Potential for Remote Sensing to Detect and Predict Herbicide Injury on Waterhyacinth (*Eichhornia crassipes*)

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Many large-scale management programs directed toward the control of waterhyacinth rely on maintenance management with herbicides. Improving the implementation of these programs could be achieved through accurately detecting herbicide injury in order to evaluate efficacy. Mesocosm studies were conducted in the fall and summer of 2006 and 2007 at the R. R. Foil Plant Science Research Center, Mississippi State University, to detect and predict herbicide injury on waterhyacinth treated with four different rates of imazapyr and glyphosate. Herbicide rates corresponded to maximum recommended rates of 0.6 and 3.4 kg ae ha^{-1} (0.5 and 3 lb ac^{-1}) for imazapyr and glyphosate, respectively, and three rates lower than recommended maximum. Injury was visually estimated using a phytotoxicity rating scale, and reflectance measurements were collected using a handheld hyperspectral sensor. Reflectance measurements were then transformed into a Landsat 5 Thematic Mapper (TM) simulated data set to obtain pixel values for each spectral band. Statistical analyses were performed to determine if a correlation existed between bands 1, 2, 3, 4, 5, and 7 and phytotoxicity ratings. Simulated data from Landsat 5 TM indicated that band 4 was the most useful band to detect and predict herbicide injury of waterhyacinth by glyphosate and imazapyr. The relationship was negative because pixel values of band 4 decreased when herbicide injury increased. At 2 wk after treatment, the relationship between band 4 and phytotoxicity was best (r^2 of 0.75 and 0.90 for glyphosate and imazapyr, respectively), which served to predict herbicide injury in the following weeks.

Nomenclature: Glyphosate; imazapyr; waterhyacinth, *Eichhornia crassipes* (Mart.) Solms EICCR.

Key words: Landsat 5 TM, reflectance, spectral bands, phytotoxicity, invasive plant, aquatic plant management.

Waterhyacinth [*Eichhornia crassipes* (Mart.) Solms] is an exotic, monocotyledonous, free-floating aquatic plant that belongs to the Pontederiaceae family (Godfrey and Wooten 1979). The native range of waterhyacinth extends to tropical and subtropical regions of South America, and it was introduced into the United States in 1884 (Wunderlich 1962). Reproduction of waterhyacinth is predominantly vegetative, by which it effectively doubles the number of plants every 12.5 d (Penfound and Earle 1948) and increases dry biomass at a rate of 1.2% d^{-1} (Center and Spencer 1981). The floating plant mats produced by this rapid growth rate and the species' relation to worldwide economic losses and environmental impacts have led to waterhyacinth being considered one of the "world's worst weeds" (Holm et al. 1991). For instance, the presence of waterhyacinth in a water body limits water use for recreation and hydroelectric power generation and reduces phytoplankton production and oxygen mixing in the water column (Honnell et al. 1993; McVea and Boyd 1975; Toft et al. 2003).

Management of waterhyacinth is usually dependent on the use of herbicides such as diquat (Langeland et al. 2002), glyphosate (Van et al. 1986), and 2,4-D (Joyce and Haller 1984) to control its biomass and prevent its spread. Diquat rapidly reduces plant tissue within 3 d, controlling more than 85% of the plant stand (Langeland et al. 2002). Likewise, glyphosate and 2,4-D are reported to reduce populations of waterhyacinth to "non problematic levels" achieving more than 85% control in 14 d or less (Joyce and Haller 1984; Van et al. 1986). Imazapyr has also been suggested to have efficacy on waterhyacinth; however, no studies have been published to date.

Upon exposure to herbicides, target plants typically have phytotoxicity symptoms. Phytotoxicity is the symptomology (e.g., chlorosis) that susceptible plant species exhibit in

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Interpretative Summary

Waterhyacinth is an aquatic weed that causes ecological and economic losses worldwide. The management of waterhyacinth relies on use of herbicide applied to the foliage. This study explored the potential of using satellite Landsat 5 Thematic Mapper data to detect and predict herbicide injury on waterhyacinth affected by two herbicides: glyphosate and imazapyr. Among the spectral bands studied, only the near-infrared band was useful to detect and predict herbicide injury. The detection of herbicide injury was comparable to visual injury ratings. The prediction of herbicide injury was possible at 3, 4, 5, and 6 wk after treatment. The main limitation appeared to be when plants showed very little injury and could not be distinguished from plants that had not been exposed to the herbicides.

Although this study only simulates sensor data from Landsat 5 TM, results suggest that aquatic plant managers may be able to use remote sensing within a 2-wk period after applications of glyphosate or imazapyr to assess effectiveness of the treatment. This method needs to be validated with field data as part of an operational waterhyacinth management program.

response to herbicide injury that can be quantified using rating scales to measure the efficacy of a particular herbicide (Willard 1958). Rating scales are subjective (Willard 1958); they are based on visual estimations of herbicide injury and may vary between observers. However, these scales provide numerical data necessary to statistically evaluate the efficacy of the herbicide used.

With large-scale applications, monitoring programs rely on the evaluation of herbicide efficacy by a subjective spot assessment of efficacy (Madsen and Bloomfield 1993). Limitations of such an evaluation are that it is very labor intensive, especially in areas with limited accessibility (Jakubauskas et al. 2002), and that it is often subjective or biased according to the observer performing the survey (Madsen and Bloomfield 1993). These problems may be overcome with the use of remote sensing because it analyzes changes across a geographic area instead of at single points, while maintaining an unbiased response of herbicide effects from plants (Jakubauskas et al. 2002).

