

## Research Article

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**Keywords:**

Drone; RGB; aquatic; vegetation index; remote sensing; herbicide injury; image analysis

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# Evaluation of water hyacinth (*Eichhornia crassipes*) response to herbicides using unmanned aerial system imagery

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**Abstract**

Water hyacinth is a highly invasive aquatic species in the southern United States that requires intensive management through frequent herbicide applications. Quantifying management success in large-scale operations is challenging with traditional survey methods that rely on boat-based teams and can be time-consuming and labor-intensive. In contrast, an unmanned aerial system (UAS) allows a single operator to survey a waterbody more efficiently and rapidly, enhancing both coverage and data collection. Therefore, the objective of this research was to develop remote sensing techniques to assess herbicide efficacy for water hyacinth control in an outdoor mesocosm study. Experiments were conducted in spring and summer 2023 to compare and correlate data from visual evaluations of herbicide efficacy against nine vegetation indices (VIs) derived from UAS-based red-green-blue imagery. Penoxsulam, carfentrazone, diquat, 2,4-D, florypyrauxifen-benzyl, and glyphosate were applied at two rates, and experimental units were evaluated for 6 wk. The carotenoid reflectance index (CRI) had the highest Spearman's correlation coefficient with visually evaluated efficacy for 2,4-D, diquat, and florypyrauxifen benzyl ( $> -0.77$ ). The visible atmospherically resistance index (VARI) had the highest correlation with carfentrazone and penoxsulam treatments ( $> -0.70$ ), and the excess greenness minus redness index had the highest correlation for glyphosate treatments ( $> -0.83$ ). CRI had the highest correlation coefficient with the most herbicide treatments, and it was the only VI tested that did not include the red band. These VIs were satisfactory predictors of mid-range visually evaluated herbicide efficacy values but were poorly correlated with extremely low and high values, corresponding to nontreated and necrotic plants. Future research should focus on applying findings to real-world (nonexperimental) field conditions and testing imagery with spectral bands beyond the visible range.

**Introduction**

Water hyacinth, known as one of the world's worst weeds, is arguably the most intensively managed invasive plant species in Florida with management costs exceeding US\$3.6 million per year (FWC 2024; Hiatt et al. 2009; Holm, 1977; Langeland et al. 2014). Native to South America, this free-floating aquatic plant was introduced to North America as an ornamental in 1884 and quickly became problematic (Wunderlich 1962). Water hyacinth populations can double in size in as little as 6 d via vegetative reproduction of ramets (Degaga 2018). Ramets fragment from mother plants and spread readily through water currents, wind, boating activities, and intentional movement (Degaga 2018). Water hyacinth forms dense mats across the water surface that limit access and navigation, block and damage infrastructure such as bridges and flood control structures, provide habitat to disease vectors, decrease water quality, and reduce biodiversity (Holm, 1977; Villamagna and Murphy 2010).

Since the 1970s, water hyacinth has primarily been managed proactively to keep population levels as low as possible by frequent (daily to weekly) deployment of boat-based applicators who search for and treat incipient plant populations with aquatic herbicides. Foliar applications of diquat and 2,4-D have been the commercial standard for water hyacinth management for decades; however, other herbicides such as carfentrazone, florypyrauxifen-benzyl, glyphosate, and penoxsulam can also provide control and are applied based on site-specific management needs (Enloe et al. 2022; Gettys 2014; Mudge and Netherland 2014b). Diquat is a fast-acting herbicide and highly effective across a wide range of conditions (Kyser et al. 2021; Wersal and Madsen 2012). Auxin-mimic herbicides such as 2,4-D and florypyrauxifen-benzyl induce death by stimulating uncontrolled growth, with 2,4-D showing results in days,

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whereas florypyrauxifen-benzyl is slower to achieve the same level of control (Hildebrand, 1946; Mudge et al. 2021). Amino acid synthesis inhibitors such as glyphosate and penoxsulam result in slow symptom development that progresses over several weeks (Mudge and Netherland 2014b; Wersal and Madsen 2010).

Scientists and technicians who manage water hyacinth prefer to use fast-acting herbicides, including diquat and 2,4-D, for their quick, visible effects, and because treated areas can be easily identified within hours to a day after application (Mudge and Netherland 2014a). However, this rapid damage can sometimes lead to public concern about herbicide use (Heinzman et al. 2024). Public alarm is lessened when slower-acting herbicides such as florypyrauxifen-benzyl, glyphosate, and penoxsulam are applied due to the inconspicuous symptoms the herbicides cause after treatment. Acetolactate synthesis (ALS) inhibitors are also generally more selective toward emergent native plants, which many resource managers find desirable (Mudge and Netherland 2014b). However, ALS-inhibitor resistance is prominent among many terrestrial weed species, and some water hyacinth populations in Florida are suspected to have a reduced sensitivity to ALS inhibitors (Brown et al. 2024; Heap 2014).

