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Research Paper

In-situ plant hyperspectral sensing for early detection of soybean injury from dicamba

Yanbo Huang [a,*], Lin Yuan [b], Krishna N. Reddy [a], Jingcheng Zhang [c]

[a] United States Department of Agriculture, Agricultural Research Service, Crop Production Systems Research Unit, P.O. Box 350, Stoneville, MS, USA

[b] School of Information Engineering and Art and Design, Zhejiang University of Water Resources and Electric Power, Hangzhou 310018, China

[c] College of Life Information Science and Instrument Engineering, Hangzhou Dianzi University, Hangzhou 310018, China

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Drift of dicamba onto non-target crops is a major concern because it is highly active on susceptible crops even at low doses. Early detection of crop injury is critical in crop management. A field study was conducted to determine spectral characteristics of soybean (Progeny P4819LL) treated with dicamba. Drift deposition of dicamba was simulated by direct application at 0.05 to 1.0 times of the recommended label rate (0.56 kg [ai] ha^{-1}) to soybean at the 5- to 6-trifloliolate leaf stage, approximately 6 weeks after planting. The canopy spectral measurements were taken at 24, 48, and 72 h after treatment (HAT) using a portable spectroradiometer in the 325–1075 nm spectral range on 3 randomly selected plants within each plot with device optimisation and data calibration. The results indicated that it was difficult to clearly differentiate the dose response of soybean to different dicamba spray rates within 72 HAT. Regardless of spray rates the soybean treated with dicamba could be clearly differentiated from untreated soybean from 24 to 72 HAT through spectral vegetation index analysis with anthocyanin reflectance and photochemical reflectance indices with accuracies at 24, 48, and 72 HAT ranging from 76 to 86%. Simulated dicamba drift injured soybean and reduced its yield by 71 and 90% at 0.05 and 0.1 times recommended rate, respectively. This study demonstrated that hyperspectral remote sensing has a potential in early detection of soybean injury from exposure to off-target dicamba drift at sub lethal rates in the field.

1. Introduction

Dicamba (3, 6-dichloro-2-methoxybenzoic acid), an auxinic herbicide used for control of several broadleaf weeds in grain crops. Several broadleaf weeds, especially pigweeds (*Amaranthus* spp.), have evolved resistance to glyphosate, a widely used herbicide in glyphosate-resistant crops. Dicamba-tolerant (DT) soybean and cotton are currently under

* *Corresponding author*. 141 Experiment Station Road, P.O. Box 350, Stoneville, MS 38776, USA. Tel.: +1 662 686 5354.
E-mail address: yanbo.huang@ars.usda.gov (Y. Huang).

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Nomenclature

Abbreviations

ANOVA	analysis of variance
DT	dicamba-tolerant
GR	glyphosate-resistant
HAT	hours after dicamba treatment
NIR	near-infrared
USDA	United States Department of Agriculture
VI	vegetation index
VNIR	visible-near infrared
WAT	weeks after dicamba treatment

Vegetation indices

ARI	anthocyanin reflectance index
CARI	chlorophyll absorption ratio index
NDVI	normalised difference vegetation index
PRI	photochemical reflectance index
SIPI	structural independent pigment index
TVI	triangular vegetation index
WI	water index

development, and when they are commercialised, dicamba can be used in these crops to manage broadleaf weeds resistant to glyphosate. However, the off-target drift of dicamba could cause severe injury to non dicamba-tolerant crops. In January 2015, the USDA (United States Department of Agriculture, Washington DC, USA) announced deregulation of Monsanto Roundup Ready 2 Xtend™ soybean (Monsanto Company, St, Louis, MO, USA), which is the first industrial biotech-stacked soybean trait with tolerance to dicamba and glyphosate herbicides, and Bollgard II® XtendFlex™ cotton, which is the first triple stack herbicide-tolerance technology in cotton, with tolerance to dicamba, glyphosate, and glufosinate herbicides. Although the launch of DT trait cotton and soybean is still pending approval of new dicamba formulations by US EPA (U.S. Environmental Protection Agency, Washington DC, USA), off-target dicamba drift from routine use in DT crops onto susceptible crops is a concern. In the state of Mississippi, there was one dicamba drift complaint in each of 2012 and 2013 (Source: John Campbell, Bureau of Plant Industry, Mississippi Department of Agriculture and Commerce). It can be anticipated that with the adoption of DT crops, the complaints of off-target drift of dicamba may increase significantly.