Most of the available sensors used in remote sensing applications (e.g., Landsat 5 Thematic Mapper [TM]) construct images based on the visible (400 to 700 nm) as well as near- and mid-infrared (700 to 900, 1,550 to 1,750, and 2,000 to 2,350 nm) spectral regions (Jensen 2000). Energy in these spectral regions, or bands, is either absorbed or reflected by vegetation. These absorption or reflectance data can be used in many weed management applications. For instance, changes in red and near-infrared reflectance are reported to be useful in the detection of plant stressors such as injury due chemical and biological control as well as water and nitrogen deficiency (Adcock et al. 1990; Carter 1991, 1993; Everitt et al. 2005; Filella and Peñuelas 1994; Henry et al. 2004; Thelen et al. 2004). Primarily, energy in the red (650 to 700 nm) and mid-infrared (1,300 to 3,000 nm) regions is absorbed by chlorophyll molecules and water in plants, respectively (Carter 1993; Ustin et al. 2009,). Alternatively, light energy is reflected by internal leaf structures (e.g., spongy mesophyll cells) in the green (500 to 550 nm) and near-infrared (700 to 1,300 nm) regions (Gates et al. 1965; Gausman 1974; Jensen 2000). Another spectral region that responds to plant stress is the red edge (695 to 725 nm) which changes spectrally due to plant pigment content (Carter and Knapp 2001; Ustin et al. 2009), such as chlorophyll (Boochs et al. 1990; Filella and Peñuelas 1994). In fact, the red edge peak of 703 nm shifts toward shorter wavelengths when plants are under stress of nitrogen and water deficiencies (Boochs et al. 1990; Filella and Peñuelas 1994).

Because herbicides have the capacity to alter the physiological function of the plant (e.g., loss of plant pigments), it is expected that the spectral characteristics will also be altered. This relationship has been studied in terrestrial plants where temporal herbicide injury has been compared with changes of energy reflectance (Adcock et al. 1990). Conversely, in aquatic plant species, most studies have focused on species differentiation using reflectance and biological control stressors (Best et al. 1981; Everitt et al. 2002, 2005) without considering spectral responses to stressors such as herbicide injury. In addition, studies of aquatic plants lack temporal data (Everitt et al. 2002) and have only assessed plant stress at the leaf level (Carter 1991) instead of the canopy level. Measuring the spectral response at the canopy level is important when working with aquatic species because of the direct influence of the ratio of vegetation cover to water background (Best et al. 1981; Lehmann and Lachavanne 1997; Peñuelas et al. 1993) and its relation to changes in canopy complexity over the growing season (Madsen 1993). Therefore, it is important to fully understand how the spectral response changes over time when aquatic plants are exposed to herbicides.

In this experiment, simulated spectral data from Landsat 5 TM were used to investigate herbicide injury through time and document the control of waterhyacinth. The TM sensor was selected because it has provided continuous imagery of the earth's surface since 1982, producing multispectral imagery in seven spectral bands including the visible and infrared regions (Table 1). Moreover, its spectral resolution coincides with currently operable sensors such as Landsat 7 Enhanced Thematic Mapper (ETM+) and the Surrey Linear Imager (SLIM6). The study was not intended to simulate either temporal or spatial resolution from Landsat 5 TM.

The objectives of this study were to (1) investigate the relationship between spectral bands 1, 2, 3, 4, 5, and 7 from the Landsat 5 TM sensor and visual phytotoxicity ratings of waterhyacinth treated with glyphosate and

Table 1. Spectral band ranges and their respect spatial resolution or pixel size used by the Thematic Mapper sensor onboard Landsat 5.

Band number		Spectral resolution (nm)	Spatial resolution (m)
1	(blue)	450–520	30
2	(green)	520–600	30
3	(red)	630–690	30
4	(near-infrared)	760–900	30
5	(midinfrared)	1,550–1,750	30
6	(thermal)	10,400–12,500	120
7	(midinfrared)	2,080–2,350	30

imazapyr and (2) using the best spectral band, develop a model to predict herbicide injury on waterhyacinth treated with glyphosate or imazapyr. The goal of this research was to determine if remote sensing can be used to monitor herbicide injury and predict ultimate mortality of waterhyacinth treated with slow-acting herbicides such as imazapyr and glyphosate. Hence, further recommendations may be developed for large-scale herbicide assessments to minimize survey time.

Materials and Methods

Field Experiment. An outdoor mesocosm study was conducted during the fall of 2006 and repeated the following year in the summer of 2007 at the R. R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS (33°28′30″N, 88°46″25″, elevation 104 m [341 ft]). The study was conducted in 378-L (100-gal) tanks that were 63 cm deep, 132 cm long, and 78 cm wide. Water volume was maintained at 300 L in each tank and aeration was supplied using a regenerative air blower to promote water circulation. Tanks were arranged in a completely randomized design to evaluate eight herbicide treatments plus an untreated reference. Each treatment was repeated in four tanks. Waterhyacinth was grown in each tank until plants covered the water surface, which took approximately 4 wk. Water was amended with fertilizer[1] at a rate of 60 mg L^{-1} (0.008 oz gal $^{-1}$) every 3 wk to prevent nutrient deficiency.

Herbicides evaluated were the isopropylamine salt of imazapyr[2] and the isopropylamine salt of glyphosate.[3] Imazapyr was applied at rates of 0.6, 0.3, 0.15, and 0.07 kg ae ha^{-1} (0.26, 0.13, 0.06lb ac^{-1}) whereas, glyphosate was applied at 3.4, 1.7, 0.8, and 0.4 kg ae ha^{-1}(1.51, 0.71, 0.35 lb ac^{-1}). Rates corresponded to the maximum recommended label rate (×) and three reduced herbicide rates (0.50×, 0.25×, and 0.125×). A nonionic surfactant[4] was added (glyphosate 0.50% v/v, imazapyr 0.25% v/v) to each spray mixture as recommended by the herbicide label.