In large-scale herbicide treatments, efficacy can be variable due to plant growth stage, nondetected plants that do not receive treatment, environmental conditions, human error, or population susceptibility (Ganie et al. 2018; Madsen 1999). This commonly leads to refuge plants remaining after treatment, thereby sustaining weed populations for regrowth and reinfestation (Cacho et al. 2006). To mitigate this, management efforts should include frequent surveillance to evaluate herbicide efficacy and determine follow-up treatments to prevent refuge populations from becoming large infestations. Herbicide efficacy evaluations are traditionally conducted through visual ratings based on phytotoxicity symptoms. Phytotoxicity refers to the symptomology that plants exhibit in response to herbicide injury, such as chlorosis and necrosis. Although these ratings are subjective, they can provide adequate accuracy and necessary numerical data for statistical analysis of herbicide efficacy by researchers. However, visual phytotoxicity assessments have their limitations under field conditions. A commonly used survey method for monitoring is the line-point intercept survey, which involves recording observations at equally spaced points along transects distributed throughout the water body (Madsen 1999). Some survey areas may be inaccessible by boat or be large enough that frequent monitoring is a significant drain on resources (Jakubauskas et al. 2002). The high growth rate and mobility of water hyacinth populations also contribute to the frequency of monitoring required, adding to the cost and resources allotted to management (Jakubauskas et al. 2002).

Remote sensing technology can be a critical tool for streamlining the monitoring process of herbicide efficacy, thus significantly reducing the cost, time, and resources required compared to reliance on traditional visual monitoring (Jakubauskas et al. 2002). While low-resolution satellite imagery (e.g., Sentinel 2; Landsat 8) has been used to map water hyacinth and predict injury, its spatial resolution is too low to map water hyacinth at the area coverages maintained by a proactive management regimen (Dube et al. 2017; Pádua et al. 2022; Robles et al. 2010; Rodríguez-Garrito et al. 2023).

As an alternative to satellite imagery, an unmanned aerial system (UAS) equipped with optical cameras and automated flight planning can quickly cover large areas and obtain high-resolution

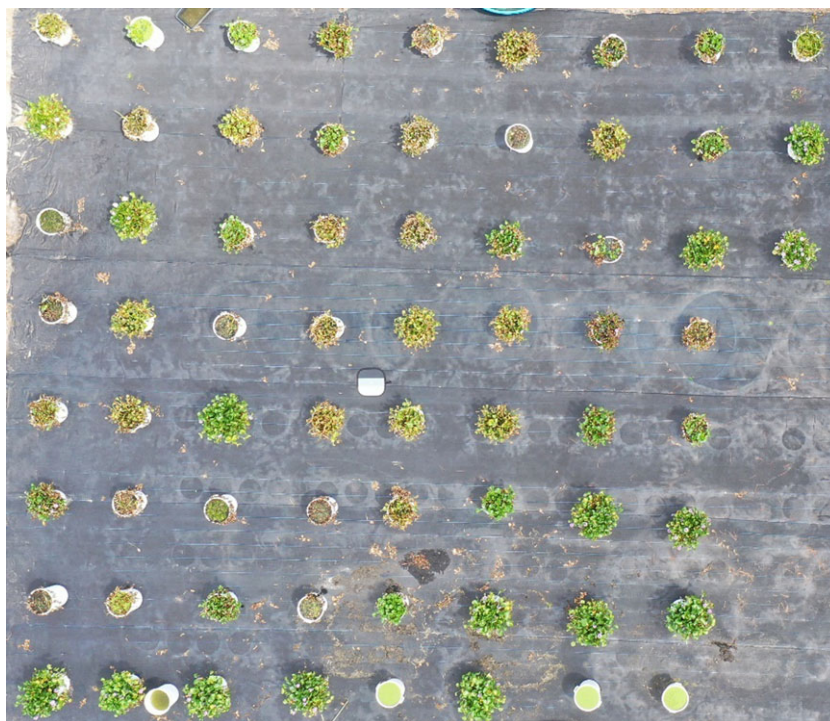
visually interpretable information (Cummings et al. 2017; Müllerová 2019). Many natural-area managers use more affordable red, green, and blue (RGB) sensors and onboard navigation sensors to directly georeference the captured images to fit their practical needs (Dronova et al. 2021; Kior et al. 2024). Curran et al. (2020) found that UAS-gathered surveys using onboard navigation systems were more spatially accurate, faster, and more efficient than manual line point-intercept surveys.

The RGB bands of an inexpensive digital camera mounted to a UAS can allow visualization of herbicide symptomology in plants (Kior et al. 2024). Changes in plant physiology qualitatively change light spectra due to the absorption of light in the visible range by photosynthetic pigments, water, and the internal structures of leaves (Kior et al. 2024). For example, herbicides that affect photosynthetic activity can result in changes in reflectance in the red spectral range, which can be detected by cameras (Kior et al. 2024). Kior et al. (2024) reported that RGB spectral bands can estimate plant biomass and chlorophyll content with high efficiency. These bands can be used in various calculations to generate vegetation indices (VIs), which are designed to estimate key aspects of plant health. These indices have been shown to correlate with chlorophyll content, herbicide-induced injury, and biomass in previous studies (Abrantes et al. 2021; Liu et al. 2021; Lussem et al. 2018). While several studies in row cropping systems have been carried out to correlate VIs from inexpensive RGB cameras with plant health, to our knowledge, no studies have applied this methodology to monitor aquatic invasive plant management activities. Aerial monitoring of herbicide injury to aquatic invasive plants could significantly improve management efforts by reducing fieldwork demands and providing timely insights for making management decisions. Given the success of RGB VIs in assessing herbicide impact on terrestrial plants, we propose that water hyacinth injury can also be effectively monitored using this approach. Therefore, the objective of this study is to develop models for predicting herbicide efficacy on water hyacinth in response to six different herbicides using VIs derived from RGB imagery captured by a UAS.