For effective weed management, the detection and assessment of crop injury from herbicides are required. Conventionally, the detection and assessment were conducted through field sampling and measurement of plant biological responses to spray amount. However, such methods are tedious and labour-intensive endeavours. Remote sensing technology has been widely developed and applied in agriculture (Huang & Thomson, 2015; Huang, Thomson, Lan, & Maas, 2010; Pinter et al., 2003) and can provide a rapid, cost-effective method for detecting and assessing crop injury caused by herbicide drift. Henry, Shaw, Reddy, Bruce, and Tamhankar (2004) indicated that a number of vegetation indices formulated from the band information extracted from hyperspectral reflectance could distinguish between healthy and injured soybean and corn plants to which glyphosate and paraquat had been applied. Huang, Thomson, Ortiz, Reddy, Ding, & Zablotowicz et al. (2010) examined the effect of glyphosate drift from aerial application on non-glyphosate-resistant (non-GR) cotton by spray drift sampling and aerial multispectral remote sensing. Ortiz, Thomson, Huang, Reddy, and Ding (2011) studied the effect of glyphosate drift from aerial application on non-GR soybean, cotton and corn, using vegetation indices generated from aerial multispectral remote sensing. Huang, Reddy, Thomson, and Yao (2015) assessed soybean injury from glyphosate using airborne multispectral remote sensing. Early detection of crop injury from herbicide is important for farmers to know the injury potential before the symptom becomes visible so that they can take timely corrective actions to prevent yield losses. Studies have indicated that crop injury from glyphosate could be detected starting at 24 h after treatment (HAT) for soybean (Huang, Thomson, Molin, Reddy, & Yao, 2012; Yao, Huang, Hruska, Thomson, & Reddy, 2012) and cotton (Zhao & Huang et al., 2014; Zhao & Guo et al., 2014). So far, little research has been done on the capability of remote sensing for early detection of soybean or cotton injury from dicamba.

This study was undertaken to evaluate plant hyperspectral reflectance measured in situ and reveal the potential and limitation of using this technique for early detection of soybean injury from dicamba. The specific objectives of the study were to characterise hyperspectral reflectance properties of soybean treated with and without dicamba, and investigate the parameters from in-field measured hyperspectral data for early detection of soybean injury from simulated dicamba drift at various doses.

2. Materials and methods

2.1. Study site

A field study was carried out on a 4.5-ha area (central latitude: 33.445062° and central longitude: −90.869967°) at the USDA Agricultural Research Service Crop Production Systems Research Farm, Stoneville, MS, USA. Maize was grown on the surroundings of the experimental area as a border to minimise spray drift from periodic applications of herbicides in neighbouring fields as well as to minimise dicamba drift to neighbouring fields (Fig. 1). Maize was planted in late March 2014 in the middle of the experimental area to divide it into two subplots. In the two isolated areas, 32-row plot of soybeans (Progeny P4819LL, Progeny Ag Products, Wynne, AK, USA) were planted on May 7, 2014. The soybean field in the east side of the entire field was used as the experimental field while the soybean field in the west side was used as backup (in case of unsuccessful dicamba treatment) and as a reference.

2.2. Experimental design and operation

The experimental field was divided into thirty-two plots planted with soybean (Fig. 1). Each plot consisted of eight rows with rows 0.97 m wide and 24 m long. In the thirty-two plots,

