$f(x, (b, d, e)) = d / (1 + (x/e)^b) \quad (7)$$

$$f(x, (b, c, d, e)) = c + (d - c) / (1 + (x/e)^b) \quad (8)$$

where ' $f(x(b,c,d,e))$ ' is the dependent variable (grain yield (kg ha^{-1})); ' x ' is the independent variable (recommended dose of herbicide (%)); ' b ', ' c ', ' d ', and ' e ' are the parameters. The parameter ' e ' refers to the dose producing a response half-way between the upper limit ' d ' and lower limit ' c ', i.e., the parameter ' e ' is the 50% lethal dose (LD_{50} ; %); and parameter ' b ' is the slope of the curve. The LD_{50} were obtained through an arithmetic calculation according to the parameters generated in the model equations.

The relationship of the RGB vegetation indices with plant injury and soybean grain yield was assessed by linear and quadratic regression analyses. As input data for the VIs, the arithmetic mean of the pixels within a corresponding area of 11.44 m^2 in each experimental plot was calculated (Figure 1). Finally, dose-response curves of a three (Equation (7)) or four parameters (Equation (8)) log-logistic model were carried out for VIs that were mostly correlated with plant injury and grain yield.

All described statistical analyses were done through R v.3.6.2 (The R Foundation for Statistical Computing, Vienna, Austria) by using the caret (Kuhn 2020), tidyverse (Wickham et al. 2019) and drc (Ritz et al. 2015) packages.

3. Results

3.1. Drift effect on grain yield

The three parameters log-logistic model (Equation (7)) fitted properly to describe the effect of dicamba and DMA and CHO 2,4D in soybean grain yield (Figure 2), as both herbicide and treatment dose effects were significant ($p < 0.05$).

Dicamba treatments reduced soybean grain yield from the 5% dose, decreasing on average by 12%, 13%, 41%, and 77% with 1%, 5%, 10%, and 20% treatment doses, respectively. In contrast, no grain was produced when plants received from 40% to 100% of dicamba. For both 2,4D DMA and CHO formulations, grain yield significantly decreased only after the doses of 20% (Figure 2). The LD_{50} for dicamba; 2,4D DMA and 2,4D CHO was around 13%, 55%, and 48% of the recommended label dose of these auxin herbicides (Figure 2).