## Materials and Methods

### Growth and Treatment Parameters

Experiments were conducted at the University of Florida's Center for Aquatic and Invasive Plants in Gainesville, Florida (29.72°N, 82.42°W), during the spring and summer of 2023. Plants were grown in 151-L white, high-density polyethylene mesocosms with a 56-cm diam and a 71-cm depth, spaced approximately 1 m apart (Figure 1). Each mesocosm contained well water amended with 0.08 g L<sup>-1</sup> of water-soluble fertilizer (24-8-16, Miracle-Gro® All Purpose Plant Food; Scotts Company, Marysville, OH) and 0.01 g L<sup>-1</sup> of chelated iron (Grow More Iron Chelate 10%; Grow More, Gardena CA). Mature water hyacinth plants 23 to 30 cm tall sourced from Rodman Reservoir (29.52°N, 81.88°W) were transferred to experimental units (five plants per mesocosm) and left to establish for 1 mo prior to herbicide application, at which time each mesocosm had 100% plant cover. Fertility was monitored using an electrical conductivity meter (GroLine Waterproof EC/TDS Tester; Hanna Instruments, Smithfield, RI) and fertilized with the same amount of fertilizer each time to maintain electrical conductivity measurements of 0.04 mS cm<sup>-1</sup>. Insect pests were managed as needed using carbaryl (Sevin SL; Bayer CropScience LLC, St. Louis, MO) and bifenthrin (UP-Star Gold Insecticide,



**Figure 1.** The study site is at the University of Florida Center for Aquatic and Invasive Plants, 6 wk after the spring treatment, at 30 m above ground level (0.76 cm/px). Three-panel gray scale reflectance target is pictured in the center of the study.

UPL, Cary, NC). During the first run, the average temperature was 22.5 C, and the average humidity was 74.5%, with weather conditions ranging from sunny to scattered clouds. In the second run, the average temperature was 27.2 C, and the average humidity was 81%, with weather conditions ranging from sunny to strong thunderstorms (NCEI 2023). Mesocosm water quality reflected similar parameters typical of a Florida eutrophic lake.

Each mesocosm was randomly assigned to receive a treatment, and the study had a factorial arrangement of treatments plus a nontreated control and four replications. Factors included herbicide active ingredient (2,4-D, diquat, carfentrazone, florpyrauxifen-benzyl, glyphosate, and penoxsulam) and rate (typical field use rate and maximum labeled rate) (Table 1). Herbicides were applied using a CO<sub>2</sub>-pressurized backpack sprayer equipped with two XR11004 nozzles (TeeJet Technologies, Glendale Heights, IL) spaced 18 inches (45.72 cm) apart to achieve an effective swath width of 36 inches (91.44 cm), ensuring uniform spray coverage. The herbicides were selected to demonstrate a range of modes of action and symptom development profiles commonly used for water hyacinth management, with application rates reflecting both standard field rates and maximum label rates (Madsen et al. 1995; Mudge et al. 2021; Wersal and Madsen 2010, 2012). Nozzle size was chosen to accurately deliver 935 L ha<sup>-1</sup> of solution at the applicator's walking speed while minimizing off-target drift. Calibration was checked before and after treatment to ensure consistency throughout the treatment. The first run of the experiment was initiated on April 14, 2023 (spring), and the second run on July 6, 2023 (summer).

### Data Collection

Efficacy was visually estimated weekly by the same person for 6 wk after treatment (WAT). Visually evaluated efficacy was based on

phytotoxicity: growth, stunting, and visible damage compared with nontreated control plants based on a scale from 0% to 100% where 0% = healthy unaffected plants and 100% = complete death. Corresponding images were captured at noon during cloud-free periods using a DJI Mavic 2 Pro quadcopter (DJI, Shenzhen, China) equipped with a Hasselblad L1D-20c RGB camera (Hasselblad, Gothenburg, Sweden) featuring a 20-megapixel CMOS optical sensor. If weather reports indicated cloudy conditions at noon, images were taken in the next closest cloud-free period to noon. A single image was designed to encompass the entire study region due to the small study area and low flight altitude. The study design was completely randomized to ensure that distortions around the edge of the image did not disproportionately affect any specific treatment group. The sensor was positioned at a nadir over the center of the entire experiment at an altitude of 30 m above ground level, producing a ground sampling distance of 0.76 cm px<sup>-1</sup>. The camera has a 77-degree field of view, an aperture range of f/2.8 to f/11, a focal length of 35 mm, and an ISO range of 100 to 3,200. Each captured image was 5,472 × 3,648 pixels.

### Image Calibration

To standardize RGB values across images, mean pixel values for each RGB band were extracted from a PhotoVision 24-inch One-Shot Digital Calibration Target three-panel grayscale reflectance target (PhotoVision Inc, Mint Hill, NC) placed at the center of the site using the histogram tool in ImageJ (U.S. National Institutes of Health, Bethesda, MD). Color curves in GIMP (Kylander 1999) were then used to adjust the tonal range and color balance by mapping input RGB values to reference values from the target manufacturer, and this process was applied to each image to account for variations in lighting conditions.



