Review

Not peer-reviewed version

Advanced Plant Phenotyping Technologies for Enhanced Detection and Mode of Action Analysis of Herbicide Damage Management

Zhongzhong Niu , Xuan Li , Tianzhang Zhao , Zhiyuan Chen , Jian Jin *

Posted Date: 29 January 2025

doi: 10.20944/preprints202501.2113.v1

Keywords: plant phenotyping; hyperspectral imaging; multispectral analysis; machine learning in agriculture; deep learning for disease detection; precision agriculture; agricultural robotics; plant disease management; sensor-based technologies; spatial and temporal analysis

Review

Advanced Plant Phenotyping Technologies for Enhanced Detection and Mode of Action Analysis of Herbicide Damage Management

Zhongzhong Niu, Xuan Li, Tianzhang Zhao, Zhiyuan Chen and Jian Jin *

Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907, USA
* Correspondence: jinjian@purdue.edu

Abstract: Weed control is fundamental to modern agriculture, underpinning crop productivity, food security, and the economic sustainability of farming operations. Herbicides have long been the cornerstone of effective weed management, significantly enhancing agricultural yields over recent decades. However, the field now faces critical challenges, including stagnation in the discovery of new herbicide modes of action (MOAs) and the escalating prevalence of herbicide-resistant weed populations. High research and development costs, coupled with stringent regulatory hurdles, have impeded the introduction of novel herbicides, while the widespread reliance on glyphosate-based systems has accelerated resistance development. In response to these issues, advanced image-based plant phenotyping technologies have emerged as pivotal tools in addressing herbicide-related challenges in weed science. Utilizing sensor technologies such as hyperspectral, multispectral, RGB, fluorescence, and thermal imaging, plant phenotyping enables precise monitoring of herbicide drift, analysis of resistance mechanisms, and development of new herbicides with innovative MOAs. The integration of machine learning algorithms with imaging data further enhances the ability to detect subtle phenotypic changes, predict herbicide resistance, and facilitate timely interventions. This review comprehensively examines the application of image phenotyping technologies in weed science, detailing various sensor types and deployment platforms, exploring modeling methods, and highlighting unique findings and innovative applications. Additionally, it addresses current limitations and proposes future research directions, emphasizing the significant contributions of phenotyping advancements to sustainable and effective weed management strategies. By leveraging these sophisticated technologies, the agricultural sector can overcome existing herbicide challenges, ensuring continued productivity and resilience in the face of evolving weed pressures.

Keywords: plant phenotyping; hyperspectral imaging; multispectral analysis; machine learning in agriculture; deep learning for disease detection; precision agriculture; agricultural robotics; plant disease management; sensor-based technologies; spatial and temporal analysis

---

1. Introduction

1.1. Challenges in Herbicide Development and Usage

Weed control is fundamental to modern agriculture, crucial for maximizing crop yields, ensuring food security, and maintaining the economic viability of farming operations [1]. Weeds aggressively compete with crops for vital resources such as sunlight, water, and nutrients, leading to significant reductions in productivity and quality [2]. Herbicides have long been the cornerstone of effective weed management due to their efficiency and ease of application over large agricultural areas, substantially contributing to increases in agricultural productivity over the past several decades [3]. However, the continued success of herbicides is now facing significant challenges.

 doi:10.20944/preprints202501.2113.v1

One of the most pressing issues is the stagnation in the discovery of new herbicide modes of action (MOAs). Remarkably, no new MOA has been introduced in the past 30 years [4]. This stagnation poses a severe threat to sustainable weed management because novel MOAs are critical for combating herbicide-resistant weed populations [5]. Without new MOAs, the effectiveness of herbicides diminishes over time as weeds evolve resistance mechanisms, rendering existing herbicides less effective or even obsolete [6].

High research and development (R&D) costs significantly hinder the discovery and commercialization of new herbicides with novel modes of action (MOAs), often requiring over a decade and investments exceeding $250 million [7]. These substantial and risky expenditures, compounded by stringent regulatory requirements and uncertain market returns, lead many companies to modify existing compounds rather than innovate new ones. Simultaneously, the widespread adoption of glyphosate-resistant (GR) crops has resulted in an over-reliance on glyphosate-based herbicides [8]. This extensive use has accelerated the emergence of glyphosate-resistant weed species, which undermine the benefits of GR crops and pose significant challenges to global crop production. Consequently, there is an urgent need for alternative weed management strategies to address these growing concerns.

Given these challenges, there is a critical need for innovative solutions in weed management.

- Monitoring and detecting herbicide damage on crop is essential to minimize its detrimental effects. By utilizing advanced monitoring technologies such as sensor networks and remote sensing, farmers and agronomists can quickly identify instances of drift and implement timely mitigation strategies.
- Analyzing herbicide resistance in weeds is also paramount. Understanding the genetic and biochemical mechanisms behind resistance can inform the development of management strategies to mitigate its spread. This includes rotating herbicides with different MOAs and integrating non-chemical control methods.
- The development of new herbicides with novel MOAs remains a high priority. Discovering new targets for herbicide action can rejuvenate the herbicide pipeline and provide fresh tools to combat resistant weeds. Identifying herbicide MOAs and analyzing their interactions at the molecular level can lead to the design of more effective and selective compounds.