Table 2. Phytotoxicity rating scale used to assess herbicide injury in waterhyacinth. Adapted from Nelson et al. (2001).

Rating	Description
1	No visible effect; green, healthy tissues; no herbicide damage; identical to check (control)
2	Very mild symptoms; slight color change (mild yellowing or browning); plants will recover
3	Mild symptoms; off-color plant tissues; more severe discoloration than no. 2 rating
4	Clear symptoms; probably won't result in plant death
5	Clear symptoms; possible permanent damage to plant tissues; will result in decrease biomass
6	Distinct damage on 25% of plant tissues (but less than 50%)
7	Severe damage on 50% of plant tissues (but less than 75%)
8	Very severe damage; 75% of tissues affected (but less than 100%)
9	Necrotic, collapsing tissues; damage on 100% of plants; total destruction of plant stand

Treatments were applied using a CO_2-pressurized backpack sprayer and a single-nozzle boom. The spray apparatus was calibrated to deliver 187 L ha^{-1} over the plant canopy at a constant speed and pressure of 3.2 km h^{-1} and 276 kPa respectively. An even flat fan nozzle[5] was used to obtain plant coverage during herbicide application. A protective barrier was placed around each tank during herbicide application to prevent drift and off-target injury.

Data Collection. Herbicide injury was evaluated weekly by one observer using a phytotoxicity rating scale for 6 wk after treatment (WAT; Nelson et al. 2001; Table 2). The scale rates visible injury from 1 (no visible injury) to 9 (destruction of the plant). Information at each category or rank in the scale details and describes common visual symptoms exhibited by injured plants such as chlorosis and necrosis in combination with an estimated percentage of plant tissue affected. The performance of the phytotoxicity rating scale was assessed using percentage of control visual ratings and chlorophyll concentration. Percentage of control was determined in 10% increments where 0% = no control and 100% = complete plant death; chlorophyll concentration was estimated from the first true leaf of a random plant in each treatment using a handheld chlorophyll meter[6] every week (Spencer et al. 2007).

Hyperspectral reflectance data were obtained weekly from each treatment using a handheld spectroradiometer.[7] This device measures reflectance in 2,151 spectral bands between 350 and 2,500 nm with a 1.4-nm bandwidth at a field of view of 25°. A total of 25 hyperspectral signatures were collected randomly from each treatment using the bare fiber of the sensor. The sensor was held at nadir (0.3 m) over the plant canopy throughout data collection to obtain signatures from an area of 137 cm^2 for each signature. Data were collected at noon (± 1 h) on cloudless days using sunlight as the energy source. Hyperspectral signatures from plants that collapsed to the bottom of the tank due to herbicide effects were recorded as zero or no spectral response. Previous studies have used this type of spectroradiometer to obtain ground-truth spectral data from herbicide-injured plants (Henry et al. 2004) and simulated reflectance data from multispectral sensors such as Landsat 7 ETM+, Terra Moderate Resolution Imaging Spectroradiometer (MODIS), and NOAA-14 Advance Very High Resolution Radiometer (AVHRR) (Miura et al. 2002).

Landsat 5 TM Data Simulation. All hyperspectral signatures for each treatment across all weeks were transformed to simulate a Landsat 5 TM multispectral data set. The spectroradiometer used measures of reflectance within the same spectral resolution as bands 1, 2, 3, 4, 5, and 7 of Landsat 5 TM (Table 1). The transformation was performed using a mathematical code developed in MatLab software[8] that applies a spectral filter to the hyperspectral signatures reducing the total number of bands to the desired bands1, 2, 3, 4, 5, and 7 of Landsat 5 TM. A transformation matrix was constructed in MatLab with each column containing weights of the spectral filters in an orthogonal manner to create the relative spectral response for each spectral band (NASA 2008). Band 6, which is considered the thermal band, was not extracted because its spectral range is not measured by the spectroradiometer used in this study (350 to 2,500 nm). The resultant simulated data set contained pixel values for each band on a scale of 0 to 255, because Landsat 5 TM has a radiometric resolution of 8 bits ($2^8 = 256$, range = 0 to 255).

Data Analysis. All statistical analyses were performed using SAS v. 9.1 software[9] using a significance level of 0.05. The performance of the phytotoxicity scale was evaluated against percent age of control ratings and chlorophyll measurements using a correlation procedure (PROC CORR).

Spectral band and phytotoxicity data were pooled across seasons according to each response variable measured. The reason for pooling the data was to have a data set that represents the growing season of waterhyacinth and a time span of when plant control techniques are implemented. Each band was correlated (PROC CORR) to phytotoxicity ratings to determine which spectral band was the best to monitor herbicide injury according to a significant yielded Spearman's correlation coefficient (*r*) obtained. The best spectral band was then subjected to linear and polynomial regression analysis using PROC GLM at each WAT. Regression models were sequentially fit beginning with a linear model. Polynomial terms were then added one at a time and lack of fit determined using partial *t*-tests. Only the highest and consistent coefficients of determination (r^2) and their corresponding regression equations were used in the prediction of herbicide phytotoxicity for each herbicide. Predicted and observed phytotoxicity values were regressed to evaluate their relationship. The best spectral band and phytotoxicity values were also analyzed using a one-way analysis of variance where significant difference between treatment means at 1, 3, and 6 WAT over each season were determined using a Fisher's Protected LSD test. This analysis led to differentiation between noninjured and injured plants at early, middle, and late injury stages following herbicide applications. These times were also chosen because they corresponded to the temporal resolution of Landsat 5 TM, which is 16 d.

