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Short Title: UAS for Water Hyacinth Injury

Evaluation of Water Hyacinth (*Eichhornia crassipes*) Response to Herbicides Using Unmanned Aerial System (UAS) Imagery

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Abstract

Water hyacinth is a highly invasive, aquatic species in the southern US that requires intensive management through frequent herbicide applications to minimize harmful impacts. Quantifying management success in large-scale operations is challenging with traditional survey methods, which rely on boat-based teams and can be time-consuming and labor-intensive. In contrast, unmanned aerial systems allow 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 unmanned aerial system (UAS)-based red-green-blue (RGB) imagery. Penoxsulam, carfentrazone, diquat, 2,4-D, florpyrauxifen-benzyl, and glyphosate were applied at two rates, and experimental units were evaluated for six weeks. The Carotenoid Reflectance Index (CRI) had the highest Spearman's correlation coefficient with visually evaluated efficacy for 2,4-D, diquat, and florpyrauxifen benzyl (> -0.77). The Visible Atmospherically Resistance Index (VARI) had the highest correlation for carfentrazone and penoxsulam treatments (> -0.70), and the EXGR 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 vegetation indices were satisfactory predictors of mid-range visually evaluated herbicide efficacy values but were poorly correlated with extremely low and high values, corresponding to non-treated and necrotic plants. Future research should focus on applying findings to real-world (non-experimental) field conditions and testing imagery with spectral bands beyond the visible range.

Nomenclature: 2,4-D; carfentrazone; diquat; florpyrauxifen-benzyl; glyphosate; penoxsulam; water hyacinth, *Eichhornia crassipes* (Mart.) Solms

Keywords: drone; RGB; aquatic; vegetation index; remote sensing; herbicide injury; image analysis

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 $3.6M per year (FWC 2024; Hiatt et al 2019; Holm et al. 1991; 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 six days by vegetative reproduction of ramets (Hailu 2019). Ramets fragment from mother plants and readily spread through water currents, wind, and anthropogenically through boating activities and intentional movement (Hailu et al. 2019). Water hyacinth forms dense mats across the water's 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 et al. 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 that 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 active ingredients such as carfentrazone, florpyrauxifen-benzyl, glyphosate, and penoxsulam can also provide control and are utilized based on site-specific management needs (Enloe et al. 2018; Gettys 2014; Mudge and Netherland 2014). 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 like 2,4-D and florpyrauxifen-benzyl induce death by stimulating uncontrolled growth, with 2,4-D showing results in days, while florpyrauxifen-benzyl is slower to achieve the same level of control (Hildebrand et al. 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 et al. 2014; Wersal et al. 2010).

Fast-acting herbicides, including diquat and 2,4-D, are preferred by applicators for their quick, visible effects and allow for easy identification of treated areas within hours to one day after application (Mudge and Netherland 2014a). However, this rapid damage can sometimes lead to

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public concern about herbicide use (Heinzman et al. 2024). Slower-acting herbicides like florpyrauxifen-benzyl, glyphosate, and penoxsulam are believed to reduce public alarm due to the inconspicuous symptoms they cause after treatment. Acetolactate synthesis (ALS)-inhibitors are also generally more selective towards emergent native plants, which is desirable to many resource managers (Mudge and Netherland 2014). However, ALS-inhibitor resistance is prominent among many terrestrial weed species, and there are currently some water hyacinth populations in Florida with suspected reduced sensitivity to ALS-inhibitors (Brown et al 2024; Heap 2007)).

In large-scale operational herbicide treatments, efficacy can be variable due to plant growth stage, non-detected plants that do not receive treatment, environmental conditions, human error, or population susceptibility (Ganie et al. 2017; Madsen et al. 2000). This commonly leads to refuge plants remaining after treatment, sustaining populations for regrowth and reinfestation (Cacho et al. 2006). To mitigate this, management efforts should be followed by frequent surveillance to evaluate herbicide efficacy and 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 subjective, these ratings 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

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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; Padua et al. 2022; Robles et al. 2010; Rodriguez et al. 2023).