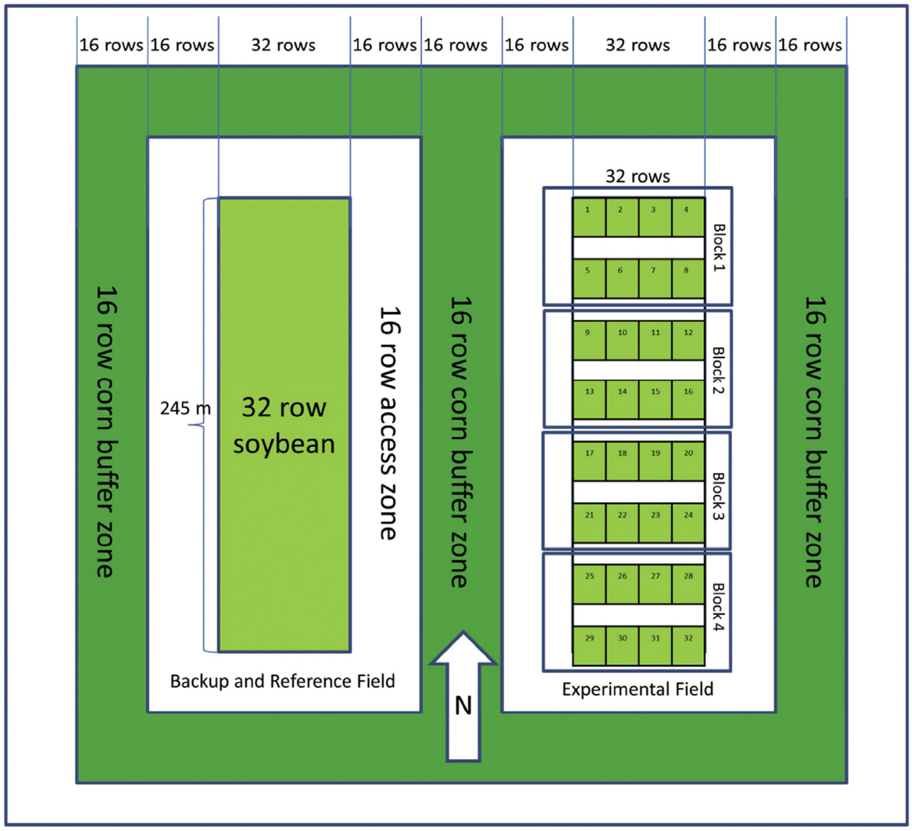


Fig. 1 – Study site layout.

four blocks were formed with eight plots in each block. The experimental design was a randomised complete block design with four replications of dicamba treatments to the plots with seven rates: 0.0X (control), 0.05X, 0.1X, 0.2X, 0.3X, 0.5X and 1X, where X was 0.56 kg [ai] ha^{-1} of dicamba. Fig. 2 shows the complete experimental design.

Herbicide treatments are typically recommended before the flowering stage of crops. However, dicamba is an herbicide that provides strong inhibitions and is sensitive to many broadleaf plants. Therefore, it is a good practice to use dicamba in a soybean field at early leaf stage (3–6 trifloliolate leaf stage) to compress young weeds to boom up the crop growth over weeds. Then, the less powerful herbicides can be used to control the regrowth of weeds to avoid crop injury in surrounding of the soybean field from the vapour spray of dicamba.

On June 17, 2014, six weeks after soybean planting when the soybeans were at the 5- to 6-trifloliolate leaf stage, the soybean plots were treated with the dimethylamine salt of dicamba, RIFLE® (Loveland Products, Inc., Greeley, CO, USA) using a tractor mounted sprayer with Tee Jet 4003 standard flat-spray nozzles delivering 140 l ha^{-1} of water at 193 kPa. No post-emergence herbicides were applied up to 3 weeks after dicamba treatment for taking various measurements. Three weeks after dicamba treatment, other post-emergence herbicides were applied as needed to keep the plots weed-free, and the field was furrow irrigated as needed. Soybean yield was measured and recorded at the time of harvest on September 9, 2014.

2.3. *Hyperspectral plant sensing*

An ASD Handheld 2 Portable Spectroradiometer (ASD Inc., Boulder, CO, USA) was used to measure on the top of the soybean canopy in each control and treated plot in the experimental field at 24, 48, and 72 HAT (hours after dicamba treatment), and 2 WAT (weeks after dicamba treatment) when the injury symptoms become completely visible to human eyes, which were on clear days and measured between 11:00 AM to 1:00 PM, to provide visible-near infrared (VNIR) spectra of soybean plants in the range of 325–1075 nm with 25° field of view. For radiometric calibration, a 0.3 m $\times$ 0.3 m Spectrolon® white reference target with 99% nominal reflectance (Labsphere, North Sutton, NH, USA) was used. Before measurement, the spectroradiometer was optimised with white reference and dark current measurement. During measurement, three random points in the middle of each plot on the plant canopy were measured with a white reference measurement after the plant canopy measurements for the plot.

2.4. *Data processing and analysis*

With the ASD measurements of soybean canopy and white reference target, the spectral reflectance of soybean canopy on the control and treated plants were generated for further data processing and analysis. In data processing, because of excessive weed infestation in the southern side of the soybean field, the spectral data measured over the soybean canopy in the plots of block 4 (see Fig. 2) were excluded from data