Table 3. Relationship of phytotoxicity rating scale rankings (P) with chlorophyll (chl) and percentage of control ratings with their respective Spearman's correlation coefficients (*r*).

Herbicide	Variable relationship	*r*
Glyphosate	P × chl	−0.91*
	P × % control	0.94*
Imazapyr	P × chl	−0.90*
	P × % control	0.96*

* Indicates a significant correlation at $P < 0.05$.

Results

Estimated chlorophyll concentration and visual ratings were significantly correlated ($P < 0.01$; $r > 0.90$) to phytotoxicity ratings (Table 3). Phytotoxicity was negatively correlated to chlorophyll, whereas the relationship was positive with percentage of control ratings. Therefore, results obtained with the phytotoxicity scale were considered applicable for our purpose.

Among the six bands evaluated to detect herbicide injury on waterhyacinth, only bands 2, 4, and 5 were significantly correlated ($P < 0.05$) to phytotoxicity ratings (Table 4). A stronger correlation for both herbicides (*r* of −0.78 and −0.74 for glyphosate and imazapyr respectively) was obtained when band 4 was used in comparison to bands 2 and 5. The relationship between band 4 and phytotoxicity was linear for all WAT. However, bands 2 and 5 had inconsistent polynomial relationships through time. Often the relationship between bands (2 and 5) and phytotoxicity switched between a cubic and quadratic. Therefore, band 4 was considered to be the best spectral band to detect phytotoxicity, as a linear model is much simpler to utilize when predicting an outcome.

Table 4. Relationship between phytotoxicity rating scale rankings (P) and pixel values of spectral bands 1, 2, 3, 4, 5, and 7 with their respective Spearman's correlation coefficients (r).

Herbicide	Variable relationship	r
Glyphosate	P × band 1	−0.13
	P × band 2	−0.61*
	P × band 3	−0.04
	P × band 4	−0.78*
	P × band 5	−0.57*
	P × band 7	−0.30
Imazapyr	P × band 1	−0.01
	P × band 2	−0.60*
	P × band 3	0.12
	P × band 4	−0.74*
	P × band 5	−0.37*
	P × band 7	−0.02

* Indicates a significant correlation at $P < 0.05$.

The relationship between band 4 and phytotoxicity was negative; when pixel values decreased, phytotoxicity ratings increased for each herbicide (Figures 1 and 2). The strongest relationship between these two variables was found at 2 WAT (r^2 of 0.75 and 0.90 for glyphosate and imazapyr respectively) (Figures 1 and 2). After 2 WAT, the relationship between these two variables decreased regardless of the herbicide used.

Herbicide Injury Detection. Significant differences between treated and untreated plants were detected at 1, 3, and 6 WAT when either band 4 or phytotoxicity ratings were used (Table 5). In general, the severity of herbicide injury increased as rates of both herbicides increased ($P < 0.05$). Consequently, it is observed that an increase of phytotoxicity corresponded to lower pixel values for band 4 (Table 5). On the other hand, the detection of herbicide injury may vary according to the method used (e.g., phytotoxicity vs. band 4) and herbicide rate with respect to WAT and season.

For instance, there was a significant difference ($P < 0.05$) between nontreated and treated plants at 1, 3, and 6 WAT for both seasons when glyphosate was used according to phytotoxicity ratings (Table 5). However, the detection of injury using pixel values of band 4 is more evident in higher rates of glyphosate at any WAT (Table 5). When the herbicide imazapyr was used, phytotoxicity ratings were able to significantly distinguish treated from nontreated plants at 1, 3, and 6 WAT in the summer treatment and at 3 and 6 WAT in the fall treatment (Table 5). Contrary to glyphosate injury, the detection of injury using pixel values of band 4 was more evident after 1 WAT in higher rates of imazapyr.

Herbicide Injury Prediction. Because the strongest relationship between phytotoxicity ratings and pixel values of band 4 occurred at 2 WAT for both herbicides, linear equations were constructed using this time period (Figures 1 and 2) to predict its corresponding herbicide injury in the following weeks. The following formulas, which are also depicted in Figures 1 and 2, were used to predict herbicide injury using band 4:

$$\text{Predicted glyphosate injury} = 13.95 - 0.20\,(\text{pixel value at band spectra of 760 to 900 nm}) \quad [1]$$

$$\text{Predicted imazapyr injury} = 14.90 - 0.25\,(\text{pixel value at band spectra of 760 to 900 nm}) \quad [2]$$

The relationship between predicted and observed phytotoxicity values at 3, 4, 5, and 6 WAT for both herbicides was linear and significant ($P < 0.05$) yielding r^2 of 0.39 and 0.46 for glyphosate and imazapyr, respectively (Figure 3). Therefore, the prediction of phytotoxicity was possible with the use of Equations 1 and 2 for each corresponding herbicide. Among the total predicted values for both herbicides at 3, 4, 5, and 6 WAT, up to 75% fall within the phytotoxicity scale range of 1 to 9. In addition, when the observed phytotoxicity rating is low (< 2), predicted phytotoxicity is likely to be either over- or underestimated (Figure 3).