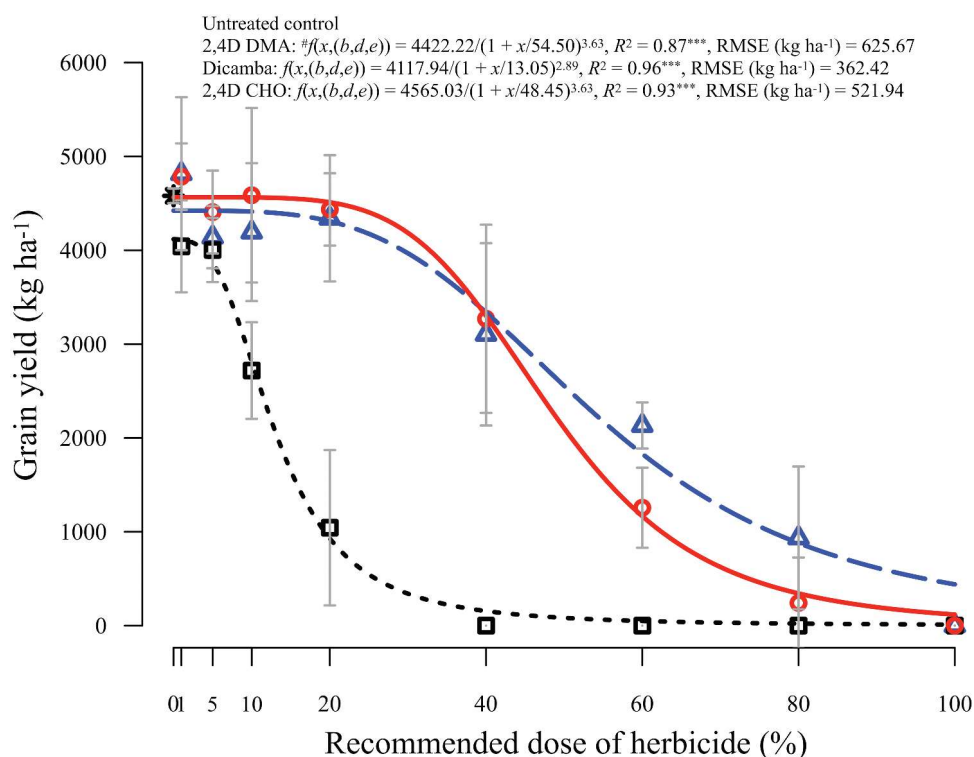


Figure 2. Dose-response curves of a three parameters[#] log-logistic model describing the soybean grain yield (kg ha⁻¹) according to applied doses (%) of dicamba (100% = 560 g a.e. ha⁻¹); 2,4D dimethylamine (DMA) (100% = 1005 g a.e. ha⁻¹) and 2,4D choline (CHO) (100% = 1005 g a.e. ha⁻¹). Gray bars correspond to the standard error. [#]Three parameters log-logistic model: $f(x,(b,d,e)) = d/(1 + (x/e)^b)$, where 'f(x,(b,d,e))' is grain yield (kg ha⁻¹); 'x' is the herbicide dose (%); 'b' is the curve slope; and parameter 'e' refers to the dose producing a response half-way between the upper limit 'd' and lower limit 'c' (which by definition is equal to zero), i.e., the parameter 'e' is the 50% lethal dose (LD_{50} ; %). ***Significance by t-test ($p = 0.01$). Grain yield for untreated control was 4581 kg ha⁻¹. (page 11).

3.2. Plant injury

The levels of soybean plant injury related with dicamba and 2,4D treatments were significantly ($p < 0.05$) described by quadratic models, shown a close relationship ($R^2 \geq 0.82$) with the applied doses of these herbicides at 8, 15 and 56 DAT (Figure 3). For dicamba, the model higher performed at 8 DAT with $R^2 = 0.93$ and with a lower Root Mean Square Error (RMSE = 8.88%). Although models for 2,4D herbicides achieved high results at 8 DAT, the performance for both DMA and CHO formulations was pronounced ($R^2 = 0.98$ and 0.97 , respectively) at 15 DAT as its RMSE reduced to 5.62% and 4.84%, respectively.

In general, the soybean plant injury was increased as the dose of herbicides increases. However, the injury levels shifted concerning the evaluation time after treatment (Figure 3). At 8 DAT, plant injury was similar for 2,4D and dicamba treatments, mainly up to the dose of 80% (Figure 3). At 15 DAT, dicamba presented higher plant injury than 2,4D and reached close to 100% plant injury at the dose of 40%, while 2,4D DMA and CHO reached approximately 60% injury. At 56 DAT, the plant injury decreased to 2,4D; however, it noticed increasing injury for dicamba since the lower evaluated dose (Figure 3).

The soybean grain yield as a function of plant injury associated with the effects of herbicides treatment was also described in most instances by quadratic regression (Figure 4). The results showed that soybean grain yield was strongly correlated ($R^2 \geq 0.83$) to plant injury regarding the tested herbicides and evaluation time after treatment. For dicamba and 2,4D CHO, the models fitted higher ($R^2 = 0.95$ and 0.91 , respectively) at 56 DAT. For 2,4D DMA, the higher ($R^2 = 0.89$) performance was achieved at 15 DAT. At 8 and 15 DAT for the same level of injury dicamba decreased the grain yield more than 2,4D.

3.3. Vegetation indices

The six RGB vegetation indices obtained from the UAV-based imagery fitted significantly ($p < 0.05$) for soybean plant injury and grain yield concerning the treatments with dicamba, and both 2,4D herbicides. The results suggested that the VIs were strongly correlated with each other considering herbicides and evaluation time after treatments (Figures 5 and Figures 6).

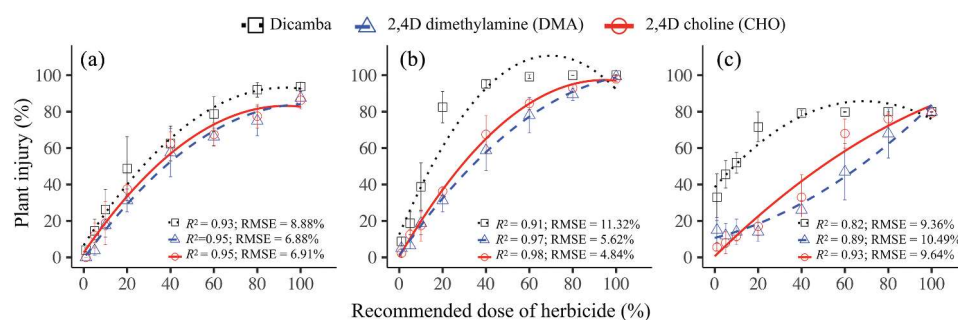


Figure 3. Relationship between soybean plant injury (%) and doses (%) of dicamba; 2,4D dimethylamine (DMA) and choline (CHO) formulations at (a) 8 days after treatment (DAT), (b) 15 DAT, and (c) 56 DAT. Bars correspond to the standard error. (page 11).

