



## Using vegetation index and modified derivative for early detection of soybean plant injury from glyphosate

Haibo Yao<sup>a,\*</sup>, Yanbo Huang<sup>b</sup>, Zuzana Hruska<sup>a</sup>, Steven J. Thomson<sup>b</sup>, Krishna N. Reddy<sup>b</sup>

<sup>a</sup> Mississippi State University, Geosystems Research Institute, Stennis Space Center, MS, USA

<sup>b</sup> United States Department of Agriculture, Agricultural Research Service, Crop Production Systems Research Unit, Stoneville, MS, USA

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### ABSTRACT

Glyphosate is a non-selective, systemic herbicide highly toxic to sensitive plant species. Its use has seen a significant increase due to the increased adoption of genetically modified glyphosate-resistant crops since the mid-1990s. Glyphosate application for weed control in glyphosate-resistant crops can drift onto an off-target area, causing unwanted injury to non-glyphosate resistant plants. Thus, early detection of crop injury from off-target drift of herbicide is critical in crop production. In non-glyphosate-resistant plants, glyphosate causes a reduction in chlorophyll content and metabolic disturbances. These subtle changes may be detectable by plant reflectance, which suggests the possibility of using optical remote sensing for early detection of drift damage to plants. In order to determine the feasibility of using optical remote sensing, a greenhouse study was initiated to measure the canopy reflectance of soybean plants using a portable hyperspectral image sensor. Non-glyphosate resistant soybean (*Glycine max* L. Merr.) plants were treated with glyphosate using a pneumatic track sprayer in a spray chamber. The three treatment groups were control (0 kg ae/ha), low dosage (0.086 kg ae/ha), and high dosage (0.86 kg ae/ha), each with four 2-plant pots. Hyperspectral images were taken at 4, 24, 48, and 72 h after application. The extracted canopy reflectance data was analyzed with vegetation indices. The results indicated that a number of vegetation indices could identify crop injury at 24 h after application, at which time visual inspection could not distinguish between glyphosate injured and non-treated plants. To improve the results a modified method of spectral derivative analysis was proposed and applied to find that the method produced better results than the vegetation indices. Four selected first derivatives at wavelength 519, 670, 685, and 697 nm could potentially differentiate crop injury at 4 h after treatment. The overall false positive rate was lower than the vegetation indices. Furthermore, the derivatives demonstrated the ability to separate treatment groups with different dosages. The study showed that hyperspectral imaging of plant canopy reflectance could be a useful tool for early detection of soybean crop injury from glyphosate, and that the modified spectral derivative analysis had a better performance than vegetation indices.

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### 1. Introduction

During routine herbicide applications, herbicide drift can occur when herbicide particles move onto off-target areas under weather conditions that are favorable for drift. When the herbicide lands on the off-target plant surfaces, unwanted plant damage can occur. Thus, in herbicide applications, one of the main objectives is to minimize off-target drift that may result in injury to other crops. This task can be implemented through proper training, careful planning of herbicide applications, good maintenance of equipment, and field experience. However, when drift does occur, it is important to be able to detect the onset of the crop injury due to

herbicide drift, and preferably be able to determine the relationship between the crop injury and dosage. In the past decade, one herbicide, glyphosate, has been widely adopted for weed management in agricultural fields due to the increased utilization of genetically modified crops that are resistant to glyphosate. Glyphosate is a non-selective herbicide used for control of weeds before planting crops and postemergence (POST) applications in genetically modified GR (glyphosate-resistant) crops. Glyphosate use has seen a significant increase. For example, the amount of glyphosate (Active Ingredient) on all soybean crops in the US has increased from 4896 (thousand lb) in 1996 to 96,725 (thousand lb) in 2006 (Center for Food Safety, 2008).

Glyphosate is highly active on sensitive plant species even at low doses. Once applied, the inhibition of plant growth is immediate due to the depletion of aromatic amino acids essential for plant growth. In addition to growth reduction, glyphosate causes chlorosis and necrosis. The consequence is yield reduction or complete

\* Corresponding author. Address: Mississippi State University, Geosystems Research Institute, 1021 Balch Boulevard, Stennis Space Center, MS 39529, USA. Tel.: +1 228 688 3742; fax: +1 228 688 7100.

E-mail address: [haibo@gri.msstate.edu](mailto:haibo@gri.msstate.edu) (H. Yao).

destruction of a susceptible plant. Glyphosate is usually applied to foliage through ground or aerial applications. Typical applications include postemergence application for weed control. For instance, glyphosate is normally applied in GR soybean fields for weed control in the early growth season. However weather conditions during this period of time in the year are normally windy (depends on location, Henry et al., 2004), and this increases the possibility of glyphosate drift. Glyphosate drift onto non-target crops is common in agricultural regions. The drift to non-GR crops may cause injury and reduce yields. When drift occurs, farmers are interested in the coverage and extent of the drift damage in order to project the severity of unintended injury as early as possible. With this information farmers can take appropriate actions to best protect their interests. Such actions include replanting or seeking insurance compensation. Thus, the study of glyphosate drift is important to the farming industry. Stated another way, an early warning system for herbicide drift detection in weed management would greatly benefit farmers.

A number of injury identification methods have been evaluated on crops injured by off-target drift of glyphosate. Typically, visual examination provides a way to assess drift occurrence and extent of injury. This approach is possible when injury is obvious to the naked eye. In this case, aerial photography can be used to aid in detection over large areas. Another approach to assess severity of plant damage is to measure physical factors such as plant height (Rowland, 2000) and/or by measurement of chlorophyll and shikimate levels (Reddy et al., 2010; Ding et al., 2011a). Above all, these methods can provide damage assessment when the injury is obvious, well after application (7–14 days after the drift incident). These damage assessment methods are tedious and cause delay in early critical decision-making. Alternative approaches for early warning/detection of glyphosate drift damage would be highly desirable.