Discussion

Simulated Spectral Bands and Phytotoxicity. According to this study, bands 2, 4, and 5 were correlated to phytotoxicity when all data were pooled across WAT. However, relationships between bands 2 and 5 to phytotoxicity were not consistent throughout the study when regression models were fit to weekly data. The utilization of bands 2 and 5 would require the fitting of different polynomial models over time to predict phytotoxicity. The use of several models for predictive purposes would be very cumbersome, time consuming, and inefficient; more importantly it would not lend itself as a practical tool for aquatic plant management. These results contradict those reported for terrestrial plants where the spectral region that coincides with bands 2 and 5 has been suggested to be sensitive to stress (Carter 1993, Carter and Knapp 2001). Our results indicate that band 4 was the only spectral band from Landsat 5 TM that was consistently related to phytotoxicity using a simple linear regression model.

Band 4 and Herbicide Phytotoxicity. Band 4 is the only spectral band from Landsat 5 TM that consistently detected herbicide injury over time, differentiated non-injured from injured plants, and successfully predicted phytotoxicity in waterhyacinth (Figure 3). The observed relationship between band 4 and waterhyacinth phytotox-

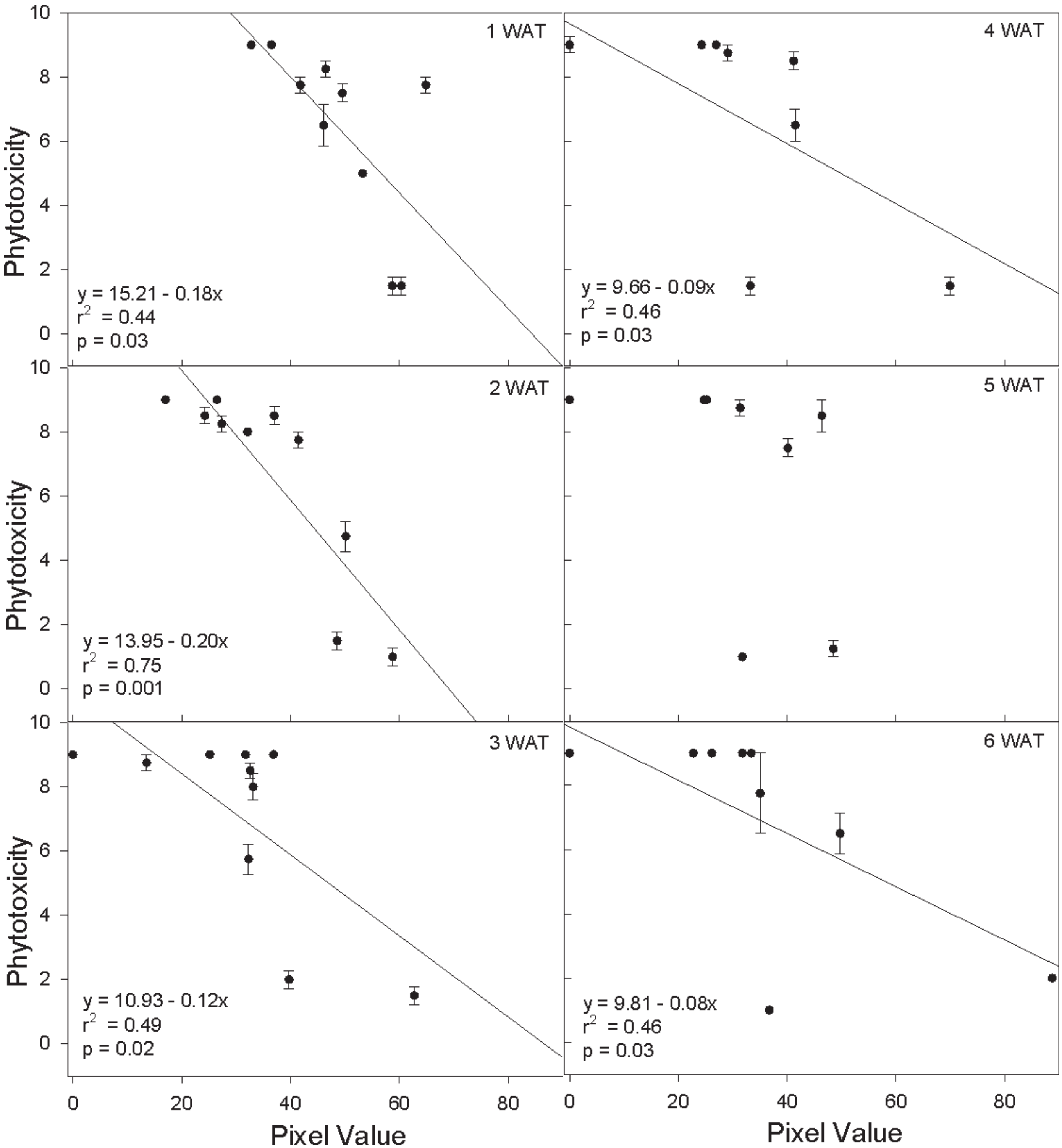

Figure 1. Linear relationship between band 4 and phytotoxicity when waterhyacinth is affected by glyphosate for every week after treatment (WAT). Values are expressed as means ($\pm$ SE) for each variable. Regression line was drawn only when the relationship was significant at a significance level of 0.05.

