Early Detection of Soybean Plant Injury from Glyphosate by Measuring Chlorophyll Reflectance and Fluorescence

Yanbo Huang (Corresponding author)
United States Department of Agriculture, Agricultural Research Service
Crop Production Systems Research Unit, Stoneville, Mississippi 38776, USA
Tel: 1-662-686-5354 E-mail: yanbo.huang@ars.usda.gov

Steven J. Thomson, William T. Molin & Krishna N. Reddy
United States Department of Agriculture, Agricultural Research Service
Crop Production Systems Research Unit, Stoneville, Mississippi 38776, USA

Haibo Yao
Mississippi State University, Geosystems Research Institute
Stennis, Mississippi 38776, USA

Received: December 19, 2011 Accepted: December 9, 2012 Online Published: April 1, 2012
doi:10.5539/jas.v4n5p117 URL: http://dx.doi.org/10.5539/jas.v4n5p117

Abstract

Early detection of crop injury from off-target drift of herbicide is critical in crop production. Subtle changes in canopy reflectance could present useful information to detect the onset of crop stress. This study was conducted in a greenhouse to evaluate a portable spectroradiometer and a portable chlorophyll fluorometer for the detection of crop injury caused by glyphosate spray. Non-glyphosate resistant soybean (*Glycine max* L. Merr.) plants were sprayed with glyphosate using a pneumatic track sprayer in a spray chamber. Four plants received a rate of 0.86 kg ae/ha glyphosate and four plants received 0.086 kg ae/ha. Additional four non-sprayed plants were used as controls. After the glyphosate spray, the chlorophyll reflectance of the plants was measured with the spectroradiometer at 4, 24, 48, and 72 hours to determine the plant response to herbicide. Simultaneously, fluorescence induction kinetics of the crop under stress was measured with the portable chlorophyll fluorometer. Results of the statistical mean separation indicated that the plant chlorophyll reflectance measurement could be used to differentiate crop stress from glyphosate at 24 hours after spray among treatments and to identify the effect of herbicide at 24 hours after spray in each treatment. Moreover, linear discriminant analysis with the reflectance data showed that the crop stress of the soybean plants from glyphosate could be identified at 24 hours or more post application. Results of the statistical mean separation also indicated that use of plant chlorophyll fluorescence measurement could not differentiate crop stress until 48 hours after spray among treatments while it could identify the effect of herbicide 24 hours after spray in each treatment. These findings demonstrate that chlorophyll reflectance and fluorescence measurements both could be used for early detection of crop stress.

Keywords: Chlorophyll reflectance, Chlorophyll fluorescence, Crop injury, Herbicide application

1. Introduction

Crops can be injured by off-target drift from non-selective herbicides. A key to managing herbicide application is to minimize off-target spray drift that may cause crop injury. It is thus critical to detect the onset of the crop injury and determine the relationship between the crop injury and dosage.

Glyphosate is a non-selective, systemic herbicide highly toxic to sensitive plant species, the use of which has seen a significant increase in the last decade. For example, in the state of Mississippi, the number of glyphosate applications per year has increased from 1.2 in 1995 to 2.6 in 2006 for soybean, and from 1.1 in 1996 to 3.1 in 2005 for cotton (NASS, 2011).The increased use of glyphosate also increases the risk of non-target crop injury. When glyphosate is applied to glyphosate-resistant (GR) crops, drift on to non-GR crops may cause injury and reduce yields. Glyphosate drift onto non-target crops from ground or aerial applications is common in agricultural regions, including the Mississippi Delta region.

For effective management of herbicide application, early detection of crop injury is important. Crop injury caused by off-target drift of glyphosate has been studied through a number of injury identification methods. Rowland (2000) used the stand height to identify the degree of glyphosate injury in corn. Remote sensing methods have been developed in an attempt to detect crop injury more effectively. The methods include multispectral imaging (Thelen et al., 2004; Huang et al., 2010) and spectral reflectance measurement (Henry et al., 2004). Moreover, it would be of interest to know if measurement of narrow-banded reflectance has the capability of revealing subtle changes in canopy reflectance, which could present more useful information in detecting the onset of stress from crop injury.