In the injury process, glyphosate also causes a reduction in chlorophyll content, decreases in photosynthetic rate, nitrate reductase activity, and nitrogen fixation and accumulation (Bellaloui et al., 2006). These changes in the plant may be detectable by plant reflectance measurements before symptoms of injury become visible. This suggests the possibility of using sensitive, optical remote sensing for early detection of drift damage to plants. Several remote sensing methods have been developed previously in an attempt to detect crop injury due to herbicide drift. The methods include multispectral imaging (Thelen et al., 2004; Huang et al., 2010), fiber optic-based spectral reflectance measurements (Henry et al., 2004; Huang et al., 2012), and chlorophyll fluorescence measurements (Huang et al., 2012). Among them the chlorophyll fluorescence method is not suitable for rapid and early detection, as its measurement requires direct contact against a small section of the plant leaves for a period of time.

The multispectral imaging approach (Thelen et al., 2004; Huang et al., 2010) used imagery with several broadband reflectance measurements including at least a red and a near-infrared band. The multispectral images could provide good spatial resolution in the data. However, the broadband multispectral imagery lacks spectral resolution in the reflectance measurement, and standard bands customarily assigned to multispectral systems are not necessarily appropriate for optimal damage detection. Consequently, a major drawback of this method is that the fine spectral reflectance feature of a plant at the red edge (~700 nm) of the electromagnetic spectrum range cannot be explored. The red edge is an important spectral range for plant vigor and stress monitoring.

The fiber optic-based method (Henry et al., 2004; Huang et al., 2012) provides an opportunity for narrowband reflectance measurements. The narrowband reflectance has the capability of revealing subtle changes in plant reflectance, which could present more useful information in detecting the onset of crop injury.

However, the fiber optic-based reflectance measurement lacks spatial information in the data. It is a single point measurement of the plant. The reflectance data is a mixed signal of all the reflectance within the fiber's field of view. Huang et al. (2012) avoided the spectral mixture problem by pointing the optical fiber directly over a single leaf. However this approach limited the viewing area. Canopy reflectance, which is crucial for crop stress detection, could not be measured in this way. To better differentiate various plant parts and background, it is desirable to use spectral data with both high spatial and spectral resolution. In this case, an imaging spectrometer or hyperspectral imaging system (Yao et al., 2008) can be used to provide such high quality data. Hyperspectral imaging systems have been widely used in agriculture applications, with data from space-borne (Gong et al., 2003), airborne (Yao and Tian, 2003), and terrestrial-based platforms (Ye et al., 2008).

When using hyperspectral image data for vegetation and plant monitoring, vegetation index (VI) is widely applied. Many vegetation indices have been used in different applications. Among them, the most important vegetation index is the Normalized Difference Vegetation Index (NDVI) (Rouse et al., 1973) calculated by using the data at the red and near infrared wavelengths. Hyperspectral images make it possible to build more refined vegetation indices by using distinct narrow-bands. A common practice in calculating hyperspectral indices is the use of individual image bands. For most of the time one specific image band pair is selected based on crop characteristics. For example, one study (Haboudane et al., 2002) used CASI (Compact Airborne Spectral Imager) images to calculate VIs. Image bands centered at 550, 670, 700, and 800 nm were used to calculate several vegetation indices for crop chlorophyll content prediction. The reason for selecting 700 nm was because it is located at the edge between the region where vegetation reflectance is dominated by pigment absorption and the beginning of the red edge region where reflectance is more influenced by the structural characteristics of the vegetation. To apply VIs for glyphosate injury detection on soybean plant, Huang et al. (2012) used 4 VIs, NDVI, RVI (Ratio Vegetation Index), SAVI (Soil Adjusted Vegetation Index), and DVI (Difference Vegetation Index). It was found that crop stress due to glyphosate injury could be detected 24 h after application. However this data was based on spectral measurements over part of a single leaf instead of using canopy reflectance.

Derivative analysis is another approach to analyze hyperspectral data (Thorpe et al., 2004). Derivative analysis is promising for use with remote sensing data (Tsai and Philpot, 1998). Higher order derivatives should be relatively insensitive to illumination variations, especially with hyperspectral data, due to its small spectral sampling interval. The most commonly used derivatives are first and second order. Since derivative analysis is quite sensitive to noise, spectral data smoothing is normally applied. Examples of the filtering techniques include Savitzky–Golay filtering and mean low-pass filtering. One study (Smith et al., 2004) suggested that derivative analysis in the red edge range (690–750 nm) could be used for plant stress detection. However, another study (Estep and Carter, 2005) found that when certain derivatives were used for plant nitrogen and water stress detection, there was no advantage of using the derivatives compared to narrow-band vegetation indices. The study used AVIRIS (Airborne Visible/Infrared Imaging Spectrometer) data over corn plots having different nitrogen fertilization treatments. The applied first derivatives were at 495, 568, 696, 982, and 1025 nm. Since these wavelengths were pre-defined from other literature, they might not be suitable for all applications.

This paper utilized a high resolution portable hyperspectral imaging system to study glyphosate damage on soybean plants. The study was conducted in a greenhouse to evaluate crop canopy reflectance data for the detection of crop injury caused by applied

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