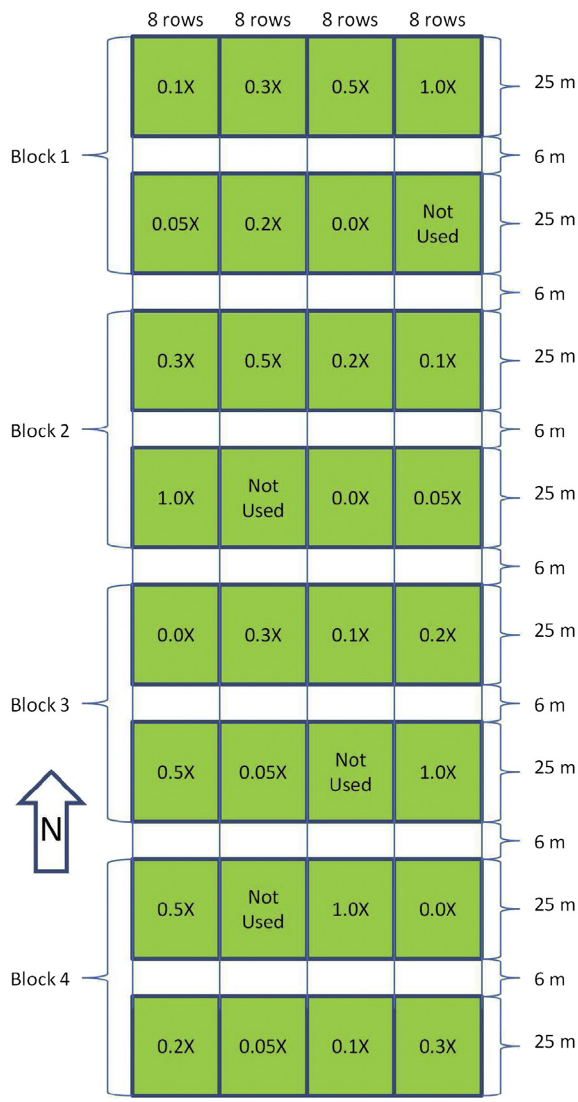


Fig. 2 – Experiment design on the soybean field.

analysis to avoid data contamination with various weed signatures.

In addition to spectral reflectance data, several important vegetation indices (VIs) were extracted to assess their potential to differentiate treated and untreated soybeans (Table 1). The NDVI (normalised difference vegetation index) (Rouse, Haas, Schell, & Deering, 1974) and TVI (triangular vegetation index) (Broge & Leblanc, 2000) were included, given that they are most known and widely used VIs in indicating vegetation growing status and stress conditions. Both VIs require only broad-band reflectance, which could be delivered by most common and inexpensive sensors (Broge and Leblanc., 2000; Rouse et al., 1974; Weng, 2011). Considering that the damage of plants caused by herbicide might disturb their pigment system, four other VIs were selected to reflect these changes. The PRI (photochemical reflectance index) is able to reflect the photosynthetic efficiency of the plant through tracking the status of xanthophylls cycle (Gamon, Peñuelas, & Field, 1992). The CARI (chlorophyll absorption ratio index) is sensitive to variation of chlorophyll content (Kim, Daughtry, Chappelle, &

Table 1 – A summary of vegetation indices for spectral analysis.

Vegetation index	Full name	Formula	Literature
NDVI	Normalised difference vegetation index	$NDVI = (R_{NIR} - R_R)/(R_{NIR} + R_R)$; where R_{NIR} represents near-infrared reflectance in 760–900 nm, R_R represents red reflectance in 630–690 nm, and refer to the channels of Landsat TM sensor	Rouse et al. (1974)
TVI	Triangular vegetation index	$TVI = 0.5\ [120\ (R_{NIR} - R_G) - 200\ (R_R - R_G)]$ where R_{NIR} represents near-infrared reflectance in 760–900 nm, R_R represents red reflectance in 630–690 nm, R_G represents green reflectance in 520–600 nm, and refer to the channels of Landsat TM sensor	Broge and Leblanc (2000)
PRI	Photochemical reflectance index	$PRI = (R_{531} - R_{570})/(R_{531} + R_{570})$	Gamon et al., 1992
CARI	Chlorophyll absorption ratio index	$CARI = ((670a + R_{670} + b)/(a^2 + 1)^{1/2})\ (R_{700}/R_{670})$; where $a = (R_{700} - R_{550})/150$, $b = R_{550} - (a \times 550)$	Kim et al., 1994
SIPI	Structural independent pigment index	$SIPI = (R_{800} - R_{445})/(R_{800} - R_{680})$	Peñuelas et al., 1995
ARI	Anthocyanin reflectance index	$ARI = (R_{550})^{-1} - (R_{700})^{-1}$	Gitelson et al., 2001
WI	Water index	$WI = R_{900}/R_{970}$	Peñuelas et al., 1993

McMurtrey, 1994), whereas SIPI (structural independent pigment index) responds well to carotenoid content (Peñuelas, Baret, & Filella, 1995). The ARI (anthocyanin reflectance index) is an efficient indicator of anthocyanin (Gitelson, Merzlyak, & Chivkunova, 2001). In addition, the WI (water index) was also included to indicate the water status of the plant (Peñuelas, Filella, Biel, Serrano, & Save, 1993), which is also closely associated with plant conditions.

To analyse the differences among group means of dicamba treatment rates to these VIs at 24, 48, and 72 HAT, and 2 WAT, ANOVA (analysis of variance) was conducted. Further, to evaluate the sensitivity of these VIs to dicamba treatment rates, correlation analysis was conducted between them at 24, 48, and 72 HAT, and 2 WAT as well.