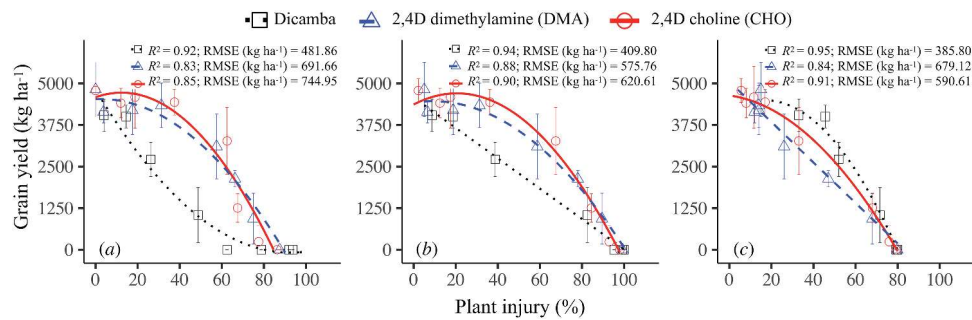


Figure 4. Soybean grain yield (kg ha^{-1}) as a function of plant injury (%) associated with dicamba; 2,4D dimethylamine (DMA) and 2,4D choline (CHO) at (a) 8 days after treatment (DAT), (b) 15 DAT, and (c) 56 DAT. Bars correspond to the standard error. (page 12).

For plant injury, the relationship with the indices was generally higher at 15 DAT. The range of R^2 (0.85 to 0.94) and RMSE values (8.76% to 14.27%) of the regression analyses suggested that five VIs (ExG, ExGR, MGRVI, MPRI, and RGBVI) could estimate plant damage with satisfactory performance. Curiously, MGRVI and MPRI showed a very narrow performance improvement (upper R^2 and lower RMSE) concerning dicamba and 2,4D DMA at 8 DAT (Figure 5). Importantly, these indices were the ones that most correlated with the symptoms of plant injury caused by dicamba and 2,4D over the three evaluated dates after treatments.

Similarly to plant injury results, when evaluating the relationship between VIs and final grain yield, it was observed that the R^2 for ExG, ExGR, MGRVI, MPRI, and RGBVI was positive and had high values (Figure 6). Considering the high relationship between plant injury and grain yield as showed in Figure 4 and the results from VIs versus plant injury (Figure 5), it could be expected that both MGRVI and MPRI models achieved superior performance contrasting to the other VIs. At 8 DAT, the use of MGRVI to predict soybean grain yield from the effects of herbicides resulted in higher accuracy ($R^2 = 0.90, 0.90$ and 0.88 and $\text{RMSE} = 535.51 \text{ kg ha}^{-1}, 546.22 \text{ kg ha}^{-1}$ and $652.24 \text{ kg ha}^{-1}$ for dicamba, DMA, and CHO 2,4D, respectively). The performance by applying the MPRI was as high as for the MGRVI index (Figure 6). Accurate grain yield predictions were generally obtained when images were taken at 15 DAT, although higher results for 2,4D DMA treatments curiously occurred at the earlier stage (8 DAT).

After identifying that in general MGRVI, MPRI, and ExG established a superior relationship with plant injury and grain yield, dose-response was tested for the three- (Equation (7)) and four-parameter (Equation (8)) log-logistic models. Dose-response curves of VIs are then illustrated in Figure 7 for the MGRVI and ExG four-parameter models as in most instances the parameter 'b' (curve slope) of the three-parameter models was not significant ($p > 0.05$). Details on the parameters of the log-logistic models describing the dose-response of MGRVI, MPRI, and ExG to the tested herbicides are described in the supplemental material. MPRI curves were not shown because its fit performance did quite similar to MGRVI, where MGRVI models reached higher R^2 (Table 1). Therefore, discussion was made on MGRVI due to this index being an MPRI transformation supported by assumptions that squaring band reflectance values helps increase red, green, and blue reflectance differences (Bendig et al. 2015).