As an alternative to satellite imagery, unmanned aerial systems (UAS) equipped with optical cameras and automated flight planning can quickly cover large areas with 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 for direct georeferencing of the captured images to fit their practical needs (Dronova et al. 2021; Kior et al. 2024). Curran et al. (2020) found that unmanned aerial 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 impact 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 utilized 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; Lieu et al. 2021; Lussem et al. 2018). While there have been several studies in row cropping systems correlating VIS from inexpensive RGB cameras with plant health, there is a lack of studies applying this methodology to monitor aquatic invasive plant management activities. Aerial monitoring of herbicide injury for aquatic invasive plants could significantly improve efficiency by reducing fieldwork demands and providing timely insights for 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 unmanned aerial systems.

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. Each mesocosm contained well-water amended with 0.08-g L^{-1} of water-soluble fertilizer (24-8-16, Miracle-Gro® All Purpose Plant Food, Scotts Company) and 0.01-g L^{-1} of chelated iron (Grow More Iron Chelate 10%, Grow More). Mature, 23 to 30 cm tall, water hyacinth plants sourced from Rodman Reservoir (29.52°N, 81.88W°) were transferred to experimental units (five plants per mesocosm) and left to establish for one month 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 (ppm) Tester, Hanna Instruments] and fertilized with the same amount of fertilizer each time to maintain electrical conductivity measurements of 4 S cm^{-1}. Insect pests were managed as needed using carbaryl (Sevin SL, Bayer CropScience LLC) and bifenthrin (UP-Star Gold Insecticide). During the first run, the average temperature was 72.5°F, and the average humidity was 74.5%, with weather conditions ranging from sunny to scattered clouds. In the second run, the average temperature was 81°F, and the average humidity was 81%, with weather conditions ranging from sunny to strong thunderstorms (National Centers for Environmental Information (NCEI) 2023). Mesocosm water quality reflected similar parameters typical of a Florida eutrophic lake.

Treatments were randomly assigned to each mesocosm, and the study had a factorial arrangement of treatments plus a non-treated control and four replications. Factors were 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 CO2-pressurized backpack sprayer equipped with two11004 nozzles (XR nozzle, TeeJet® Technologies, Spraying Systems, 1891 Business Park Dr, Springfield, IL 62703, USA) spaced 18 inches apart to achieve an effective swatch width of 36 inches, 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 (Mudge et al. 2021; Wersal and Madsen 2012; Wersal and Madsen 2010; Madsen et al. 1995). Nozzle size was chosen to accurately deliver 935 L ha-1 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 weeks after treatment (WAT). Visually evaluated efficacy was based on phytotoxicity: growth, stunting, and visible damage compared to the non-treated control based on a scale from 0 to 100% (0 = healthy unaffected plants and 100 = complete death). Corresponding images were captured at noon during cloud-free windows, using a DJI Mavic 2 Pro quadcopter equipped with a Hasselblad L1D-20c RGB camera featuring a 20-megapixel CMOS optical sensor. If weather reports indicated cloudy conditions at noon, images were taken in the next closest cloud-free window 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 (AGL), producing a ground sampling distance (GSD) of 0.76 cm px^{-1}. The camera has a 77-degree field of view (FOV), an aperture range of f/2.8 to f/11, a focal length of 35mm, and an ISO range of 100–3200. Each captured image was 5472 × 3648 pixels.

Image Calibration

To standardize RGB values across images, mean pixel values for each RGB band were extracted from a PhotoVision 24” One-Shot Digital Calibration Target three-panel grayscale reflectance target placed at the center of the site using the histogram tool in ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health). Color curves in GIMP (Kylander and Olof et al. 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.

Image Processing

In QGIS (QGIS Development Team 2024), circular polygons with an area of approximately .25 m^2 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 vegetation indices (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; Lieu et al. 2021; Lussem et al. 2018).