**Table 1.** Herbicide treatments and application rates for water hyacinth control in spring and summer studies.

Herbicide <sup>a</sup>	Standard	Maximum	Trade name and manufacturer <sup>b</sup>
	kg ai or ae ha <sup>-1</sup>		
2,4-D	2.20	4.48	2,4-D Amine; Alligare
Diquat	2.20	4.48	Tribune; Syngenta
Carfentrazone	0.47	0.95	Stingray; Sepro
Florpyrauxifen-benzyl	0.19	0.38	ProcellaCOR SC; Sepro
Glyphosate	2.20	4.48	Roundup Custom; Bayer
Penoxsulam	0.20	0.39	Galleon SC; Sepro

<sup>a</sup>A nonionic surfactant (Induce; Helena Chemical Company, Collierville, TN) was applied at 2.5 mL L<sup>-1</sup>. Florpyrauxifen-benzyl was applied with a methylated seed oil concentrate (Leci-Tech; Loveland Products, Inc., Loveland, CO) at 10 mL L<sup>-1</sup>.

<sup>b</sup>Manufacturers locations: Alligare LLC, Opelika, AL; Bayer CropScience LLC, St. Louis, MO; Sepro Corporation, Carmel, IN; Syngenta Crop Protection LLC, Greensboro, NC.

**Table 2.** Vegetation Index names, references, and corresponding equations.

Vegetation index	Reference	Equation <sup>a</sup>
Triangular greenness indices	Hunt et al. 2013	$TGI = G^a - .39R - .61B$
Visible atmospherically resistant index	Gitelson et al. 2002	$VARI = \frac{G-R}{G+R-B}$
Excess green index	Meyer and Neto 2008	$ExGI = 2G - R - B$
Modified green red vegetation index	Bendig et al. 2015	$MGRVI = \frac{G^2 - R^2}{G^2 + R^2}$
RGB vegetation index	Bendig et al. 2015	$RGBVI = \frac{G^2 - RB}{G^2 + RB}$
Green leaf index	Louhaichi et al. 2001	$GLI = \frac{2G - R - B}{2G + R + B}$
Modified photochemical reflectance index	Li et al. 2014	$MPRI = \frac{G-R}{G+R}$
Modified carotenoid reflectance index	Gitelson et al. 2002; Abrantes et al. 2021	$CRI = \frac{1}{B} - \frac{1}{G}$
Excess greenness minus red index	Meyer and Neto 2008	$ExGR = ExGI - 1.4R - G$

<sup>a</sup>R, G, and B correspond to the red, green, and blue bands of an image.

## Image Processing

Using QGIS software (QGIS Development Team 2024), circular polygons with an area of approximately 0.25 m<sup>2</sup> were created to delineate each mesocosm, isolating vegetation from the background. The Zonal Statistics tool was then used to extract the mean RGB pixel values within each polygon, with digital numbers ranging from 0 to 255 (where 255 represents the highest intensity and 0 represents the absence of that color).

## Image Analysis

The extracted RGB values were used to compute VIs in RStudio (RStudio Team 2024) based on equations in Table 2. Selected VIs were chosen based on their demonstrated correlations with herbicide efficacy or crop yield in previous studies (Abrantes et al. 2021; Liu et al. 2021; Lussem et al. 2018).

## Data Analysis

Data analysis was performed with RStudio software (v.4.4.2; RStudio Team 2024). The following R packages were used: DHARMA (Hartig 2017), GGPLOT2 (Wickham 2016), RSTATIX (Kassambara 2019), TIDYVERSE (Wickham et al. 2019), and MULTCOMP (Hothorn et al. 2016). Analysis of variance detected no difference in the interactions among rate, season, and

treatment; therefore, data were pooled across these parameters to reflect a variety of rates and timings at which water hyacinth may be treated. Data were filtered to the 3 wk when peak efficacy of each herbicide was demonstrated (1 to 3 WAT for diquat, 2,4-D, and carfentrazone; 2 to 4 WAT for florpyrauxifen-benzyl and glyphosate; and 4 to 6 WAT for penoxsulam) as determined by prior studies (Madsen et al. 1995; Mudge et al. 2021; Wersal and Madsen 2010, 2012). Nontreated control plant data were also paired with the treated data for the corresponding monitoring weeks. The VIs were correlated with visually evaluated efficacy using Spearman's correlation coefficient due to its robustness to outliers and ability to handle ranked data. The best VI for each herbicide was chosen by selecting the VI with the highest correlation. Additionally, the VI with the highest correlation with visually evaluated efficacy when all herbicide data were combined was chosen for analysis to create a combined model. Data were then subjected to a linear regression using a random selection of 80% of the data with visual efficacy as the response and the best vegetation index as the independent variable. The linear relationship between the observed and predicted visual efficacy values was then evaluated using the remaining 20% of the data to ensure model robustness. The decision to use linear regression was based on an initial visual inspection of scatter plots showing a linear relationship between the variables, as well as supportive R<sup>2</sup> values from various vegetation index models indicating that linear models adequately captured the underlying relationship.