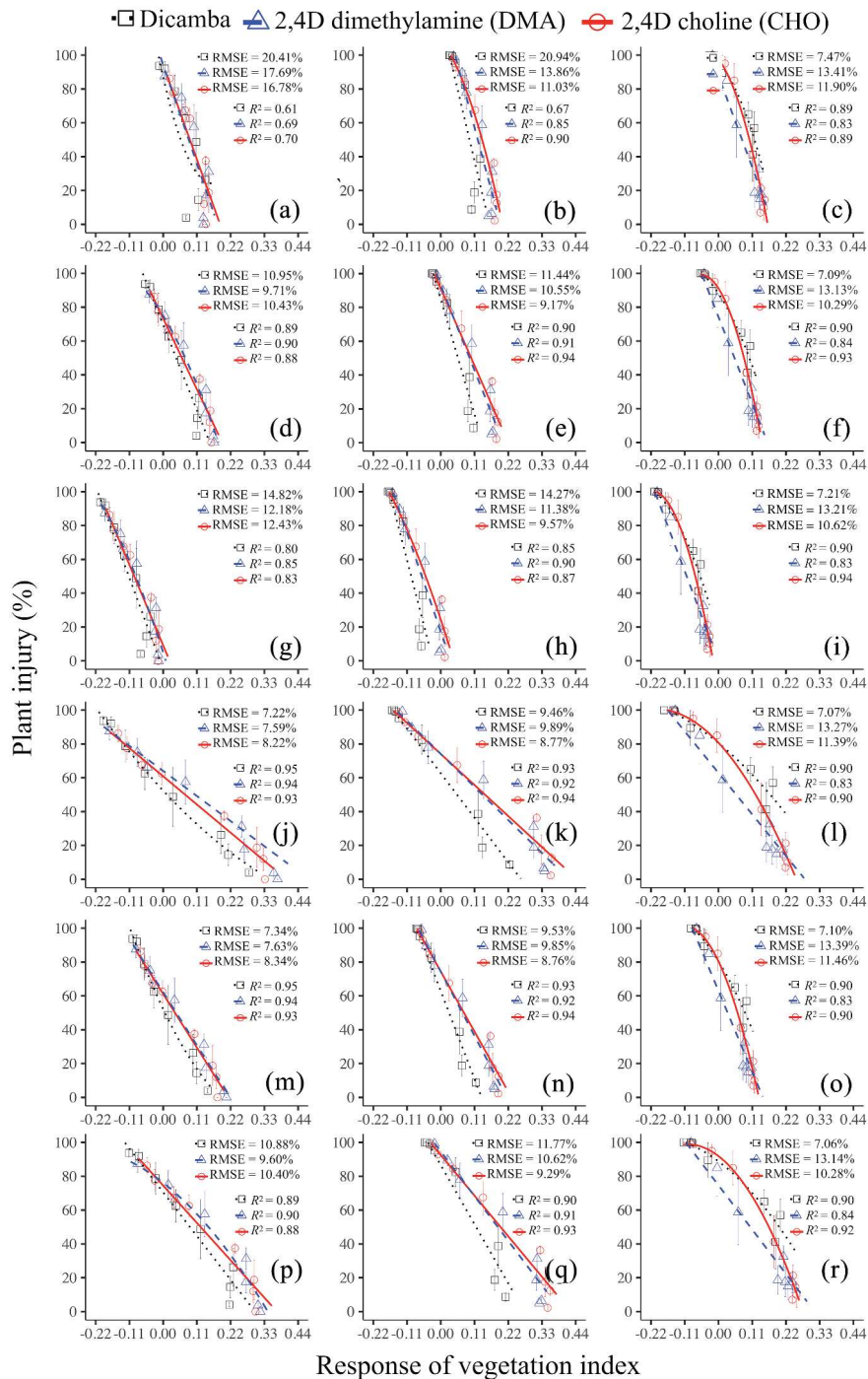


Figure 5. Relationship between soybean plant injury (%) and RGB vegetation indices according to applied doses (%) of dicamba; 2,4D dimethylamine (DMA) and 2,4D choline (CHO): left figures (a) Carotenoid Reflectance Index 1 (CRI₁), (d) Excess Green (ExG), (g) Excess Green minus Excess Red (ExGR), (j) Modified Green-Red Vegetation Index (MGRVI), (m) Modified Photochemical Reflectance Index (MPRI) and (p) Red-Green-Blue Vegetation Index (RGBVI) are results from 8 days after treatment (DAT); central figures (b) CRI₁, (e) ExG, (h) ExGR, (k) MGRVI, (n) MPRI and (q) RGBVI are results from 15 DAT; right figures (c) CRI₁, (f) ExG, (i) ExGR, (l) MGRVI, (o) MPRI and (r) RGBVI are results from 56 DAT. (page 13).