Data Analysis

Data analysis were performed in R Studio (v.4.4.2) (R Core Team, 2024, PBC, Boston, MA). The following R packages were used: DHARMa (Hartig et al. 2016), ggplot2 (Wickham 2016), rstatix (Kassambara 2019), tidyverse (Wickham et al. 2016), and multcomp (Hothorn et al. 2002). Analysis of variance detected no difference for the interactions between rate, season, and treatment therefore data was pooled across these parameters to reflect a variety of rates and timings at which water hyacinth may be treated. Data were filtered to the three weeks displaying peak efficacy for each herbicide (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 (Mudge et al 2021; Wersal and Madsen 2012; Wersal and Madsen 2010; Madsen et al. 1995). Non treated control 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^2 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 vegetation indices (VIs) and visually evaluated efficacy were strong and negative across various herbicides and for the combined models ($p < 0.0001$) (Table 3). The vegetation index 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, Visibly Atmospheric Resistance Index (VARI) was selected for carfentrazone and penoxsulam, and 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 (Table 3). All linear models had significant negative relationships between the VI and visually evaluated efficacy (Figure 2) with R^2 values ranging between 0.47 and 0.75.

The Carotenoid Reflectance Index demonstrated the highest correlations with visually evaluated efficacy for half of the treatments, indicating its robustness as a predictor of herbicide efficacy on 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 to 550 nm was linearly related to the total pigment content in leaves. Abrantes et al. (2021)

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adapted this VI for assessing herbicide injury in soybeans with an RGB camera and found CRI to have significant relationships with visually evaluated efficacy for herbicide treatments of soybean. Of the VIs tested, the CRI was the only index that 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 the exclusion of the red band may have been beneficial. Newete et al. (2014) similarly found that a VI calculated using green and green-blue wavelengths (Photochemical Reflectance Index), though not as robust as VIs that included the near infrared band, was significantly correlated with water hyacinth stress.

The Visible Atmospherically Resistant Index (VARI), developed to estimate green vegetation fraction in wheat canopies with minimal sensitivity to atmospheric effects (Gitelson 2002), is one of the most widely used vegetation indices in agriculture within the visible spectrum (Xue et al. 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 vegetation indices had the best results for both treatments (Figure 2). While penoxsulam can cause progressive injury up to 10 weeks after treatment (Wersal et al. 2010), this study was limited to 6 weeks. Additionally, carfentrazone has a history of inconsistent control of water hyacinth (Wersal et al. 2012). The peak symptomology for both herbicides was exhibited as chlorosis compared to the necrosis exhibited by the other herbicide treatments used in this study.

The Excess Greenness Index was developed by Woebbecke et al. (1995) for separating green plants from soil and residue for image analysis and has been widely cited in various agricultural applications (Gitelson et al 2002; Lamm et al 2002; Mao et al 2003). However, Meyer et al. (2004b) 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 Excess Greenness minus Redness (EXGR) index to minimize this problem. Abrantes et al. (2002) 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 (Figure 2, Figure 4). Glyphosate has been shown to reduce anthocyanin production, which could have resulted in a more prominent drop in ‘Redness’ 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 dataset.

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 only reliably predicted visually evaluated efficacy in the medium ranges but 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 fast-acting herbicides used in the study, such as diquat, which had already resulted in high levels of injury before the first data acquisition date. Contamination from algae and duckweed may have increased “greenness” in these cases and skewed the VI values higher. Non-treated 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 have also limited predictability of low injury (Robles 2010). Rampazzo et al. (2022) found that UAV-derived VI estimates of 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 estimate visually evaluated efficacy for other herbicide treatments as well as 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 vegetation indices 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 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 accessible to 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 Carotenoid Reflectance Index, Visible Atmospherically Resistant Index, and Excess Greenness Minus Redness Index are strongly correlated with visually evaluated efficacy of water hyacinth and can be easily calculated from these aerial surveys in GIS. These vegetation indices 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 approach for monitoring 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.

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Competing Interests

Competing interests: The author(s) declare none.