1.2. Role of Phenotyping and Advanced Technologies

Image-based plant phenotyping, which involves the identification and quantification of observable plant characteristics (phenotypes) through imaging technologies, has become a pivotal tool in addressing herbicide challenges [9–13]. This approach focuses on capturing detailed images using techniques such as hyperspectral, multispectral, fluorescence and thermal imaging to assess how plants interact with their environment and genetic makeup. In herbicide research, image phenotyping plays a critical role in understanding how different herbicides affect plant growth and development, as well as in identifying and characterizing herbicide resistance [14–16].

One of the paramount challenges is monitoring and detecting herbicide drift to minimize its detrimental effects. Herbicide drift can lead to unintended exposure of non-target crops and ecosystems, causing damage that ranges from reduced crop yields to loss of biodiversity [17]. By employing advanced monitoring technologies like sensor networks and remote sensing within image phenotyping frameworks, farmers and agronomists can quickly identify instances of drift. Hyperspectral imaging, for example, can detect subtle changes in plant reflectance patterns indicative of herbicide exposure, allowing for timely mitigation strategies to be implemented [10,18].

Analyzing herbicide resistance in weeds is another critical need in modern agriculture. Understanding the genetic and biochemical mechanisms behind resistance informs the development of management strategies to mitigate its spread. Image phenotyping plays a vital role in this regard by identifying phenotypic markers associated with resistance mechanisms [18,19]. Techniques such as chlorophyll fluorescence imaging measure disruptions in photosynthetic processes caused by herbicides, which can indicate resistance [16]. By integrating machine learning algorithms with

 doi:10.20944/preprints202501.2113.v1

imaging data, complex patterns of resistance can be deciphered, facilitating the rotation of herbicides with different modes of action (MOAs) and the integration of non-chemical control methods [ref].

The development of new herbicides with novel MOAs remains a high priority to combat resistant weed populations. Discovering new targets for herbicide action can rejuvenate the herbicide pipeline and provide fresh tools against resistant weeds. Image phenotyping contributes to this effort by enabling the study of herbicide mechanisms of action through detailed phenotypic responses. For instance, thermal imaging can detect changes in plant canopy temperature resulting from herbicide-induced stress, offering insights into how plants metabolize and respond to different compounds at physiological levels [20]. Identifying herbicide MOAs and analyzing their interactions at the plant level through imaging techniques can lead to the design of more effective and selective compounds [21].

Advancements in image phenotyping, such as high-throughput phenotyping platforms and the integration of multiple sensor technologies, enhance the ability to address these challenges [22]. Automated systems utilizing robotics and conveyor belts allow for the rapid processing of large numbers of plant samples, essential for screening herbicide efficacy and resistance on a broad scale [23,24]. Machine learning algorithms further amplify the capabilities of image phenotyping by analyzing complex datasets to identify patterns and predict herbicide resistance or susceptibility with high accuracy [10,25,26].

1.3. Outline

The article is structured into three main sections, meticulously detailing the application of image phenotyping technologies to address herbicide challenges in weed science. **Section 1** provides an overview of plant phenotyping technologies applied in weed science, discussing various sensor types—including hyperspectral, multispectral, RGB, fluorescence, and thermal imaging. **Section 2** explores the applications of imaging techniques in herbicide challenges, focusing on monitoring and detecting herbicide drift, analyzing herbicide resistance in weeds, and aiding in the development of new herbicide and discusses unique findings in plant phenotyping related to herbicides, highlighting significant breakthroughs and innovative applications. **Section 3** examines the limitations and future work for plant phenotyping, addressing current challenges and suggesting potential research directions.

2. Overview of Plant Phenotyping Technologies

The development of image-based phenotyping has significantly advanced the field of herbicide response assessment. These non-destructive methods has enabled high-throughput screening and detailed analysis of plant responses to herbicide treatment, crucial for evaluating the sometimes severe damage caused by herbicides. Such technologies necessitate non-destructive data collection techniques to assess these impacts accurately. Utilizing a range of sensors and platforms, imaging-based phenotyping captures intricate data on plant traits, providing unique advantages tailored to specific research needs.

2.1. RGB and Multispectral Sensing

RGB imaging, utilizing standard digital cameras capturing red, green, and blue color, forms the foundation of many image-based phenotyping studies. Its accessibility, low cost, and ease of use has made it a valuable tool, particularly for initial screening and assessing gross phenotypic changes in response to herbicide application. RGB images can effectively capture visible traits such as plant growth, overall vigor, the presence and extent of chlorosis (loss of green color), and necrosis (tissue death). These visual symptoms are often indicative of herbicide stress and can be used to assess herbicide efficacy and resistance. For example, Ramirez-Rojas et al. (2020) [27] used RGB imaging to evaluate the effects of mesotrione on corn plants, observing chlorosis as a key indicator of herbicide impact. While RGB imaging provides valuable visual information, its limited spectral information

 doi:10.20944/preprints202501.2113.v1

restricts its capacity for quantitative assessment of subtle physiological changes or biochemical alterations within plants.