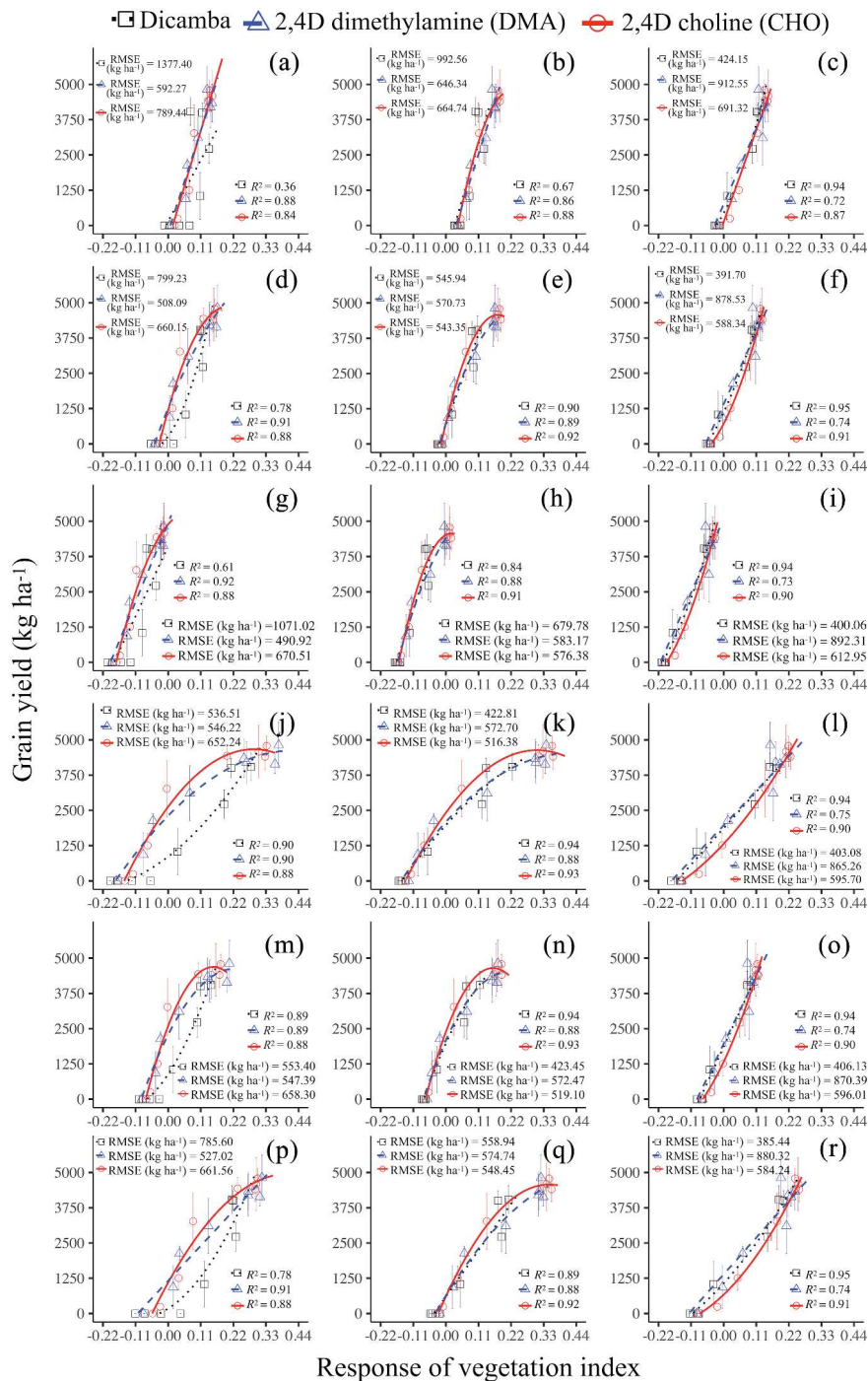


Figure 6. Relationship between soybean grain yield (kg ha^{-1}) and RGB vegetation indices according to applied doses (%) of dicamba; 2,4D dimethylamine (DMA) and 2,4D choline (CHO): left figures (a) Carotenoid Reflectance Index 1 (CRI_1), (d) Excess Green (ExG), (g) Excess Green minus Excess Red (ExGR), (j) Modified Green-Red Vegetation Index (MGRVI), (m) Modified Photochemical Reflectance Index (MPRI) and (p) Red-Green-Blue Vegetation Index (RGBVI) are results from 8 days after treatment (DAT); central figures (b) CRI_1 , (e) ExG, (h) ExGR, (k) MGRVI, (n) MPRI and (q) RGBVI are results from 15 DAT; right figures (c) CRI_1 , (f) ExG, (i) ExGR, (l) MGRVI, (o) MPRI and (r) RGBVI are results from 56 DAT. (page 13).

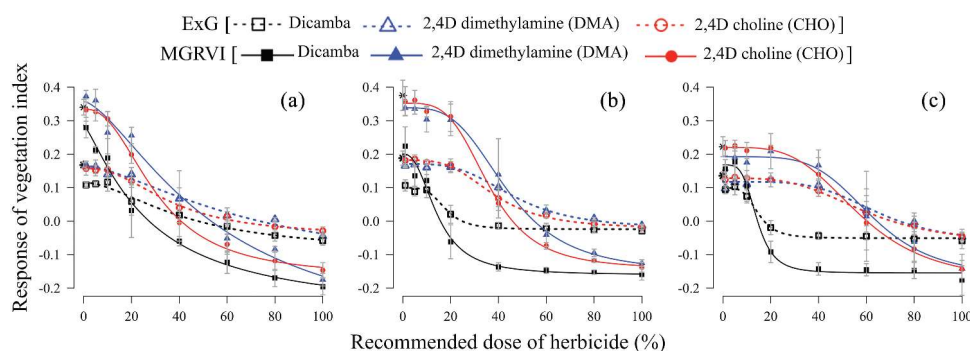


Figure 7. Dose-response curves of a four parameters[#] log-logistic model describing the response of vegetation indices Modified Green-Red Vegetation Index (MGRVI) and Excess Green (ExG) associated with applied doses (%) of dicamba (100% = 560 g a.e. ha⁻¹); 2,4D dimethylamine (DMA) (100% = 1005 g a.e. ha⁻¹) and 2,4D choline (CHO) (100% = 1005 g a.e. ha⁻¹) at (a) 8 days after treatment (DAT) (b) 15 DAT (c) 56 DAT. Bars correspond to the standard error. [#]Four parameters log-logistic model: $f(x,b,c,d,e) = c + (d - c)/(1 + (x/e)^b)$, where 'f(x(b,c,d,e))' is the response of vegetation index; 'x' is the herbicide dose (%); 'b' is the curve slope; and parameter 'e' refers to the dose producing a response half-way between the upper limit 'd' and lower limit 'c', i.e., the parameter 'e' is the 50% lethal dose (LD₅₀; %). (page 14).