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

Herbicide[1]	Standard	Maximum	Product and Manufacturer
	kg ai or ae ha^{-1}		
2,4-D	2.20	4.48	2,4-D Amine, Alligare LLC, Opelika, AL, USA
Diquat	2.20	4.48	Tribune™, Syngenta Crop Protection LLC, Basel, Switzerland
Carfentrazone	0.47	0.95	Stingray®, Sepro Corporation, Carmel, IN,USA
Florpyrauxifen-benzyl	0.19	0.38	ProcellaCOR SC™, Sepro Corporation, Carmel, IN, USA
Glyphosate	2.20	4.48	Roundup® Custom, Bayer CropScience LLC, Kaiser-Wilhelm-Allee, Leverkusen
Penoxsulam	0.20	0.39	Galleon® SC, Sepro Corporation, Carmel, IN, USA

[1]non-ionic surfactant (Induce®, Helena Chemical Company, Collierville, TN, USA) at 0.25% v v^{-1}. Florpyrauxifen-benzyl (FPB) was applied with a methylated seed oil (MSO concentrate with Leci-Tech, Loveland Products, Inc., Loveland, CO, USA) surfactant at 1% v v^{-1}.

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

Vegetation Index	Reference	Equation
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	$RGBVI = \frac{G^2 - R\mathrm{B}}{\mathrm{G}^2 + R\mathrm{B}}$
Green Leaf Index	Louchaichi et al. 2001	$GLI = \frac{2G - R - B}{2G + R + B}$
Modified Photochemical Reflectance Index	Li, Li and Sun 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	$ExGR = ExGI - 1.4R - G$

[a]R,G, and B correspond to the Red, Green and Blue Bands of an image.

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Table 3. Spearman's correlation coefficients between visually evaluated efficacy and vegetation indices by herbicide

Herbicide	TGI[ab]	VARI	EXGI	MGRVI	RGBVI	GLI	MPRI	CRI	EXGR
2,4-D	-0.621[c]	-0.745	-0.657	-0.741	-0.752	-0.755	-0.741	**-0.779**[d]	-0.773
Carfentrazone	-0.650	**-0.701**	-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	**-0.890**	-0.809
Florpyrauxifen-benzyl	-0.700	-0.729	-0.711	-0.713	-0.806	-0.792	-0.713	**-0.813**	-0.591
Glyphosate	-0.584	-0.813	-0.666	-0.809	-0.749	-0.793	-0.809	-0.720	**-0.834**
Penoxsulam	-0.650	**-0.811**	-0.690	-0.807	-0.736	-0.780	-0.807	-0.661	-0.792
Combined	-.668	-.787	-.705	-.780	-.778	-.793	-.780	-.765	**-.794**

[a]Vegetation indices calculated from the Red, Green, and Blue bands of the image according to calculations listed in Table 2.
[b]TGI is Triangular Greenness Index, VARI is Visible Atmospherically Resistant Index, EXGI is Excess Green Index, MGRVI is Modified Green Red Vegetation Index, RGBVI is RGB Vegetation Index, GLI is Green Leaf Index, MPRI is Modified Photochemical Reflectance Index, CRI is Modified Carotenoid Reflectance Index, and EXGR is Excess Greenness Minus Red Index
[c]all correlations were significant with $p < 0.0001$
[d]Bold values indicate highest correlation for that herbicide.

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

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

[a]R, G, and B correspond to digital numbers from the red, green, and blue bands of a digital camera and VE refers to visually evaluated efficacy

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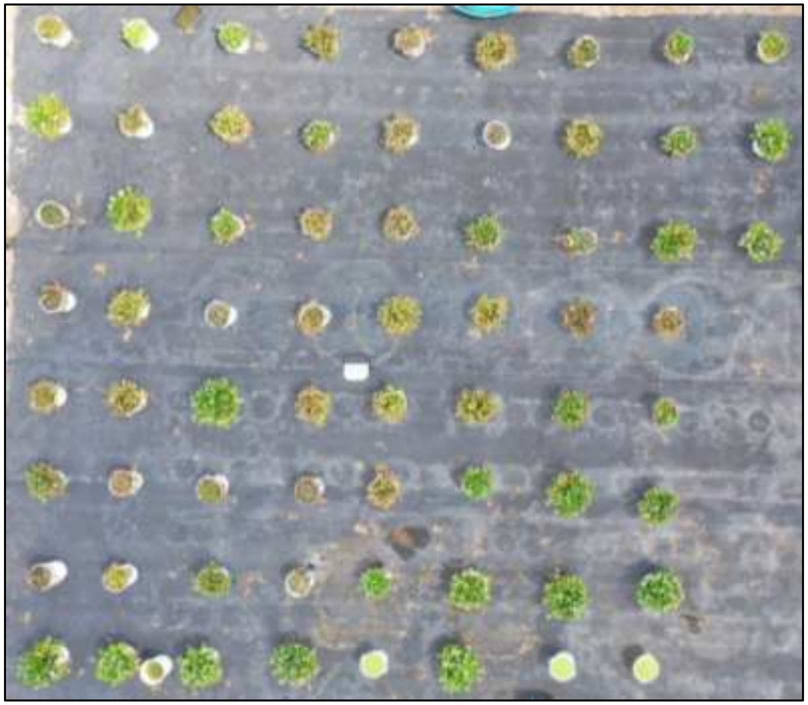