This study was conducted in a greenhouse to evaluate data from a portable spectroradiometer for the detection of crop injury caused by applied glyphosate by measuring crop chlorophyll reflectance. Chlorophyll fluorescence was measured on crop leaves along with reflectance measurement. The objectives of this study were, to determine the effectiveness of chlorophyll reflectance and fluorescence analysis for detecting the onset of crop injury caused by applied glyphosate, and to investigate the relationship between crop injury and chlorophyll reflectance and fluorescence.

2. Materials and Methods

2.1 Experiment Facility and Equipment

The experiment was conducted in a greenhouse. Non-glyphosate resistant soybean (cultivar SO80120LL) plants were raised in pots. A spray chamber was used to treat the plants with different doses of glyphosate (Ding et al 2011).

A portable spectroradiometer (LI-COR LI1800, LI-COR, Inc., Lincoln, Nebraska) was used to measure the chlorophyll reflectance of the soybean leaves. The spectral range of the spectroradiometer was from 400 nm to 1100 nm with the spectral resolution of 2 nm. A portable chlorophyll fluorometer (Handy Pea, Hansatech Instruments Ltd, Norflok, UK) was used to measure the chlorophyll fluorescence of the soybean leaves.

2.2 Experiment Configuration and Design

Twelve pots of non-glyphosate-resistant soybeans were used. Four plants were for low dose treatment (0.086 kg ae/ha), and four were for high dose treatment (0.86 kg ae/ha). The remaining four plants were used as controls (no glyphosate treatment).

In the spray chamber a TeeJet 8002E nozzle (TeeJet Technologies, Wheaton, Illinois) was used to spray the high and low doses of glyphosate at spray rate of 187 L/ha. Pressure was set at 138 kPa, release height was 36 cm, and forward speed was 3.7 km/h. At the time of treatment, the soybeans were at three-trifoliolate leaf stage.

2.3 Chlorophyll Reflectance Data Processing

Using the measured spectral reflectance of the plant leaves, narrow-band vegetation indices were calculated to enhance detection of plant vigor. The calculated indices include:

1. NDVI (Normalized Difference Vegetation Index) (Rouse et al., 1973):

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED}}$$

where NIR is the value of the reflectance in the Near Infrared band; RED is the value of the reflectance in the RED band.

2. RVI (Ratio Vegetation Index) (Jordan, 1969):

$$\mathrm{RVI} = \frac{\mathrm{NIR}}{\mathrm{RED}}$$

3. SAVI (Soil Adjusted Vegetation Index) (Huete, 1988):

$$\mathrm{SAVI} = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED} + \mathrm{L}} \times (1 + \mathrm{L})$$

L is a parameter that indicates the effect of the soil background behind the plant leaves; presented as 0.5 for this study.

4. DVI (Difference Vegetation Index) (Tucker, 1979):

$$\mathrm{DVI} = \mathrm{NIR} - \mathrm{RED}$$

Bandwidth of RED and NIR was 4 nm located at 662 nm and 734 nm centers, respectively. These two wavelengths correspond to maximum chlorophyll absorption (662 nm) and the maximum chlorophyll reflectance

(734 nm) on the long-wavelength shoulder of the chlorophyll red-edge, which was observed from the data (Figure 1).

2.4 Chlorophyll Fluorescence Data Processing

The chlorophyll fluorometer generates a Kautsky fluorescence induction (Kautsky and Hirsch, 1931) curve. The kinetics of the induction curve appears universally in photosynthetic organisms including microalgae and cyanobacteria. A set of parameters can be extracted from the curve to induce a fast chlorophyll fluorescence response from a dark adapted leaf sample (Figure 2), including:

1) Fo (Fluorescence origin)

The starting value of the curve.

2) Fm (Fluorescence maximum)

The maximum fluorescence value obtained for a continuous light intensity.

3) Fv (variable fluorescence)

The variable component of the recording related to the maximum capacity for photochemical quenching:

$$\mathrm{Fv} = \mathrm{Fm} - \mathrm{Fo}$$

4) Fv/Fm

Fv/Fm is a highly effective and sensitive parameter which may be used as the indicator of plant stress.

5) Tfm

The parameter to indicate the time when the maximum fluorescence value (Fm) is reached.