Moreover, considering that the values of VIs are prone to be affected by some background conditions (e.g., growth period, growing status, soil exposure, etc.), a ratio metric was calculated further as:

$$\text{Ratio} = \frac{\text{VI} - \text{VI}_{\text{normal}}}{\text{VI}_{\text{normal}}} \quad (1)$$

where VI represents a specific vegetation index of treated plots and $\text{VI}_{\text{normal}}$ represents the VI of corresponding untreated plots, which here serves as a baseline.

To facilitate the practical use of this ratio metric, a stepwise strategy was applied on the most sensitive VI to determine the optimal threshold for differentiating treated and untreated crops, as well as varying treatment rates at each stage. Such method aims to avoid a subjective threshold value in analysis. To implement this, 100 evenly spaced intervals were set within the data range (i.e., from minimum to maximum) for each VI. Using the entire data, the overall accuracy was calculated by traversing all intervals. The cut-off value was defined as a point when the highest accuracy was reached. To facilitate the understanding of the stepwise thresholding strategy, Fig. 3 showed an example for PRI at 24 HAT. At all stages, such stepwise optimised thresholding was applied first to generate the cut-off value for separating treated and untreated samples, and then to identify the cut-off value for differentiating different treatment levels. The entire data analysis were processed using MATLAB software (MathWorks Inc., Natick, MA, USA).

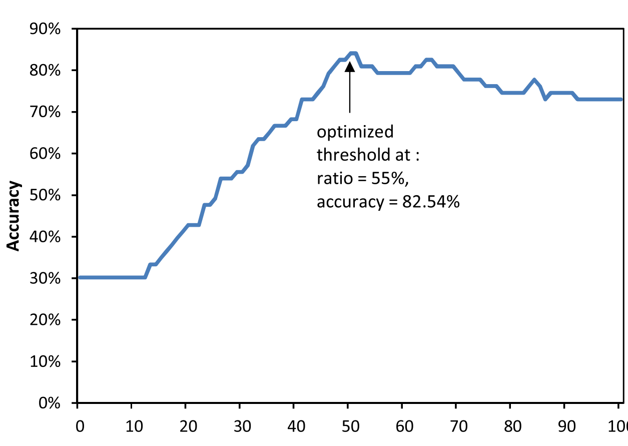


Fig. 3 – An example about stepwise thresholding strategy (PRI at 24 HAT).

3. Results and discussion

Initial data analysis indicated that, in general, the treated soybeans had a higher reflectance values than the untreated soybeans in the visible region of the spectrum from 400 to 700 nm. In the near-infrared (NIR) range of 700–1000 nm, the treated soybeans had a lower reflectance value than the untreated soybeans. This pattern is illustrated in Fig. 4 with the spectra ratios of dicamba treatment rates to control (0.0X) regardless of HAT and WAT. It is noticeable that all the ratio curves have two peaks, with one in green (around 500 nm) and another in red (around 650 nm) bands. These spectral positions are major absorption regions of several pigments systems, such as *a* and *b* of CARI, carotenoid, and anthocyanin, which could be the indicators of typical plant biophysical damage induced by dicamba. Although some abnormality exists, for example in the NIR range the ratio of 0.3X at 48 HAT is greater than 1 and the ratio of 0.2X at 72 HAT is greater than 1, which means that in the spectral range the spectra of treated soybean has a higher reflectance than the spectra of the untreated soybean, such stable pattern (indicated by the similar shape of spectral ratio curves in Fig. 4) can definitely be used to differentiate treated and untreated soybeans. However, it was found, by inspection of Fig. 4, that the spectra with different dicamba treatment rates do not really line up with the increase of the treatment rates. While at 2 WAT when the injury symptoms become completely visible to human eyes, the spectral response began to show a clear pattern to the treatment rates (Fig. 4). Therefore, the differentiation of the effects of different dicamba treatment rates remains a challenge until 2 WAT. So, it is important to evaluate methods to determine an optimal approach to early detection of soybean injury from dicamba in response to different rates and find out the capability and limits of hyperspectral remote sensing for solving this problem.

As in Table 2, despite significant correlations achieved between treatment rates and most VIs at different stages, the correlations were relatively low at 24, 48, and 72 HAT. However, the correlation improved significantly at 2 WAT. This result suggests that it was difficult to directly use VIs to estimate treatment rate of herbicide at early stages (i.e., within 72 HAT). Dicamba, being an auxinic herbicide, produces profound effects on the growth and structure of plants. The initial injury symptoms of dicamba include twisting and curling of stems and petioles, and cupping and crinkling of leaves, stem swelling, and disruption of phloem transport. These symptoms are followed by chlorosis, growth inhibition, wilting, and necrosis. Apparently, dicamba exhibits a flat-dose response that can cause injury at sub-lethal rates. At early stages, symptomology appears to be similar in soybean regardless of rates and by 2 WAT, severity of injury becomes more pronounced at higher rates.