Figure 1. The study site is at the University of Florida Center for Aquatic and Invasive Plants, six weeks after the spring treatment, at 30 m AGL (0.76cm/px). Three-panel grey scale reflectance target is pictured in the center of the study.

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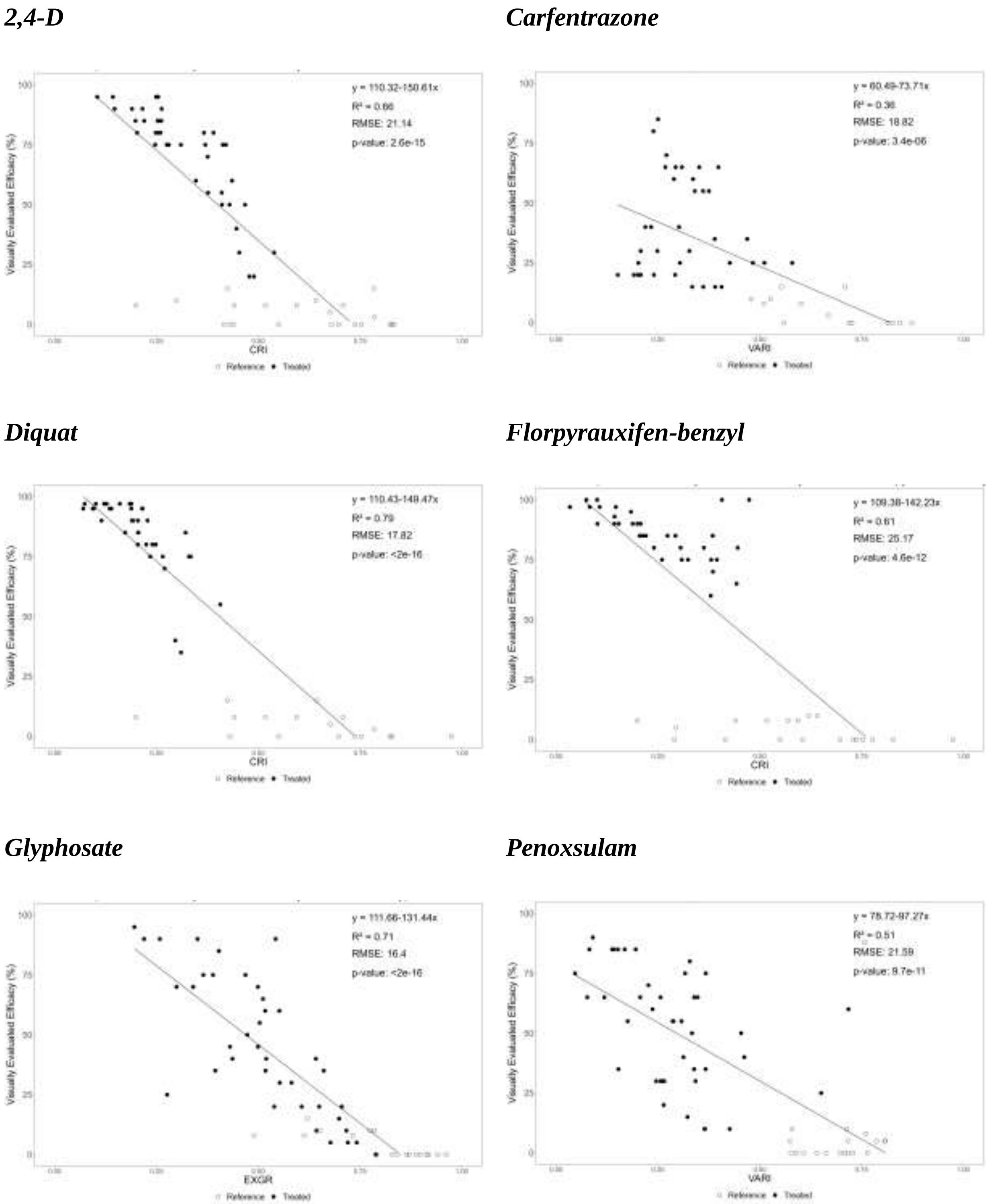