## Results and Discussion

### Vegetation Indices for Herbicide Visually Evaluated Efficacy

Correlations between the VIs and visually evaluated efficacy were strong and negative across various herbicides and for the combined models ( $p < 0.0001$ ) (Table 3). The VI with the strongest correlation for each herbicide to predict efficacy was selected. However, many of the VIs demonstrated similar levels of correlation, suggesting that multiple indices may be similarly effective in predicting visually evaluated efficacy. The carotenoid reflectance index (CRI) was selected for 2,4-D, diquat, and florpyrauxifen-benzyl; the visibly atmospheric resistance index (VARI) was selected for carfentrazone and penoxsulam; and the excess greenness minus redness index (EXGR) was selected for glyphosate. Since visually evaluated efficacy showed the strongest correlation with EXGR when all treatment data were aggregated, this VI was chosen to create a combined model (Tables 3 and 4). All linear models had significant negative relationships between the VI and visually evaluated efficacy (Figure 2) with R<sup>2</sup> values ranging between 0.47 and 0.75.

The CRI demonstrated the highest correlations with visually evaluated efficacy for half of the treatments, indicating its robustness as a predictor of herbicide efficacy against water hyacinth. This VI was developed for nondestructive total carotenoid estimation in agricultural contexts from the principles that healthy vegetation has high reflectance in the green band (Gitelson et al. 2002). Gitelson et al. (2002) found that reciprocal reflectance in the range 510 nm to 550 nm was linearly related to the total pigment content in leaves. Abrantes et al. (2021) adapted this VI for assessing herbicide injury to soybeans with an RGB camera and found CRI to have significant relationships with visually evaluated efficacy of herbicide treatments. Of the VIs tested, the CRI was the only index that

**Table 3.** Spearman's correlation coefficients between visually evaluated efficacy and vegetation indices by herbicide.<sup>a-d</sup>

Herbicide	TGI	VARI	EXGI	MGRVI	RGBVI	GLI	MPRI	CRI	EXGR
2,4-D	−0.621	−0.745	−0.657	−0.741	−0.752	−0.755	−0.741	<b>−0.779</b>	−0.773
Carfentrazone	−0.650	<b>−0.701</b>	−0.672	−0.698	−0.683	−0.696	−0.698	−0.509	−0.658
Diquat	−0.743	−0.720	−0.734	−0.705	−0.813	−0.763	−0.705	<b>−0.890</b>	−0.809
Florpyrauxifen-benzyl	−0.700	−0.729	−0.711	−0.713	−0.806	−0.792	−0.713	<b>−0.813</b>	−0.591
Glyphosate	−0.584	−0.813	−0.666	−0.809	−0.749	−0.793	−0.809	−0.720	<b>−0.834</b>
Penoxsulam	−0.650	<b>−0.811</b>	−0.690	−0.807	−0.736	−0.780	−0.807	−0.661	−0.792
Combined	−0.668	−0.787	−0.705	−0.780	−0.778	−0.793	−0.780	−0.765	<b>−0.794</b>

<sup>a</sup>Abbreviations: CRI, modified carotenoid reflectance index; EXGI, excess green index; EXGR, excess greenness minus red index; GLI, green leaf index; MGRVI, modified green red vegetation index; MPRI, modified photochemical reflectance index; RGBVI, red-green-blue (RGB) vegetation index; TGI, triangular greenness index; VARI, visible atmospherically resistant index.

<sup>b</sup>Vegetation indices were calculated from the red, green, and blue bands of the image according to calculations listed in Table 2.

<sup>c</sup>All correlations were significant with  $\rho < 0.0001$ .

<sup>d</sup>Bold values indicate highest correlation for that herbicide.

**Table 4.** Equations for predicting visually evaluated efficacy when water hyacinth (*Eichhornia crassipes*) is affected by herbicide.<sup>a</sup>

Herbicide	Equation	Monitoring period
2,4-D	$VE = -3493.85\left(\frac{1}{B} - \frac{1}{G}\right) + 119.89$	1 to 3 WAT
Carfentrazone	$VE = -123.79\left(\frac{G-R}{G+R-B}\right) + 25.38$	1 to 3 WAT
Diquat	$VE = -3696.65\left(\frac{1}{B} - \frac{1}{G}\right) + 119.54$	1 to 3 WAT
Florpyrauxifen-benzyl	$VE = -3645.59\left(\frac{1}{B} - \frac{1}{G}\right) + 121.46$	2 to 4 WAT
Glyphosate	$VE = -0.53(G - 2.4R - B) - 42.27$	2 to 4 WAT
Penoxsulam	$VE = -143.75\left(\frac{G-R}{G+R-B}\right) + 34.39$	4 to 6 WAT
Combined EXGR	$VE = -0.55(G - 2.4R - B) - 36.19$	–

<sup>a</sup>Abbreviations: EXGR, excess greenness minus red index; R, G, and B correspond to digital numbers from the red, green, and blue bands of a digital camera; VE, visually evaluated efficacy; WAT, weeks after treatment.