6) Area

The area above the curve between Fo and Fm. It highlights changes in the shape of the induction kinetic between Fo and Fm.

7) PI (Performance index)

PI is essentially an indicator of sample vitality generated from the measurement of chlorophyll fluorescence.

2.5 Statistical Analysis

Four narrow-band vegetation indices from chlorophyll reflectance data and 7 parameters from the chlorophyll fluorescence curves were analyzed using the SAS GLM procedure (SAS Institute Inc., Cary, NC) for mean separation of the indices among high, low and 0 (control) doses and among 4, 24, 48, and 72 hours after treatment (0.05 confidence probability). In addition, linear discriminant analysis for the treatments was implemented for each time period after treatment. A leave-one-out cross validation schema was used in the analysis. The discriminant analysis was based on the four vegetation indices calculated from reflectance data.

3. Results and Discussion

Four narrow-band vegetation indices from chlorophyll reflectance data were analyzed. Tables 1 shows the results of the mean separation of the vegetation indices for 4, 24, 48 and 72 hours after treatment, respectively. The table indicates that 4 hours after treatment all vegetation indices were not significantly different from among high dose, low dose, and control. 24 hours after treatment the high-dose SAVI and DVI were significantly different from the control but not significantly different from the low-dose indices. However, the high-dose NDVI and RVI were significantly different from the low-dose indices but not controls. Seventy-two hours after treatment the high-dose NDVI, RVI, SAVI and DVI were all significantly different from the controls but not the low-dose indices. The 48 hours post-treatment results required further analysis because of anomalies present in the data. Table 2 shows the results of the mean separation of the vegetation indices from 4, 24, 48 to 72 hours after treatment for high-dose, low-dose and control treatment, respectively. The table indicates that for high-dose treatment NDVIs at 4 and 24 hours after treatment were significantly different from the ones at 48 and 48 hours after treatment. RVI at 4 hours was significantly different from the one at 24 hours which, in turn, was significantly differently from the ones at 48 and 72 hours. SAVI and DVI at 4 hours were not significantly different from that of 48 hours but significantly different from 24 and 72 hours. SAVI and DVI were not significantly different at all from 4 hours to 72 hours after low-dose treatment. NDVI at 4 hours were not significantly different from 24 hours but significantly different from 48 hours. RVI at 4 hours were significantly different from the ones at 24, 48 and 72 hours. All vegetation indices were not significantly different from 4 hours to 72 hours for control.

The results explain that the vegetation indices were able to differentiate between the high dose treatment and the control as early as 1 day after treatment. Also, temporally from 4 hours to 72 hours the indices could identify

high- and low-dose herbicide effect in 1 day after treatment. The indices were derived from measurement of plant chlorophyll reflectance, which was sensitive to the effect of glyphosate on plants.

Linear discriminant analysis shows results similar to the previous mean separation results (Table 3). It also indicates that results from 48 hours are inconsistent with other time period. Specifically, at 4 hour it is difficult to classify among the treatments and the controls. This is due to the herbicide damage over the plant is not severe enough for the reflectance measurement to pick up. At 24 hours and 72 hours, the control can be 100% classified. Accuracy for the low treatment is 75% and 50% for the two time period. For the high treatment, the accuracy is 50% and 100%, respectively. If the low and high treatments were pooled together as a single treatment group, the accuracy would be 100% for 24 hours and 87.5% for 72 hours. The 48 hours results have relatively low accuracy. For example, even with pooled operation, the accuracy for the treatment group is only 50%. This is possibly due to the anomalies in the data. Thus, from the above linear discriminant analysis, it demonstrated that herbicide damage to the soybean plants could be identified at 24 hours or more post application with reflectance data.