In general, the response of MGRVI and ExG decreased for both dicamba and 2,4D compared to the untreated control, although the decrease was superior for dicamba since lower doses throughout 8, 15, and 56 days after treatment (Figure 7). At 8 DAT, ExG demonstrated to be able to discriminate between the damage level caused by dicamba and 2,4D up to 5% dose (Figure 7(a)), as was successfully achieved by MGRVI for all doses only at 15 DAT (Figure 7(b)). The discrimination between the effects of dicamba and 2,4D for ExG at 15 DAT was achieved by up to 60% dose. As for 56 DAT, the VIs response significantly shifted down even for the untreated control. Moreover, separability between treatments only occurred for moderate doses (about 20% to 60%) of herbicides (Figure 7 (c)). The VIs were not suitable to assign significant differences in response between DMA and CHO 2,4D formulations.

4. Discussion

4.1. Dicamba and 2,4D effects on soybean

Dicamba can injury non-tolerant (susceptible) soybean by doses from 0.56 to 560 g a.e. ha⁻¹, which consequently induced significant losses in grain yield (Figure 2), how has been reported by several studies (Foster and Griffin 2018; D. R. O. da Silva et al. 2018; Robinson, Simpson, and Johnson 2013b). Even soybean plants receiving dicamba at 0.56 g (1% of the recommended dose) presented plant injury (Figure 3). The decreasing in grain yield for up 0.56 g a.e. ha⁻¹ dose surpassed 12%, with over to 40% at 112 g a.e. ha⁻¹ (Figure 2), meaning that a drift event of dicamba on those non-dicamba-tolerant soybean plants will cause critical damage and significant yield loss.

The effect of sublethal doses of 2,4D in the tested non-2,4D-tolerant soybean suggested a hormesis phenomenon (Belz and Duke 2014; J. R. O. Silva et al. 2019). It was

Table 1. Performance of the four parameters⁸ log-logistic models describing the dose-response of the vegetation indices Excess Green (ExG), Modified Green-Red Vegetation Index (MGRVI) and Modified Photochemical Reflectance Index (MPRI) to dicamba (DIC); 2,4D dimethylamine (DMA) and 2,4D choline (CHO) at (a) 8 days after treatment (DAT), (b) 15 DAT, and (c) 56 DAT. The performance of each model was expressed by means of the coefficient of determination (R^2) and the Root Mean Square Error (RMSE).

DAT	Dose-response model of vegetation index	R^{2***}	RMSE
8	$ExG_{DIC} = -0.09 + (0.21)/(1 + (x/42.61)^{1.75})$	0.9167	0.0201
	$MGRVI_{DIC} = -0.28 + (0.57)/(1 + (x/27.09)^{1.27})$	0.9531	0.0385
	$MPRI_{DIC} = -0.14 + (0.29)/(1 + (x/26.68)^{1.26})$	0.9516	0.0201
	$ExG_{DMA} = -0.15 + (0.31)/(1 + (x/68.99)^{1.60})$	0.9383	0.0194
	$MGRVI_{DMA} = -0.47 + (0.85)/(1 + (x/64.68)^{1.30})$	0.9553	0.0430
	$MPRI_{DMA} = -0.27 + (0.49)/(1 + (x/67.98)^{1.15})$	0.9537	0.0235
	$ExG_{CHO} = -0.04 + (0.20)/(1 + (x/36.73)^{2.32})$	0.9528	0.0166
	$MGRVI_{CHO} = -0.17 + (0.50)/(1 + (x/30.27)^{2.36})$	0.9789	0.0287
15	$MPRI_{CHO} = -0.08 + (0.26)/(1 + (x/29.43)^{2.32})$	0.9769	0.0157
	$ExG_{DIC} = -0.02 + (0.12)/(1 + (x/17.75)^{4.17})$	0.9323	0.0148
	$MGRVI_{DIC} = -0.16 + (0.36)/(1 + (x/14.42)^{2.48})$	0.9414	0.0362
	$MPRI_{DIC} = -0.08 + (0.18)/(1 + (x/14.31)^{2.41})$	0.9403	0.0185
	$ExG_{DMA} = -0.02 + (0.18)/(1 + (x/46.60)^{3.96})$	0.9503	0.0167
	$MGRVI_{DMA} = -0.14 + (0.47)/(1 + (x/43.98)^{3.96})$	0.9545	0.0414
	$MPRI_{DMA} = -0.07 + (0.24)/(1 + (x/43.68)^{3.90})$	0.9531	0.0216
	$ExG_{CHO} = -0.02 + (0.20)/(1 + (x/38.03)^{3.50})$	0.9780	0.0127
56	$MGRVI_{CHO} = -0.14 + (0.50)/(1 + (x/36.20)^{3.79})$	0.9831	0.0277
	$MPRI_{CHO} = -0.07 + (0.26)/(1 + (x/35.81)^{3.74})$	0.9808	0.0152
	$ExG_{DIC} = -0.05 + (0.15)/(1 + (x/14.68)^{4.16})$	0.9438	0.0161
	$MGRVI_{DIC} = -0.15 + (0.32)/(1 + (x/14.21)^{4.12})$	0.9508	0.0321
	$MPRI_{DIC} = -0.08 + (0.16)/(1 + (x/14.16)^{4.11})$	0.9491	0.0167
	$ExG_{DMA} = -0.06 + (0.17)/(1 + (x/64.50)^{4.86})$	0.8336	0.0266
	$MGRVI_{DMA} = -0.16 + (0.35)/(1 + (x/62.62)^{5.03})$	0.8255	0.0567
	$MPRI_{DMA} = -0.08 + (0.18)/(1 + (x/62.38)^{5.07})$	0.8221	0.0293
$ExG_{CHO} = -0.07 + (0.20)/(1 + (x/59.47)^{3.51})$	0.9421	0.0165	
$MGRVI_{CHO} = -0.18 + (0.40)/(1 + (x/57.34)^{3.77})$	0.9336	0.0379	
$MPRI_{CHO} = -0.09 + (0.21)/(1 + (x/56.94)^{3.77})$	0.9332	0.0195	