Figure 2. Linear relationship between the highest correlated vegetation indices (Table 2) with visually evaluated efficacy when water hyacinth is affected by herbicide at 1to 3 WAT for diquat, 2,4-D and carfentrazone, 2 to 5 WAT for florpyrauxifen-benzyl and glyphosate, and 3 to 6 WAT for penoxsulam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

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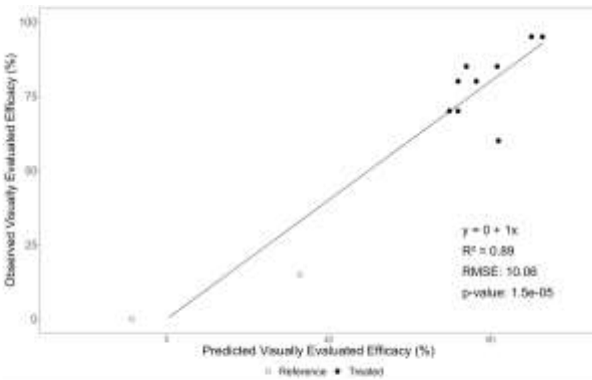
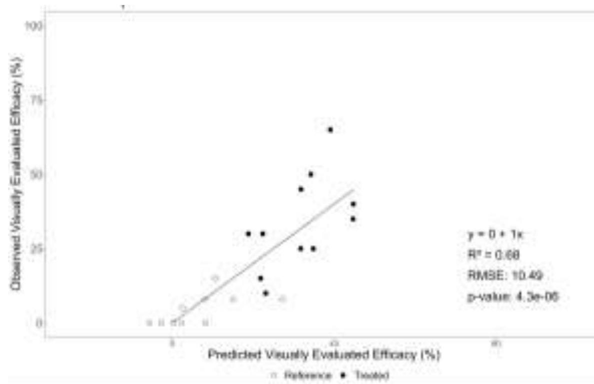
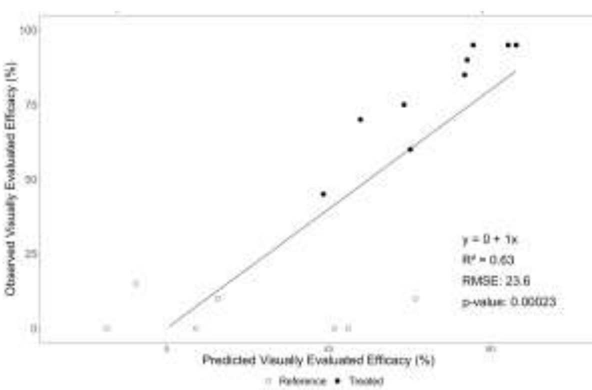
2,4-D