Seven parameters from the chlorophyll fluorescence curves were also analyzed. Table 4 shows the results of the mean separation of the fluorescence parameters for 4, 24, 48 and 72 hours after treatment, respectively. The table illustrates that in the same day of treatment and 1 day after treatment all parameters from the chlorophyll fluorescence curves were not significantly different among high-dose, low-does, and control. 48 hours after treatment the high-dose Fo, Fv/Fm, and PI were significantly different from the control and low-dose parameters. The high-dose Fv was significantly different from the control but not the low-dose parameter. Seventy-two hours after treatment the high-dose Fo, Fv, Fv/Fm, and area were significantly different from the controls and the low-dose parameters. The high-dose Fm and PI were significantly different from the control but similar to the low-dose one. Table 5 shows the results of the mean separation of the fluorescence parameters from 4, 24, 48 to 72 hours after treatment for high-dose, low-dose, and control treatment, respectively. The table indicates that for high-dose treatment PI and area at 4 hours were significantly differently from 24, 48, and 72 hours after treatment. Fo, Fm, Fv and Fv/Fm at 4 hours were significantly differently from 48 and 72 hours but similar at 24 hours, while Tfm did not show significant difference among hours after treatment. For low-dose treatment PI and area at 4 hours were still significantly different from 24, 48, and 72 hours after treatment, and Fv/Fm at 4 hours was significantly different from 24 and 72 hours but not 48 hours. Fo, Fm, Fv, and Tfm had no significant difference among hours after treatment. For control Fo, Fm, Fv, area and PI had no significant difference among hours after treatment, while Fv/Fm and Tfm at 4 hours were significantly different from 24 and 72 hours but not 48 hours.

These results explain that the chlorophyll fluorescence parameters were able to differentiate between the high dose treatment and the low dose in 48 hours after treatment. Temporally from 4 hours (0 day) to 72 hours (3 days) the parameters could identify high- and low-dose herbicide effect in 1 day after treatment. The indices were derived from measurement of plant chlorophyll reflectance, which is sensitive to glyphosate sprayed on plants. The parameters were extracted from the chlorophyll fluorescence curves, which showed a comparable sensitivity to the effect of glyphosate dose sprayed on plants than chlorophyll reflectance.

4. Conclusions

This study was conducted in a greenhouse to measure the chlorophyll reflectance and fluorescence of soybean plant leaves using a portable spectroradiometer and a portable chlorophyll fluorometer, respectively. The measured data were processed and analyzed for the detection of crop injury caused by applied glyphosate. The results of the statistical mean separation indicated that the vegetation indices SAVI and DVI derived from plant chlorophyll reflectance were able to differentiate among the high dose treatment and the control, 24 hours after treatment, at which time visual inspection could not distinguish between glyphosate injured and non-treated plants. The results further indicated that RVI could help identify high- and low-dose herbicide effect within 1 day after treatment. Moreover, linear discriminant analysis with reflectance data showed that herbicide damage to the soybean plants could be identified at 24 hours or more post application. The results of the statistical mean separation also indicated that the Kautsky fluorescence induction parameters, especially Fo and Fv/Fm, were able to differentiate between the high dose treatment and low dose treatment and between high dose treatment and the control, 48 hours after treatment. Furthermore, the results indicated that PI and area could identify high- and low-dose herbicide effect in 1 day after treatment. This study concludes that plant chlorophyll reflectance has a comparable sensitivity to glyphosate doses compared with chlorophyll fluorescence. The results of this study provides the method and information for coming studies investigate chlorophyll reflectance and chlorophyll fluorescence responses to glyphosate in greenhouse and fields for soybean or other crop, such as corn and cotton, to see if responses are crop-specific.

Acknowledgements

Thanks to Mr. Efren Ford and Mr. Richard Horton for technical assistance in raising plants and herbicide applications. Special thanks to Mr. Jason Weick for assistance with plant reflectance data acquisition.

Disclaimer

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply approval of the product to the exclusion of others that may be available.

References

Ding, W., Reddy, K. N., Zablotowicz, R. M., Bellaloui, N., & Bruns, H. A. (2011). Physiological responses of glyphosate-resistant and glyphosate-sensitive soybean to aminomethylphosphonic acid, a metabolite of glyphosate. *Chemosphere, 83*, 593-598. http://dx.doi.org/10.1016/j.chemosphere.2010.12.008

Henry, W. B., Shaw, D. R., Reddy, K. R., Bruce, L. M., & Tamhankar, H. D. (2004). Remote sensing to detect herbicide drift on crops. *Weed Technol., 18*, 358–368. http://dx.doi.org/10.1614/WT-03-098

Huete, A. R. (1988). A soil-adjusted vegetation index (SAVI). *Remote Sens. Environ., 25*, 295–309. http://dx.doi.org/10.1016/0034-4257(88)90106-X