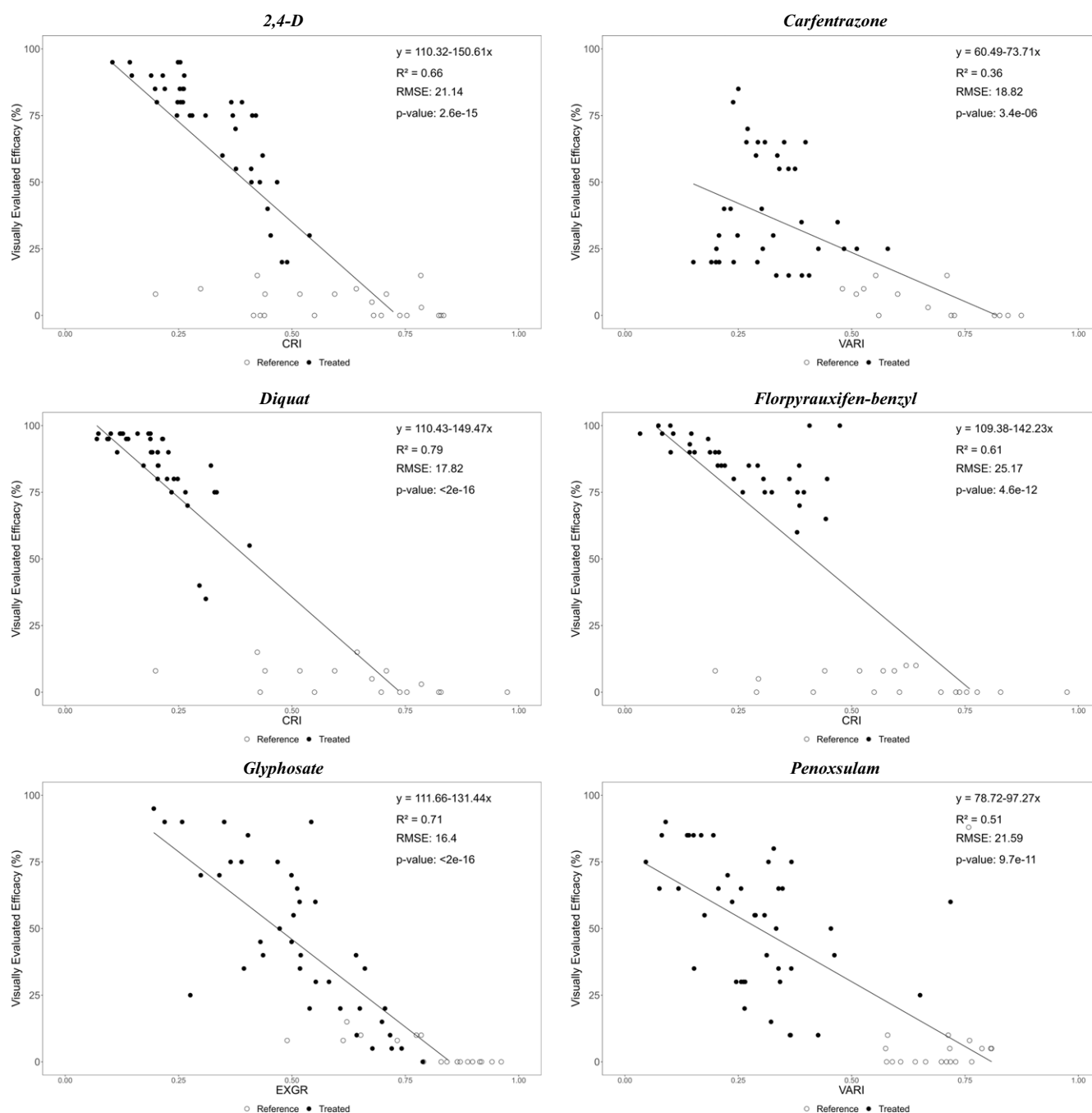
did not include the red band as part of the calculation. Water hyacinth does not produce high levels of anthocyanins (red pigment) in response to injury, which is another reason why excluding the red band may have been beneficial. Newete (2014) similarly found that a VI calculated using green and green-blue wavelengths (the photochemical reflectance index), was significantly correlated with water hyacinth stress even though it was not as robust as VIs that included the near infrared band.

The VARI, which was developed to estimate green vegetation fraction in wheat canopies with minimal sensitivity to atmospheric effects (Gitelson et al., 2002), is one of the most widely used VIs in agriculture within the visible spectrum (Xue and Su 2017). Rampazzo et al. (2022) found that VARI measurements complemented in-field estimates of soybean injury across various herbicide treatments. In the current study, VARI demonstrated the highest correlations with visually evaluated efficacy for water hyacinth treated with carfentrazone and penoxsulam. Despite their differences in mode of action and symptom development timelines, water hyacinth treated with these herbicides showed lower levels of maximum control compared to all other herbicides used in this study, which may have been why the same VIs had the best results for both treatments (Figures 2 and 3). While penoxsulam can cause progressive injury up to 10 wk after treatment (Wersal and Madsen 2010), this study was limited to 6 wk. Additionally, carfentrazone has a history of inconsistent control of water hyacinth (Wersal and Madsen 2012). The peak symptomology from both herbicides was exhibited as chlorosis compared to necrosis exhibited by the other herbicide treatments used in this study.

The excess greenness index was developed by Woebbecke and Von Bargen (1995) for separating green plants from soil and residue for image analysis and has been widely cited (Gitelson et al. 2002; Lamm et al. 2002; Mao et al. 2003). However, Meyer et al. (2004) noted that a disproportionate amount of redness from the background of the image may reduce the accuracy of this index, so Meyer and Neto (2008) developed the EXGR index to minimize this problem. Abrantes et al. (2021) found that EXGR could satisfactorily estimate herbicide damage and soybean-estimated yield loss from dicamba and 2,4-D. In our study, we found that EXGR had the highest correlation with the visual efficacy of water hyacinth in response to glyphosate, as well as the highest correlation with the aggregated dataset (Figures 2 and 4). Glyphosate has been shown to reduce anthocyanin production, which could have resulted in a more prominent drop in red color, thus showing a high response to this index (Hoagland 1980). Additionally, all herbicides lead to a reduction in greenness over time, which this VI effectively captures, likely explaining why it performed the best when applied to the aggregated data set.