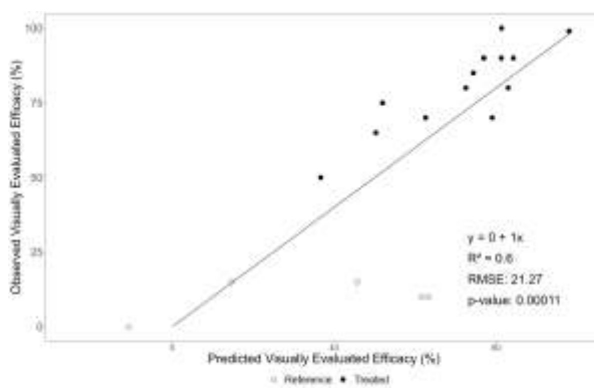
Carfentrazone



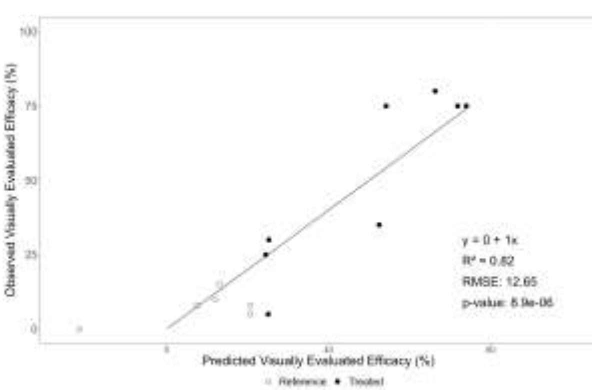
Diquat



Florpyrauxifen-benzyl



Glyphosate



Penoxsulam

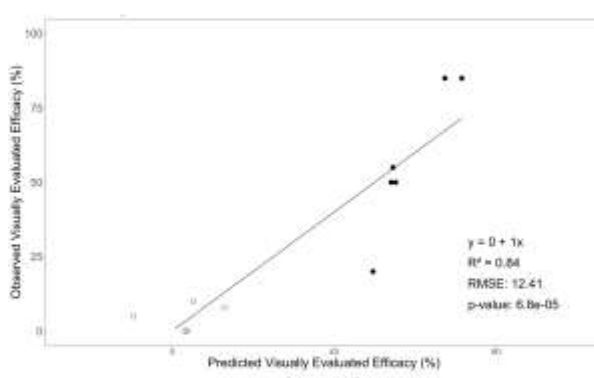


Figure 3. Linear relationship between predicted and observed visually evaluated efficacy values (Table 2) when water hyacinth is affected by herbicide treatments 1 to 3 WAT for diquat, 2,4-D and carfentrazone, 2 to 4 WAT for florpyrauxifen-benzyl and glyphosate, and 3 to 6 WAT for penoxsulam (n = 15).

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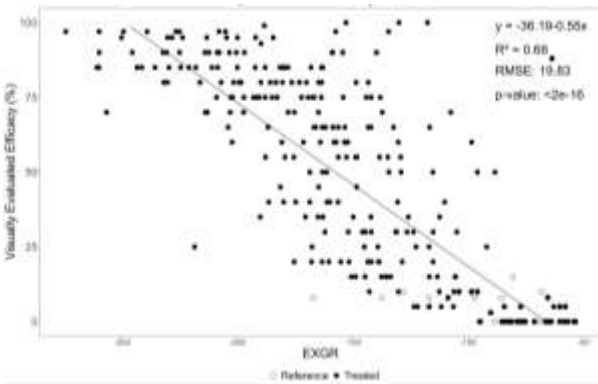
EXGR vs Visually Evaluated Efficacy ***EXGR Predictions***



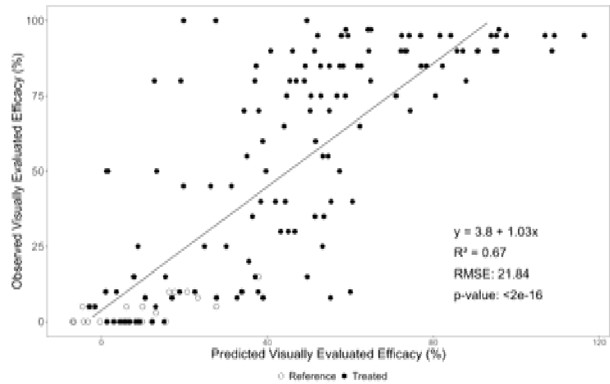


Figure 4. (Left) Linear relationship between the highest correlated vegetation index (Table 2) with visually evaluated efficacy when water hyacinth is affected by herbicide for the aggregated data (n = 342). (Right) A linear relationship between predicted and observed visually evaluated efficacy is also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

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