Applying the VIs of the untreated plots as baseline, Fig. 5 shows the ratios of each VI at (a) 24 HAT, (b) 48 HAT, (c) 72 HAT, and (d) 2 WAT. From 24 HAT to 72 HAT, a non-monotonic pattern was observed across different treatment rates for all

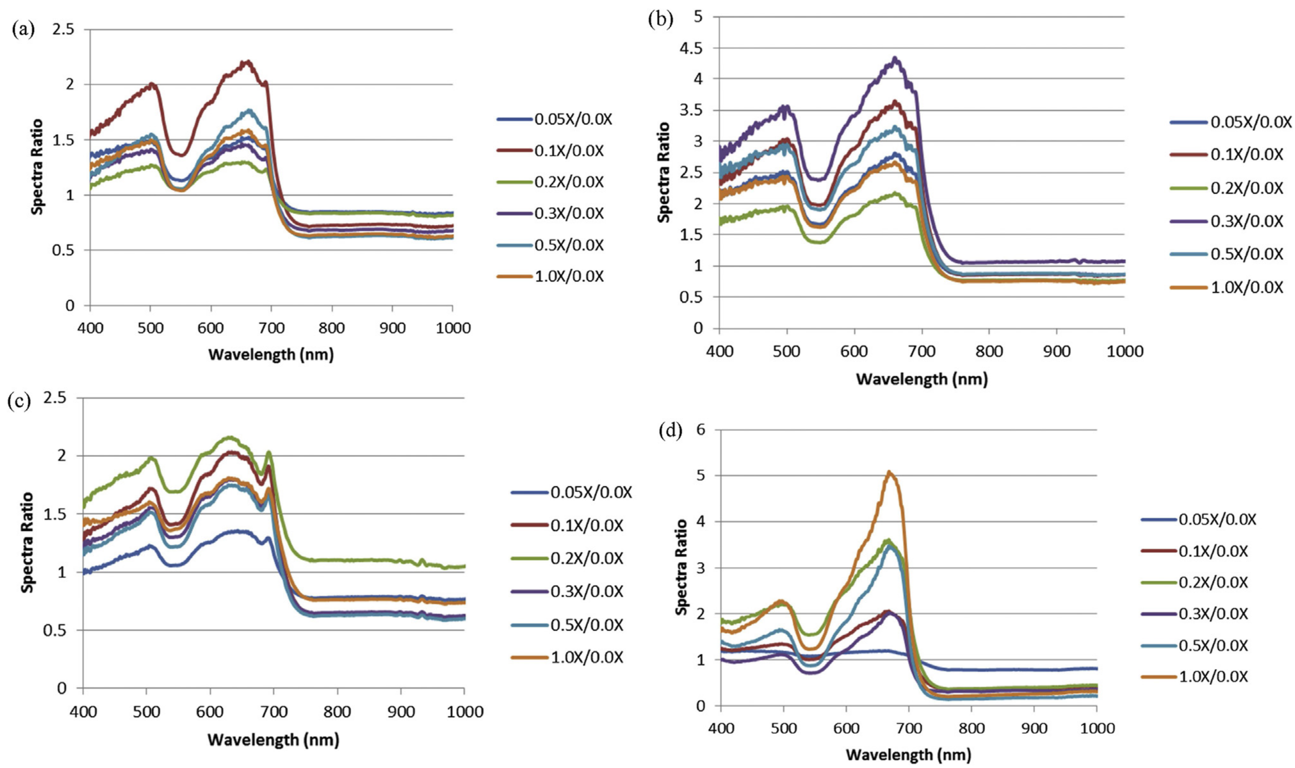


Fig. 4 – Spectra ratios of dicamba treatment rates to control (0.0X) – (a) 24 HAT (b) 48 HAT (c) 72 HAT (d) 2 WAT.

VIs, which thus hampers the differentiation among treatment rates through VIs. While at 2 WAT, a generally monotonic pattern was evident for most VIs, which allows the differentiation between different treatment rates. Among these VIs, ARI and PRI have stronger spectral response than the other VIs across all treatments and stages. Particularly, based on the response pattern of all VIs as shown in Fig. 5, the ARI seemed to be the most sensitive VI which exhibited significant and stable sensitivity to the dicamba treatments. Such results suggest that the ARI and PRI, corresponding to xanthophylls and anthocyanin systems, outperformed those VIs related with chlorophyll or carotenoid systems (i.e., NDVI, TVI, CARI, SIPI). Therefore, hyperspectral observations are necessary in calculating these narrow-bands VIs to achieve better differentiation among different treatment rates.