### Predicting Visually Evaluated Efficacy

A perfect model would have a slope of 1,  $R^2$  of 1, and RMSE of 0 (Figure 3). While all linear relationships between predicted and observed visual efficacy values had moderate to high  $R^2$  between 0.42 and 0.81, equations reliably predicted visually evaluated efficacy only in the medium ranges, but they poorly predicted visually evaluated efficacy in the extreme ranges (25% < x > 90%) (Figure 2). The upper extreme range corresponds to necrotic plants that are approaching complete control. As water hyacinth dies, the release of nutrients into the water may promote algal blooms, while the increased space makes room for other vegetation, such as duckweed, to colonize the mesocosms (Clugston 1963). This problem was exacerbated by the fast-acting herbicides, such as diquat, used in our study, which had already resulted in high levels of injury before the first data acquisition date. Contamination from algae and duckweed may have increased the greenness in these cases and skewed the VI values higher. Nontreated mesocosms represented the lower extreme of visually evaluated efficacy, with values less than 25%. Biomass production in the untreated mesocosms often presents a level of visual stress in these mature water hyacinths due to the natural senescence of older leaves that were not being accounted for with the visually evaluated efficacy observations. Additionally, the presence of flowers and various leaf angles may also have limited predictability of low injury (Robles et al. 2010). Rampazzo et al. (2022) found that VI estimates of

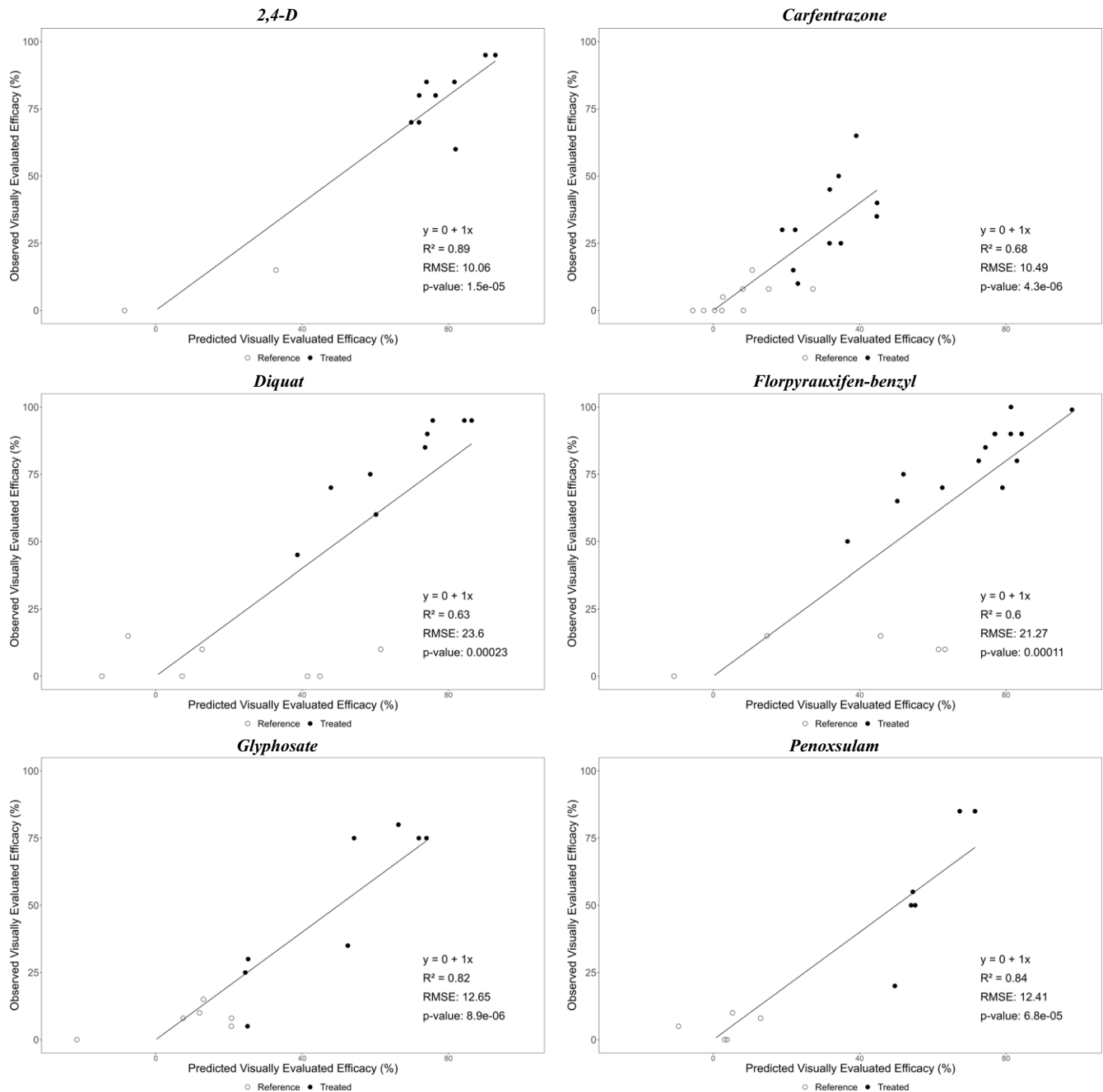


**Figure 2.** Linear relationship between the highest correlated vegetation indices (Table 2) with visually evaluated efficacy when water hyacinth is affected by herbicide at 1 to 3 wk after treatment (WAT) for diquat, 2,4-D, and carfentrazone; 2 to 5 WAT for florpyrauxifen-benzyl and glyphosate; and 3 to 6 WAT for penoxsulam ( $n = 57$ ).