Huang, Y., Thomson, S. J., Ortiz, B. V., Reddy, K. N., Ding, W., Zablotowicz, R. W., & Bright, J. R. (2010). Airborne remote sensing assessment of the damage to cotton caused by spray drift from aerially applied glyphosate through spray deposition measurements. *Biosyst. Eng., 107*, 212-220. http://dx.doi.org/10.1016/j.biosystemseng.2010.08.003

Jordan, C.F. (1969). Derivation of leaf area index from quality of light on the forest floor. *Ecology*, *50*, 663–666. http://dx.doi.org/10.2307/1936256

Kautsky, H., & Hirsch, A. (1931). Neue versuche zur kohlensäureassimilation. *Naturwissenschaften, 19*, 964-964. http://dx.doi.org/10.1007/BF01516164

NASS (National Agricultural Statistics Service). (2011). Agricultural Chemical Use Database. Available at. Accessed 4 May 2011.

Rouse, J. W., Haas, R. H., Schell, J. A., & Deering, D. W. (1973). Monitoring vegetation systems in the great plains with ERTS. In: *Third ERTS Symposium NASA SP-351,* NASA, Washington, DC, *1*, 309–317.

Rowland, C. D. (2000). Crop tolerance to non-target and labeled herbicide applications. MS thesis. Mississippi State, Mississippi: Mississippi State University, Department of Plant and Soil Sciences.

Thelen, K. D., Kravchenko, A. N., & Lee, C. (2004). Use of optical remote sensing for detecting herbicide injury in soybean. *Weed Technol., 18*, 292-297. http://dx.doi.org/10.1614/WT-03-049R2

Tucker, C. J. (1979). Red and photographic infrared linear combinations for monitoring vegetation. *Remote Sens. Environ., 8*, 127–150. http://dx.doi.org/10.1016/0034-4257(79)90013-0

Table 1. Mean separation of the vegetation indices 4 hours, 24 hours, 48 hours and 72 hours after treatment, respectively*

4 Hours after Treatment				
Dose	NDVI	RVI	SAVI	DVI
High (0.86 kg ae/ha)	0.8001 [a]	9.0594 [a]	0.6263 [a]	0.4528 [a]
Low (0.086 kg ae/ha)	0.8210 [a]	10.2070 [a]	0.6232 [a]	0.4252 [a]
Control	0.8007 [a]	9.0537 [a]	0.5922 [a]	0.3942 [a]
24 Hours after Treatment				
Dose	NDVI	RVI	SAVI	DVI
High (0.86 kg ae/ha)	0.78201 [b]	8.1903 [b]	0.5283 [b]	0.3260 [b]
Low (0.086 kg ae/ha)	0.8068 [a]	9.3939 [a]	0.6105 [ba]	0.4122 [ba]
Control	0.7981 [ba]	8.9131 [ba]	0.6698 [a]	0.5154 [a]
48 Hours after Treatment				
Dose	NDVI	RVI	SAVI	DVI
High (0.86 kg ae/ha)	0.7585 [a]	7.3075 [a]	0.4441 [a]	0.2446 [a]
Low (0.086 kg ae/ha)	0.7963 [a]	8.8592 [a]	0.5668 [a]	0.3694 [a]
Control	0.7808 [a]	8.2491 [a]	0.5141 [a]	0.3144 [a]
72 Hours after Treatment				
Dose	NDVI	RVI	SAVI	DVI
High (0.86 kg ae/ha)	0.7402 [b]	6.7761 [b]	0.5216 [b]	0.3310 [b]
Low (0.086 kg ae/ha)	0.7783 [ba]	8.0495 [ba]	0.5833 [ba]	0.3962 [ba]
Control	0.7932 [a]	8.7208 [a]	0.6584 [a]	0.4938 [a]

*mean is not significantly different with the same letter at 0.05 level

Table 2. Mean separation of the vegetation indices from 4 to 24, 48, and 72 hours for high-dose, low-dose, and no glyphosate control treatment, respectively*