⁸Four parameters log-logistic model: $f(x, b, c, d, e) = c + (d - c)/(1 + (x/e)^b)$; where ' $f(x, b, c, d, e)$ ' is the response of vegetation index; ' x ' is the herbicide dose (%); ' b ' is the curve slope; and parameter ' e ' refers to the dose producing a response half-way between the upper limit ' d ' and lower limit ' c ', i.e., the parameter ' e ' is the 50% lethal dose (LD_{50} ; %).

p -value significance codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 ''.

reported that 2,4D induced some stimulating effects on soybean plants (Schabenberger et al. 1999; Morr  2000; Belz and Duke 2014), as the increase in grain yield from 1 g a.e. ha⁻¹ for DMA and CHO 2,4D treatments (Figure 2). Despite the visual symptoms of plant injury ranging from 0 (8 DAT) to 15% (56 DAT) and from 0 (8 DAT) to 6% (56 DAT) for 2,4D

DMA and CHO (Figure 3), respectively, the soybean grain yield increased 229 kg ha^{-1} (5%) on average related to the untreated control. Although low doses may stimulate plant development, thus characterizing the hormetic effect, high doses of 2,4D significantly decreased grain yield (Egan, Barlow, and Mortensen 2014). The findings outcoming from this paper agree with other works reporting both stimulatory or inhibit effects from low to high concentrations of 2,4D and other synthetic auxin herbicides on soybean (Solomon and Bradley 2014; J. R. O. Silva et al. 2019).

The LD_{50} for dicamba considering the effect on grain yield was around 13% (72.80 g a.e. ha^{-1}) of the recommended dose. The same effect for 2,4D DMA and CHO occurred with around 55% (552.75 g a.e. ha^{-1}) and 48% (482.40 g a.e. ha^{-1}) of the recommended dose (Figure 2). These results suggest how a drift event from an auxin herbicide, especially dicamba, can be active in non-tolerant soybean genotypes. Moreover, it must be considered for the potential impact from moderate to high doses of both 2,4D DMA and CHO when applied at V6 growth stage. Recent research suggested that 2,4D (including on Choline formulation) doses higher than those inducing hormetic effects seem to compromise soybean yield components more strongly when applied in early vegetative growth stages (J. R. O. Silva et al. 2019; Rizzardi et al. 2019).

4.2. Vegetation indices for detection of plant injury and soybean yield

Vegetation indices are a remote sensing valuable tool to gather plant physiological and biochemical changes in a non-destructive way. Although some recent studies have evaluated herbicide symptoms in soybean by using traditional VIs from visible plus infrared spectral images or hyperspectral data (Zhang et al. 2019; da Silva et al. 2019), few attempted to predict effects by applying only UAV-based RGB images (i.e., visible electromagnetic radiation spectrum). In this paper, we accomplished that five RGB vegetation indices (ExG, ExGR, MGRVI, MPRI, and RGBVI) demonstrated to be useful for assessing soybean plant injury and estimate yield loss caused by low to high doses of dicamba and 2,4D.