Table 2 – Sensitivity of VIs over different stages.

VIs	Correlation coefficient			
	24 HAT	48 HAT	72 HAT	2 WAT
NDVI	0.29***	0.28***	0.24**	0.75***
TVI	0.45***	0.23**	0.18*	0.66***
PRI	0.31***	0.29***	0.33***	0.71***
CARI	0.26**	0.1	0.23**	0.08
SIPI	0.32***	0.33***	0.26**	0.72***
ARI	0.36***	0.28***	0.19*	0.55***
WI	0.36***	0.15	0.25**	0.7***

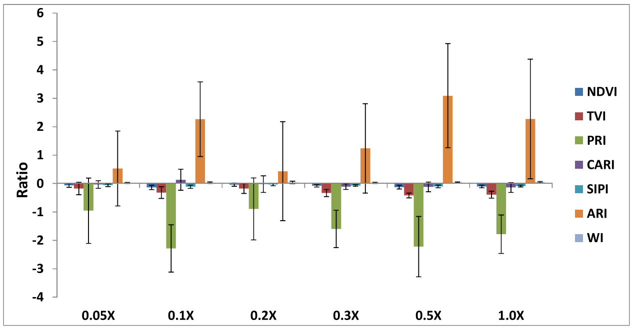
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

With the non-monotonic pattern shown in Fig. 5, from 24 HAT to 72 HAT, the stepwise optimised thresholding method was applied to determine a cut-off value to differentiate treated and untreated plots. At 2 WAT, given that the plots that received 0.05X treatment rate seemed to have experienced some regrowth, untreated corresponding to 0–0.05X vs 0.1X–1.0X and 0.1X–0.2X vs 0.3X–1.0X were determined using the stepwise optimisation thresholding method.

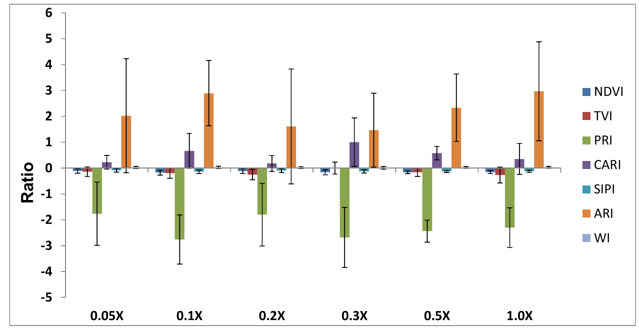
The results of ARI and PRI thresholding (Table 3) suggest that at the early stages after the application of dicamba (24 HAT – 72 HAT), the treated soybean can be clearly differentiated from untreated through spectral analysis, with accuracy varying from 76 to 86%. As the injury caused by dicamba tends to get more pronounced with time passing, a generally ascending trend of the accuracy was observed as expected. The only exception occurred during the 24 HAT and 48 HAT for PRI, which might associate with the uncertainty due to weak response at early stages. However, the symptoms caused by different treatment rates could not be identified by spectral measurements up to 2 WAT, which indicated that the spectral differentiation among dicamba treatment rates was difficult at early stages.

The spectral ratio method provided a simple yet straightforward way to detect soybean injury from dicamba, which is relatively easy to use.

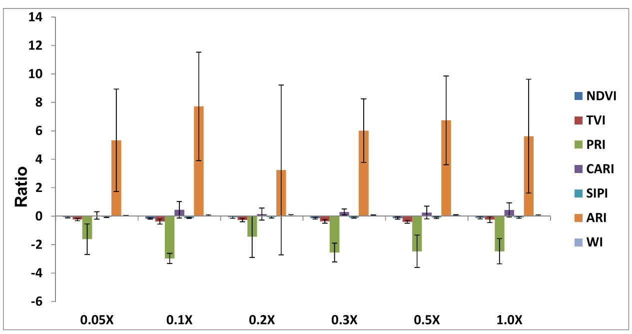
The relationship between soybean yield and dicamba rates showed (Fig. 6) that even a relatively low treatment rate (0.05X) can cause a significant yield loss (60%–75%) to the soybean. There would be almost no harvest of soybean under treatment rates over 0.2X, with yield reductions reaching 100%. Therefore, from a practical perspective, it still can be