High-Dose Treatment (0.86 kg ae/ha)				
Hours after Treatment	NDVI	RVI	SAVI	DVI
4	0.8001 [a]	9.0594 [a]	0.6263 [a]	0.4528 [a]
24	0.7821 [a]	8.1903 [b]	0.5283 [ab]	0.3260 [ab]
48	0.7585 [b]	7.3075 [c]	0.4441 [b]	0.2446 [b]
72	0.7402 [b]	6.7761 [c]	0.5216 [ab]	0.33102 [ab]
Low-Dose Treatment (0.086 kg ae/ha)				
Hours after Treatment	NDVI	RVI	SAVI	DVI
4	0.8210 [a]	10.2070 [a]	0.6232 [a]	0.4252 [a]
24	0.8068 [ab]	9.3939 [b]	0.6105 [a]	0.4122 [a]
48	0.7963 [b]	8.8592 [b]	0.5668 [a]	0.5141 [a]
72	0.7783 [c]	8.0495 [c]	0.5833 [a]	0.3962 [a]
Control				
Hours after Treatment	NDVI	RVI	SAVI	DVI
4	0.8007 [a]	9.0537 [a]	0.5922 [ab]	0.3942 [a]
24	0.7981 [a]	8.9131 [a]	0.6698 [a]	0.5154 [ab]
48	0.7932 [a]	8.7208 [a]	0.5141 [b]	0.3144 [b]
72	0.7808 [a]	8.2491 [a]	0.6584 [a]	0.4938 [a]

*mean is not significantly different with the same letter at 0.05 level

Table 3. Summary of discriminant analysis with cross-validation using linear discriminant function

Number of Observations Classified into Treatment (4 hours)				
From Treatment	Control	Low	High	Accuracy
High (0.86 kg ae/ha)	3	1	0	0%
Low (0.086 kg ae/ha)	1	2	1	50%
Control	1	1	2	25%
Number of Observations Classified into Treatment (24 hours)				
From Treatment	Control	Low	High	Accuracy
High (0.86 kg ae/ha)	0	2	2	50%
Low (0.086 kg ae/ha)	0	3	1	75%
Control	4	0	0	100%
Number of Observations Classified into Treatment (48 hours)				
From Treatment	Control	Low	High	Accuracy
High (0.86 kg ae/ha)	3	0	1	25%
Low (0.086 kg ae/ha)	1	2	1	50%
Control	1	1	2	25%
Number of Observations Classified into Treatment (72 hours)				
From Treatment	Control	Low	High	Accuracy
High (0.86 kg ae/ha)	0	0	4	100%
Low (0.086 kg ae/ha)	1	2	1	50%
Control	4	0	0	100%

ISSN 1916-9752 E-ISSN 1916-9760

Table 4. Mean separation of the fluorescence parameters for 4 hours, 24 hours, 48 hours and 72 hours after treatment, respectively*

4 Hours after Treatment							
Dose	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
High (0.86 kg ae/ha)	515.25 [a]	2875.5 [a]	2360.3 [a]	0.8218 [a]	282.5 [a]	51942 [a]	1.3113 [a]
Low (0.086 kg ae/ha)	510.50 [a]	2911.8 [a]	2401.3 [a]	0.8270 [a]	330.0 [a]	65874 [a]	1.6178 [a]
Control	504.25 [a]	3051.3 [a]	2547.0 [a]	0.8350 [a]	300.0 [a]	58499 [a]	0.8078 [a]
24 Hours after Treatment							
Dose	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
High (0.86 kg ae/ha)	703.8 [a]	2284.0 [a]	1580.3 [a]	0.6678 [a]	285.0 [a]	24592 [a]	0.1323 [a]
Low (0.086 kg ae/ha)	678.3 [a]	2919.0 [a]	2240.8 [a]	0.7685 [a]	245.0 [a]	40612 [a]	0.2718 [a]
Control	607.5 [a]	2741.5 [a]	2134.0 [a]	0.7793 [a]	207.5 [a]	36364 [a]	0.5945 [a]
48 Hours after Treatment							
Dose	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
High (0.86 kg ae/ha)	879.0 [a]	2044.0 [a]	1165.0 [b]	0.5015 [b]	697.5 [a]	27236 [a]	0.1813 [b]
Low (0.086 kg ae/ha)	504.0 [b]	2530.8 [a]	2026.8 [ba]	0.8018 [a]	270.0 [a]	41122 [a]	1.1570 [a]
Control	570.3 [b]	2942.3 [a]	2372.0 [a]	0.8025 [a]	267.5 [a]	49100 [a]	1.0725 [a]
72 Hours after Treatment							
Dose	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
High (0.86 kg ae/ha)	1016.8 [a]	1872.3 [b]	855.5 [b]	0.3633 [b]	118.8 [a]	11353 [b]	0.0398 [b]
Low (0.086 kg ae/ha)	603.5 [b]	2595.3 [ba]	1991.8 [a]	0.7680 [a]	242.5 [a]	32755 [a]	0.3195 [ab]
Control	649.3 [b]	2801.5 [a]	2152.3 [a]	0.7643 [a]	207.5 [a]	35658 [a]	0.6363 [a]