The results accomplished with the use of RGB indices in the proposed methodology can be strongly supported by correlating agronomic knowledge upon herbicides mechanism of action with the physical principles of remote sensing for vegetation. On one hand, herbicides like dicamba can lead to severe destruction of the pigment systems associated with photosynthesis, consequently affecting dry matter accumulation, thus resulting in corresponding changes in biophysical parameters (Huang, Lin Yuan, and Zhang 2016; Robinson, Simpson, and Johnson 2013b). Compounds of 2,4D were related to reducing the photosynthetically activity (D. R. O. da Silva et al. 2018) in a lower intensity than dicamba. Therefore, this reduced leaf area, inhibited the growth of soybean plants, caused fewer main stem nodes, and result in significant yield loss (Robinson et al. 2013a). On the other hand, the leaf pigments are directly correlated with the changes in the reflectance of Red-Green-Blue (RGB) part of the electromagnetic radiation spectrum. Our main results showed that symptoms of plant injury strongly correlated with ExG, MGRVI, MPRI, and RGBVI (Figure 5). These VIs were developed to address the photosynthetic efficiency of vegetation (Bendig et al. 2015; Li, Li, and Sun 2014). Furthermore, a great advantage of this remote sensing-based technique is its non-destructive way of gathering information about vegetation status.

Unlike the conventional method based on injury criteria by visual analysis, the protocol by applying RGB vegetation indices revealed some orderly VI-response patterns related to the effect of the synthetic auxins (Figure 5). Therefore, these findings could lead to quantify and distinguish between the early effects of sublethal doses of dicamba or 2,4D drift events, how was successfully achieved for ExG at 8 DAT (Figure 7(a)) and MGRVI and MPRI (data not shown) at 15 DAT (Figure 7(b)). The response of these indices show substantial changes in its values and serve as examples of typical reflectance responses to plant stress in the corresponding wavelength range (visible spectrum about 400 to 700 nm) of the RGB bands, which here were due to the effects of herbicides. The change in VIs values refers to the specificity of the mechanism of action of dicamba (Grossmann 2010), showing how soybeans are most susceptible to this synthetic auxin than 2,4D compounds (Solomon and Bradley 2014; Silva et al. 2018).

The notable shift down in dose-response curves for MGRVI and ExG (Figure 7) when comparing between dicamba and 2,4D occurred because of several typical spectral traits of green vegetation, such as green peak related to xanthophyll cycle and red valley related to chlorophyll concentration (Zhang et al. 2019). These spectral traits gradually became vague as a consequence of stronger soybean susceptibility to dicamba, so the VIs response was decreased. As a result, this spectral property suggested that the vitality of the soybean plants was already compromised at 8 DAT since lower doses of dicamba (Figure 7 (a)). Also observed at 15 DAT was a tendency shift up in dose-response curves for doses of 2,4D between 10% and 20% (Figure 7(b)), which could be a sign that injured soybean can recovery from low to moderate rates of this compound. The attenuation of the VIs response towards lower values at 56 DAT (Figure 7(c)) can be mainly credited to the background (e.g., soil) that became prominent as the vegetation fraction decreased due to soybean senescence.

The strong orderly patterns of the VIs-response suggested the possibility to spectrally quantify different herbicides doses (Zhang et al. 2019) and early predict the herbicide drift effects (Figures 5 and Figures 6), as we accomplished for dicamba and 2,4D. Particularly, our results demonstrated noteworthy limitations to early discrimination versus the effects of 2,4D dimethylamine or choline salt. This is because both are just different formulations of the same herbicide and once absorbed cause the same effect on plants. The difference between this formulation is associated with physical properties related to the drift potential at the moment of application (Marcinkowska et al. 2017).

When it comes to herbicides that indirectly affect plant pigment cycles such as dicamba and 2,4D, RGB vegetation indices can be useful to measure injury symptoms and predict grain yield in soybean crops. RGB imagery based on UAV platforms enables assessment at the desired time. Consecutive measurements on the same plots showed high repeatability and consistency of the vegetation indices tested in the present study. Temporal planning of field and UAV sampling were performed with flexibility pointing to the feasibility to assess the effects of a drift from off-target auxin herbicides when main events occurred. The protocol described here allowed the analysis of a soybean field with a low-cost UAV system and having the corresponding RGB vegetation indices. This analysis will be useful to indicate distinct parts of the field with associate levels of soybean injury and the corresponding impact on grain yield.

5. Conclusion

This study demonstrated the feasibility of the combined use of agronomic metrics and UAV-based vegetation indices derived from a low-cost RGB consumer-grade camera for plant injury detection and to assess the impact of synthetic auxin herbicides, as dicamba and 2,4D, in a soybean crop.

The vegetation indices MGRVI and ExG achieved high-performance for real-time assessment of plant injury and soybean grain yield from both dicamba and 2,4D effects. Furthermore, this remote sensing technique can be used to assess typical changes in plant spectral response to some herbicides. This methodology has the potential to provide an objective measurement of symptoms and grain yield loss, as well as for distinguishing between the herbicides that caused the damage.

With expectations for the introduction of new genetic soybean events and concomitantly new synthetic auxin compounds, our results pointed out that the proposed methodology can lead to a protocol for identifying and estimating the damage to the off-target movement from these outcoming herbicides on neighbourhood fields.

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Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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