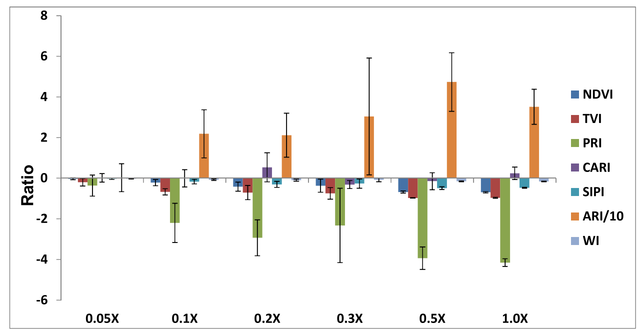
(a) 24 HAT



(b) 48 HAT



(c) 72 HAT



(d) 2 WAT

Fig. 5 – Ratios of VIs under different dicamba treatments at (a) 24 HAT, (b) 48 HAT, (c) 72 HAT and (d) 2 WAT (NDVI is narrow band NDVI where the red band is at 650 nm and the NIR band is at 850 nm). The bars indicated the means of ratio for each index whereas the lines indicate their standard deviations.

very helpful to just identify soybean plants exposed to dicamba drift from those unexposed at an early stage to any corrective actions. Besides, it should be aware that the low dosage of 0–0.05X would cause noticeable spectral response on the indices during 24 HAT to 72 HAT, but show unobvious response in 2 WAT (Fig. 5). The possible reason for this phenomenon was the regrowth of the soybean plants at late stages. Despite the plants (i.e., the leaf area, biomass) were recovered from the injury, the yield reduction occurred for the low dosage in any case (Fig. 6). Therefore, such a result indicated that for dicamba injury at low dose, the present technology could give a reasonable determination at early stages (24 HAT to 72 HAT), whereas stands a risk of misdiagnosis at late stage (2 WAT). Given the complexity of the impact of dicamba application doses on plants, more studies on biophysical response process of soybean to dicamba and the ways for capturing the unique initial signals are needed. Some other techniques and analysis, such as fluorescence sensing and image processing, are of potential in solving these challenges.

Table 3 – Ratio threshold values of ARI and PRI for differentiating dicamba treatments (N/A means that data is not available because ARI and PRI could not clearly differentiate the dose response of soybean to different dicamba spray rates within 72 HAT).

Vegetation index	Time after treatment	Treated vs untreated		Slightly vs heavily treated	
		Ratio threshold	Accuracy	Ratio threshold	Accuracy
ARI	24 HAT	0.37	76.19%	N/A	N/A
	48 HAT	0.18	84.13%	N/A	N/A
	72 HAT	1.61	85.71%	N/A	N/A
	2 WAT	5.68	90.74%	23.84	79.63%
PRI	24 HAT	0.55	82.54%	N/A	N/A
	48 HAT	1.50	79.37%	N/A	N/A
	72 HAT	3.68	84.13%	N/A	N/A
	2 WAT	1.28	92.06%	4.44	80.00%

Note: for 2 WAT, the reference group indicated 0–0.05X, the treated group indicated 0.1X–1.0X; the slight group indicated 0.1X–0.2X and the heavy group indicated 0.3X–1.0X.

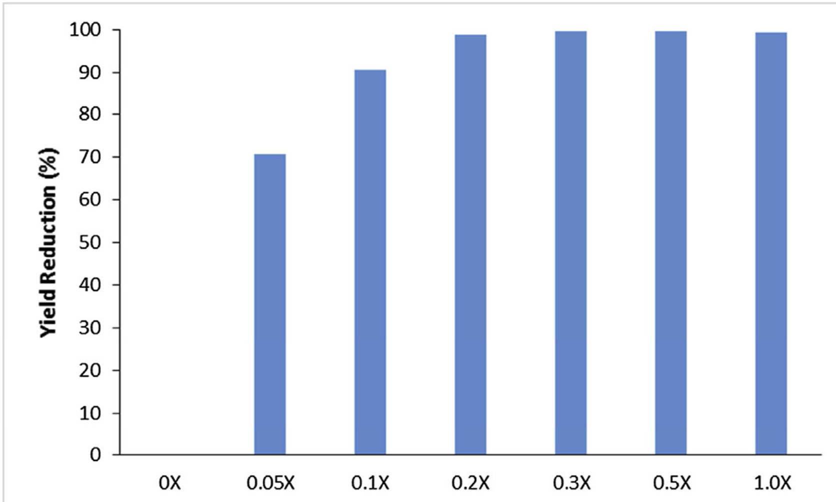


Fig. 6 – Yield reduction trend with dicamba treatment rates.

4. Conclusion

This study determined that it was difficult to clearly differentiate the dose response of soybean to different dicamba spray rates within 72 HAT, which illustrates the challenge of early detection of soybean injury from dicamba. However, it was found that the soybean treated with dicamba, regardless of spray rates, could be clearly differentiated from untreated soybean from 24 to 72 HAT through spectral vegetation index analysis, especially ARI and PRI. The results would be useful in practice for differentiating, at early stage after dicamba treatment, treated soybean from untreated soybean even though the dose response could not be clearly determined yet.

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