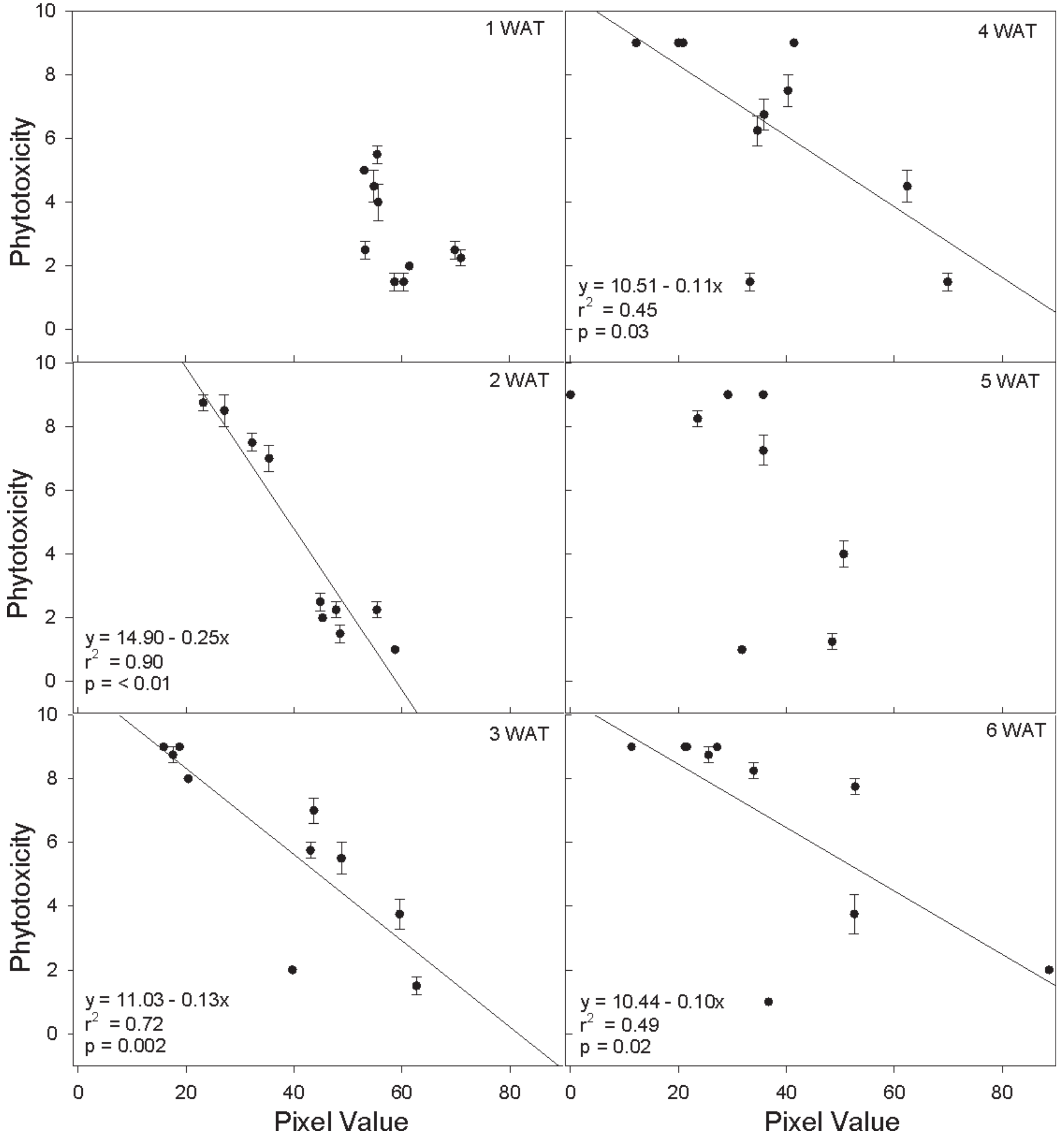

Figure 2. Linear relationship between band 4 and phytotoxicity when waterhyacinth is affected by imazapyr for every week after treatment (WAT). Values are expressed as means (± SE) for each variable. Regression line was drawn only when the relationship was significant at a significance level of 0.05.

Table 5. Phytotoxicity ratings ($n = 4$) and band 4 pixel values ($n = 25$) comparisons* at 1, 3, and 6 wk after treatment (WAT) for each study (season) when waterhyacinth was treated with glyphosate and imazapyr. Data are represented as means.

	Glyphosate						Imazapyr					
	Summer			Fall			Summer			Fall		
Treatment	1 WAT	3 WAT	6 WAT	1 WAT	3 WAT	6 WAT	1 WAT	3 WAT	6 WAT	1 WAT	3 WAT	6 WAT
	Phytotoxicity											
Reference	2 c	2 b	1 b	2 d	2 d	2 c	3 c	2 c	1 b	2 b	2 d	2 c
1×	9 a	9 a	9 a	8 ab	9 a	9 a	6 a	9 a	9 a	2 ab	7 a	9 a
0.50×	9 a	9 a	9 a	8 a	9 a	9 a	5 ab	9 a	9 a	2 ab	6 b	8 a
0.25×	8 b	8 a	8 a	7 b	8 b	9 a	4 b	9 a	9 a	3 a	6 b	8 a
0.125×	8 b	8 a	9 a	5 c	6 c	7 b	5 ab	8 b	9 a	3 a	4 c	4 b
	Band 4											
Reference	59 a	40 a	37 a	60 ab	63 a	89 a	59 a	40 a	37 a	60 bc	63 a	89 a
1×	33 e	0 d	0 b	50 c	25 c	26 cd	55 ab	16 c	27 b	61 abc	44 b	26 c
0.50×	37 d	37 a	0 b	65 a	32 b	23 d	53 b	18 bc	22 c	71 a	43 b	34 c
0.25×	46 b	14 c	35 a	46 c	33 b	32 c	56 ab	19 bc	11 d	70 ab	49 b	53 b
0.125×	42 c	33 b	33 a	53 bc	32 b	50 b	55 b	20 b	21 c	53 c	60 a	53 b