*mean is not significantly different with the same letter at 0.05 level

Table 5. Mean separation of the fluorescence parameters from 4 to 24, 48, and 72 hours for high-dose, low-dose, and no glyphosate control treatment, respectively*

High-Dose Treatment (0.86 kg ae/ha)							
Hours after Treatment	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
4	515.3[c]	2875.5[a]	2360.3[a]	0.8218[a]	282.5[ab]	51942[a]	0.8078[a]
24	703.8[cb]	2284.0[ab]	1580.3[ab]	0.6678[ab]	285.0[ab]	24592[b]	0.1323[b]
48	879.0[ba]	2044.0[b]	1165.0[b]	0.5015[bc]	697.5[a]	27236[b]	0.1813[b]
72	1016.8[a]	1872.3[b]	855.5[b]	0.3633[c]	118.8[b]	11353[b]	0.0398[b]
Low-Dose Treatment (0.086 kg ae/ha)							
Hours after Treatment	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
4	510.5[a]	2911.8[a]	2401.3[a]	0.82700[a]	330.00[a]	65874[a]	1.6178[a]
24	678.3[a]	2919.0[a]	2240.8[a]	0.7685[b]	245.00[a]	40612[b]	0.2718[b]
48	504.0[a]	2530.8[a]	2026.8[a]	0.80175[ab]	270.00[a]	41122[b]	1.1570[a]
72	603.5[a]	2595.3[a]	1991.8[a]	0.76800[b]	242.50[a]	32755[b]	0.3195[b]
Control							
Hours after Treatment	Fo	Fm	Fv	Fv/Fm	Tfm	Area	PI
4	504.3[a]	3051.3[a]	2547.0[a]	0.83500[a]	300.00[a]	58499[a]	1.3113[a]
24	607.5[a]	2741.5[a]	2134.0[a]	0.77925[b]	207.50[b]	36364[a]	0.6363[a]
48	570.3[a]	2942.3[a]	2372.0[a]	0.80250[ba]	267.50[ab]	49100[a]	1.0725[a]
72	649.3[a]	2801.5[a]	2152.3[a]	0.76425[b]	207.50[b]	35658[a]	0.5945[a]