injury appeared to be less sensitive to differentiating low levels of injury than a trained observer. Some herbicide symptoms such as the curling, twisting, and callus formation caused by auxin herbicides may be visible to an observer before chlorosis-induced color changes can be observed in imagery.

This study demonstrates the feasibility of using a low-cost UAS equipped with a digital camera to estimate the visually evaluated efficacy of water hyacinth treated with six different herbicides. The method developed in this study could be modified to visually estimate the effects of herbicide treatments, but also other emergent and floating vegetation, and it has the

potential to aid the development of a cost-effective tool for routinely monitoring water hyacinth chemical management. Open water present in the mesocosms was included in the vegetation index calculations to mimic field conditions, where more water would be exposed as a treatment progresses. However, water clarity and turbidity, which vary by water body and are likely to differ from mesocosm conditions, could make these VIs less reliable as treatments progress and more water is exposed. Therefore, future research should aim to translate this controlled study into field conditions to validate the practical application of these findings. Future analysis should also focus



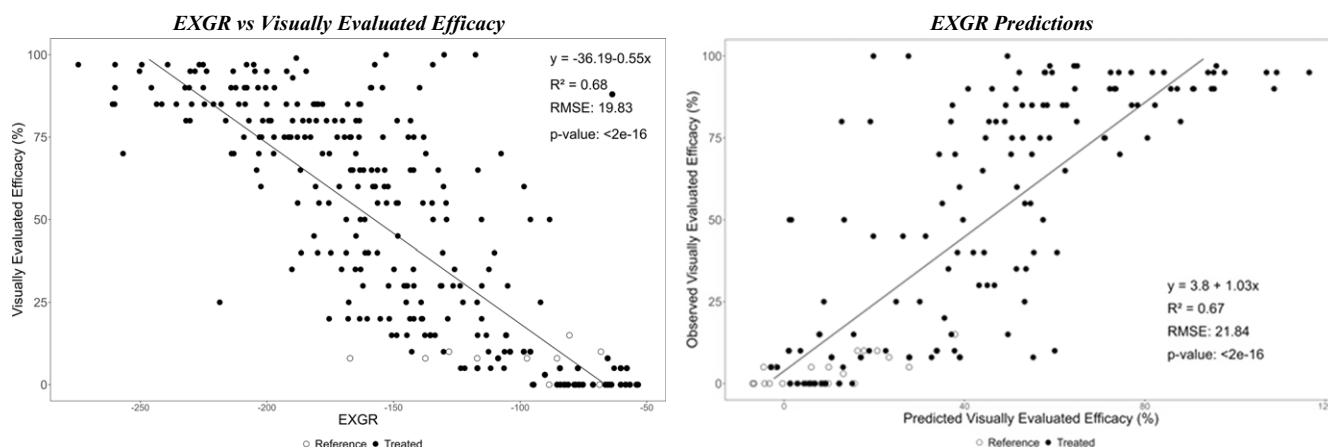
**Figure 3.** Linear relationship between predicted and observed visually evaluated efficacy values (Table 2) when water hyacinth is affected by herbicide treatments 1 to 3 wk after treatment (WAT) for diquat, 2,4-D, and carfentrazone; 2 to 4 WAT for florpyrauxifen-benzyl and glyphosate; and 3 to 6 WAT for penoxsulam ( $n = 15$ ).

on using other spectral calibration methods, such as empirical line calibration. Efforts should focus on testing imagery with bands beyond the visible spectrum and automating the GIS processing workflow to reduce turnaround time for follow-up treatment planning.

### Practical Implications

Remote sensing may improve the effectiveness of a proactive management program. Quadcopters equipped with digital cameras are inexpensive and easy to use by natural area managers, and regular aerial surveys could more quickly and efficiently capture large areas of interest than traditional monitoring

methods. Vegetation indices such as the CRI, VARI, and EXGR are strongly correlated with visually evaluated efficacy of water hyacinth and can be easily calculated from these aerial surveys in GIS. These VIs may be able to aid an image analyst in differentiating healthy and injured plants. This information could improve herbicide treatment monitoring by detecting missed water hyacinth populations or ineffective treatments for planning follow-up herbicide applications. The use of UAS imagery and VIs offers a promising way to monitor herbicide treatments in water hyacinth management. By reducing the need for intensive field monitoring and improving detection of treatment efficacy, these methods can enhance invasive species management strategies.



**Figure 4.** Left: Linear relationship between the highest correlated vegetation index (Table 2) with visually evaluated efficacy when water hyacinth is affected by herbicide for the aggregated data ( $n = 342$ ). Right: A linear relationship between predicted and observed visually evaluated efficacy for the vegetation index that had the highest correlation with visually evaluated efficacy for the aggregated data ( $n = 90$ ).

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