* Means within columns followed by same letter are not statistically different (P = 0.05) according to Fisher's Protected LSD.

icity was negative. Adcock and others (1990) observed a similar relationship between near-infrared (800 nm) and phytotoxicity when soybean (*Glycine max* L.) was treated with the herbicide paraquat. In aquatic habitats, injury due to the effect of a biological control agent over a homogeneous population of giant salvinia (*Salvinia molesta* Mitchell) was distinguished with the use of near-infrared (760 to 900 nm) (Everitt et al. 2005). A possible explanation for the relationship between band 4 and phytotoxicity is attributed to the increasing amount of dead plant tissue present as weeks progressed, caused by losses in pigment and cell integrity as a result of the herbicide damage.

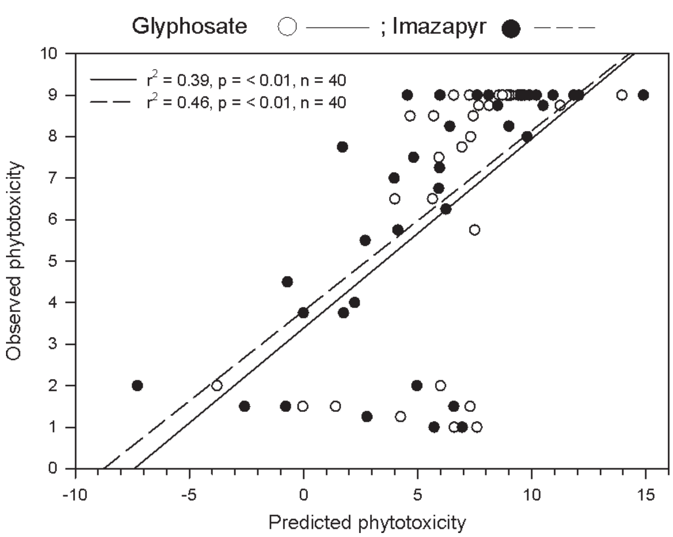

Figure 3. Linear relationship between predicted and observed phytotoxicity at 3, 4, 5, and 6 wk after treatment (WAT) when waterhyacinth was treated with glyphosate (open circles, solid line) and imazapyr (closed circles, dashed line). Values are expressed as means for each variable.

Commonly, when plants are injured with herbicides, discoloration of plant tissues appears, progressing from a general yellowing to necrosis (black, dead tissue) (Senseman 2007). Similarly, when glyphosate is used on waterhyacinth, the progress and eventual destruction of the plant stand is also gradual, with phytotoxicity proceeding from wilting and yellowing to browning and necrosis (Van et al. 1986). Discoloration causes energy in the infrared region (band 4) to be absorbed (Gausman 1974) due the alteration of plant cell integrity and pigment content (Murtha 1978). Consequently, pixel values of band 4 decreased as injury progressed. A similar relationship was observed between visual disease ratings and a near-infrared (800 nm) band when a fungicide was applied to control a fungal disease in peanut (*Arachis hypogaea* L.) (Nutter et al. 1990).

According to our results, band 4 and phytotoxicity are negatively correlated (Table 4) and correspond to the progress of herbicide injury (e.g., yellowing to necrosis). Although the relationship was significant, energy loss due to lack of plant canopy could potentially be a factor in altering the spectral response of band 4, limiting its relationship with phytotoxicity ratings and the subsequent lower r^2 values observed. It was also observed that the relationship between band 4 and phytotoxicity becomes weaker as injury progressed (Figures 1 and 2). It is suggested that a decrease of the ratio of vegetation cover to water background (Best et al. 1981), which consequently limits the reflectance on this band, may influence the relationship. Injured waterhyacinth plants were observed to sink at the bottom of the tank by 2 WAT, which increased exposed water surface area. Another limiting factor that may alter the spectral response of band 4 may be the presence of dry or wet dead tissue. Studies conducted by Carter (1993) stated that dehydration due to stress affects the spectral response of infrared reflectance. For instance, if plant tissues are dead but air-dried, reflectance in band 4 is higher than if they are dead but still wet (Gausman 1974; Murtha 1978). In our study, dead dry and wet plant tissues were not differentiated at the time of data collection, which may have affected the variation of band 4 for the same phytotoxicity rating.

Despite these limitations, band 4 could be used to detect herbicide injury and differentiate noninjured from injured plants remotely, and to collect data that were comparable to phytotoxicity visual ratings. Such detection was possible at early, middle, and late stages of herbicide injury over the two growing seasons when either band 4 or phytotoxicity ratings were used. However, the detection of injury was better after 1 WAT when imazapyr was used, perhaps because of a slower herbicidal effect in the plant. It has been reported that detection of injury using near-infrared reflectance is possible if the herbicide used can cause foliar damage (Adcock et al. 1990), as imazapyr and glyphosate do in susceptible plants. Other studies have reported that near-infrared is useful to detect injury produced by the auxin-mimic herbicides dicamba (Hickman et al. 1991).