*mean is not significantly different with the same letter at 0.05 level

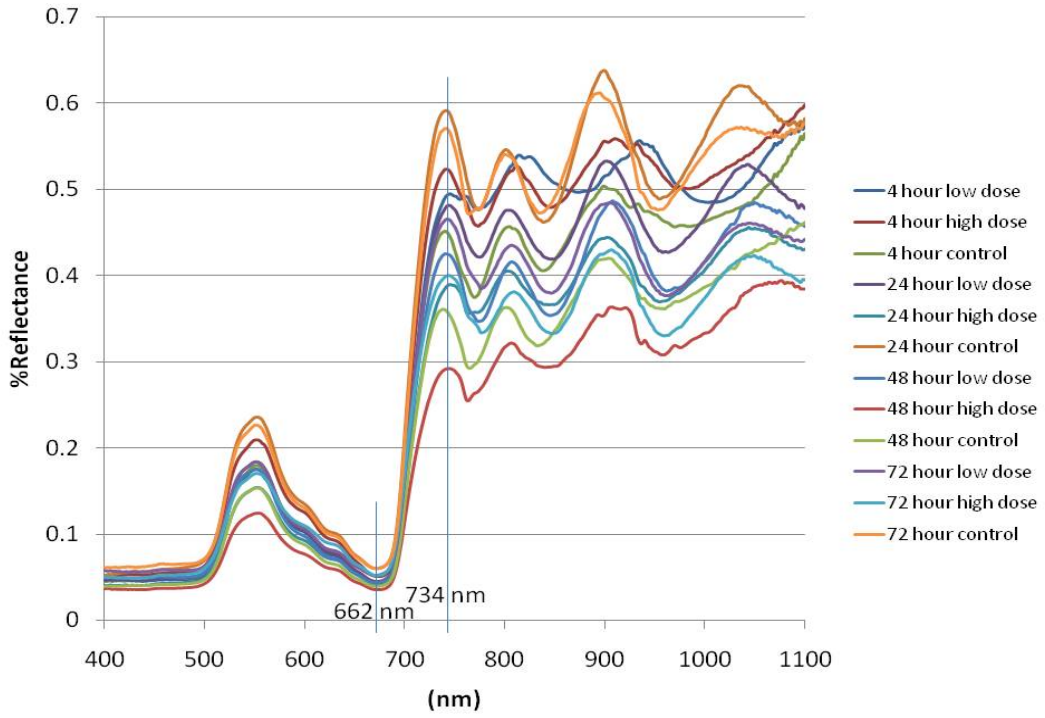

Figure 1. Average spectral reflectance curves

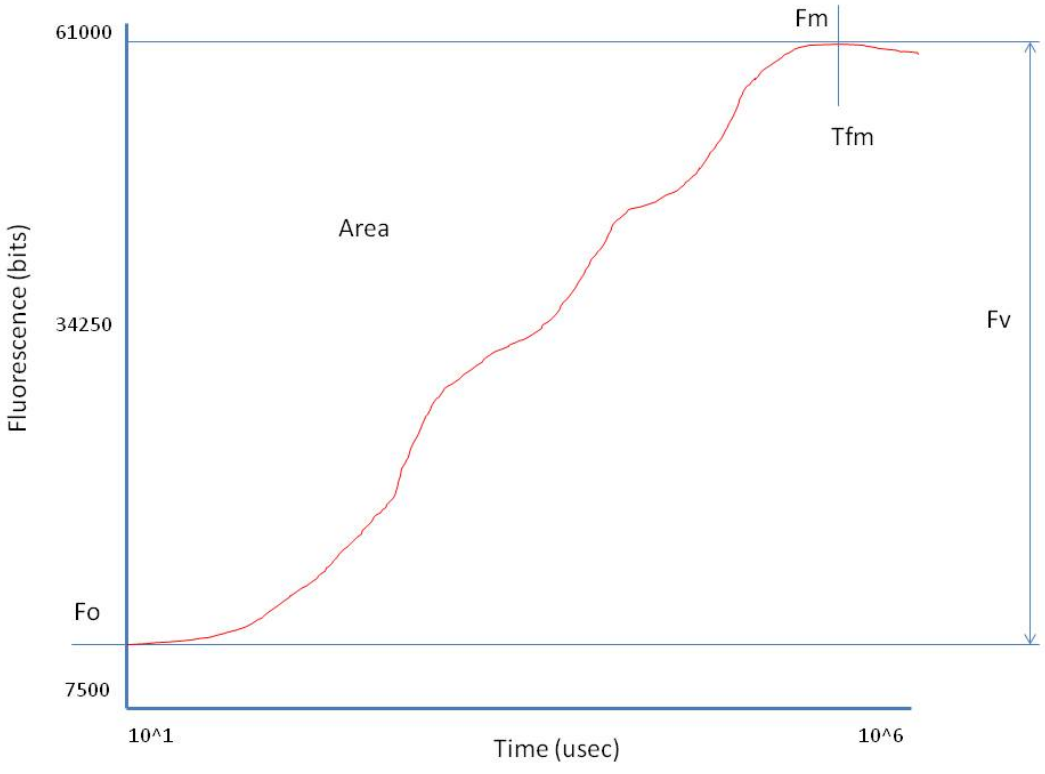

Figure 2. A typical Kautsky fluorescence induction curve

ISSN 1916-9752 E-ISSN 1916-9760