Predicting Herbicide Injury. Herbicide injury was predicted for each herbicide and compared to observed values for the same WAT. According to this relationship, it was observed that injury prediction is consistent when phytotoxicity ratings are greater than 2. Under- or overestimation may occur if phytotoxicity is lower than 2, which corresponded to untreated plants. Hickman and others (1991) found similar limitations when herbicide injury is low, but recommend the use of near-infrared reflectance if the injury is moderate to severe. It has been reported that near-infrared is sensitive if the stress is sufficient to cause a severe damage to the leaf (Carter 1993). In addition, Murtha (1978) reported that when the damage is "chronic," near-infrared reflectance may or may not change. Based on this information and since the phytotoxicity rating scale used in our study uses visual description of the plant based on color, it is possible that untreated plants were responding to a different stressor (e.g., nutrient deficiency) as well as to herbicide exposure, while visual ratings remained the same. Therefore, herbicide injury has to be developed enough (> 2) to cause changes in band 4. Other factors, such as the presence of flowers and leaf angle due the seasonality (Madsen 1993), may have limited the predictability of low-phytotoxicity plants.

Management Implications. Remote sensing using a spectral simulated data set for band 4 of Landsat 5 TM was useful to detect and predict herbicide injury on waterhyacinth affected with glyphosate and imazapyr. Based on this result, band 4 is recommended as a potential tool in a management plan to assess herbicide efficacy in homogeneous populations of waterhyacinth during two growing seasons. It is clear that as herbicide injury increases, band 4 pixel values decrease.

The use of remote sensing may improve the effectiveness and implementation of a vegetation management program by facilitating the assessment of large herbicide applications remotely. Based on the spectral response of band 4 as observed in this study, results may guide the image analyst to identify and differentiate targeted treated areas from nontreated areas in an image. Once identified, the classified treated areas can be analyzed in terms of area coverage and spread by the target plant treated. Within the same treated areas, estimations of herbicide injury severity or ultimate control may be performed using band 4 values in Equations 1 and 2, developed for glyphosate and imazapyr. The outcome needs to be compared to the phytototoxicity rating scale used in this study in order to estimate herbicide injury. By knowing the estimated phytotoxicity rating of plants within treated areas, it may help to elucidate which homogeneous populations of waterhyacinth need to be revisited to apply herbicide again and assure ultimate control.

In terms of herbicide injury prediction after 2 WAT, estimates can be made using Equations 1 or 2 to determine eventual control when glyphosate or imazapyr, respectively, are used. Predicted phytotoxicity < 2 is not considered accurate and results may under- or overestimate herbicide injury. The prediction of herbicide injury using remote sensing may minimize monitoring efforts at single points throughout the lake while maintaining acceptable assessments.

Further Recommendations. According to Hickman et al. (1991) data collected with handheld devices are as sensitive as aerial photography. Moreover, aerial photography that measures near-infrared in the same spectral region as band 4 (760 to 900 nm) from Landsat 5 TM was able to distinguish giant salvinia under biological control stress (Everitt et al. 2005). Therefore, the use of airborne or commercial satellite data should be considered by the aquatic plant management community to extend information resulting from this study. Available data from multispectral sensors with equivalent spectral resolution to Landsat 5 TM, such as Landsat 7 ETM+ and SLIM6 are suggested. Both of these sensors have same spectral resolution as Landsat 5 TM and are currently operable, providing multispectral imaging from space.

The present study used only simulated spectral data from Landsat 5 TM obtained at the canopy level and recommendations were based upon this result. If data were to be collected from space, reflectance is likely to be attenuated by the atmosphere. Therefore, developed linear equations from this study should be tested using real Landsat 5 TM data acquired from natural and homogeneous populations of waterhyacinth treated with glyphosate and imazapyr. Results may validate the performance of the developed equations obtained in this study to detect herbicide phytotoxicity, especially after 2 WAT. Moreover, it might help to elucidate the effects of other factors not measured in this study such as mixed pixels over reflectance and ratio of vegetation cover to water background, as well as temporal and spatial resolution. A successful validation and accurate performance of these linear equations may be extended to further assess phytotoxicity in nontarget plants leading to the documentation of herbicide drift or off-target effects from herbicide applications.

If imagery is not available, new handheld devices that measure near-infrared within the same spectral range as band 4 of Landsat 5 TM could potentially be used to estimate herbicide injury in plants. Although the use of near-infrared has been documented to detect herbicide injury in soybean (Thelen et al. 2004), it has not been evaluated for aquatic plants.

In addition to the previous discussion, further research is needed to investigate the use of other spectral bands that were not considered in this study. For instance, the red edge spectral region (695 to 725nm) has been recently reported to be sensitive to plant stress (Carter and Knapp 2001; Ustin et al. 2009). Previous studies reported that shifts on the red edge may provide information regarding chlorophyll content in the plant (Boochs et al. 1990; Filella and Peñuelas 1994), which is evidently affected by herbicide injury. Results may help to elucidate a better spectral band (or bands) than band 4 from Landsat 5 TM to detect herbicide injury or other plant stressors. Likewise, information may help in the development of new sensors useful in plant applications.

Sources of Materials

[1] Miracle-Gro (24-8-16), Scotts Miracle-Gro Company, Marysville, OH 43041.

[2] Habitat, BASF Corporation, Research Triangle Park, NC 27709.

[3] AquaPro, SePRO Corporation, Carmel, IN 46032.

[4] Dyne-Amic, Helena Chemical Company, Collierville, TN 38017.

[5] TeeJet 8002E, TeeJet Technologies, Wheaton, IL 60187.

[6] Minolta SPAD 502 DL meter, Spectrum Technologies, Inc., Plainfield, IL 60585.

[7] ASD Spectroradiometer, Field Spec Pro® model FR, ASD, Inc., Boulder, CO 80301.

[8] MatLab, version 7.4, The Mathworks Inc. Natick, MA.

[9] SAS for Windows, version 9.1, SAS Institute Inc. Cary, NC.

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