Multispectral imaging extends beyond the visible spectrum, employing sensors that capture light across multiple discrete wavelengths, including near-infrared (NIR) and sometimes shortwave infrared (SWIR) regions. This broader spectral range provides significantly richer data compared to RGB images, allowing for the calculation of vegetation indices (VIs). VIs are mathematical combinations of reflectance values at different wavelengths and serve as quantitative proxies for various plant properties, including biomass, chlorophyll content, and overall plant health. The use of VIs significantly enhances the objectivity and quantitative nature of herbicide phenotyping. For instance, Duddu et al. (2019) [28] used multispectral imaging to calculate the optimized soil-adjusted vegetation index (OSAVI) in fababeans, demonstrating its superior precision compared to traditional visual ratings for assessing herbicide tolerance. The enhanced information content of multispectral imaging allows for more accurate and consistent assessment of herbicide effects, facilitating more reliable comparisons across treatments and genotypes.

2.2. Hyperspectral Imaging

Hyperspectral imaging (HSI) is a non-destructive optical sensing technology that combines continuous wavelengths and imaging to capture and analyze a wide range of wavelengths across the electromagnetic spectrum [9,25,29–31]. Unlike conventional imaging systems that capture images in three broad bands corresponding to red, green, and blue, HSI systems acquire images across hundreds of narrow, contiguous spectral bands, producing a three-dimensional dataset known as a hypercube [25,30,31]. This hypercube contains both spatial and spectral information for each pixel, allowing for detailed analysis of the chemical and physical properties of plant tissues [32]. For example, glyphosate applications can alter the chemical composition of leaves, affecting light absorption patterns in glyphosate-resistant and glyphosate-susceptible plants, which can be detected using HSI [8].

The effectiveness of hyperspectral imaging (HSI) in herbicide research is critically dependent on advanced hardware components and platforms. Hyperspectral cameras, essential for capturing detailed spectral data, operate across a broad spectral range, including the visible and near-infrared (VNIR) regions, with high spectral resolution. These cameras are often mounted on unmanned aerial vehicles (UAVs) [12] or handheld devices like LeafSpec [22] for field applications, or integrated into conveyor-based systems for high-throughput screening in controlled environments. Spectrometers such as the Analytical Spectral Devices (ASD) FieldSpec provide precise measurements essential for plant analysis [31]. Stable illumination sources, like quartz-tungsten-halogen lamps, ensure consistent lighting conditions necessary for accurate spectral data capture. Calibration with reference panels and the control of environmental variables in laboratory settings are crucial for maintaining data integrity, making the hardware configuration and software integration foundational to leveraging HSI in modern agricultural practices.

Despite its advantages, hyperspectral imaging (HSI) has inherent limitations that must be considered. The high dimensionality of hyperspectral data introduces redundancy and multicollinearity among spectral bands, complicating data analysis and interpretation [35]. To effectively mine HSI data, there is a growing application of both supervised and unsupervised machine learning methods. Statistical machine learning techniques are widely used to extract features from the spectral dimensions of HSI. Additionally, neural networks, recognized as robust and powerful supervised tools, are employed to utilize both the spatial and spectral dimensions of HSI data. The unique and large data characteristics of HSI also make it challenging to apply existing pre-trained RGB deep learning models directly. Training neural networks for HSI requires substantially more computational resources compared to RGB images. Additionally, high-resolution HSI systems can be costly, potentially limiting their accessibility for widespread use in both research and practical applications.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2025 doi:10.20944/preprints202501.2113.v1

2.3. Fluorescence Imaging

Fluorescence imaging is a non-destructive optical sensing technique that measures the re-emission of light by chlorophyll molecules during photosynthesis. This method provides detailed insights into the photosynthetic efficiency and physiological status of plants by assessing parameters related to photosystem II (PSII) activity [29,33]. In herbicide research, fluorescence imaging has been extensively applied to detect herbicide-induced stress, analyze herbicide resistance in weeds, and aid the development of new herbicides with novel modes of action. The following table show some common fluorescence parameters used in fluorescence research:

Parameter	Definition	Formula	Key Applications	References
Fv/Fm	Maximum quantum yield of PSII photochemistry	$\frac{F_m - F_0}{Fm}$	Used to screen for metabolic perturbations, detect early stress responses, and identify herbicide-resistant weeds	[19,29,34,35]
$\Phi PSII$	Operating efficiency of PSII	$\frac{F_s - F_0}{F_s}$	Serves as a bioindicator of photosynthetic machinery damage and stress evaluation	[29,33,35–37]
NPQ	Reflects heat dissipation of excess energy in PSII antenna complexes	$\frac{Fm - Fm'}{Fm'}$	Evaluates photoprotection mechanisms and the degree of thermal energy dissipation under stress conditions	[29,34,36,38]
Fd/Fm	Fraction of maximum fluorescence dissipated under steady-state conditions	$\frac{F_m - F_s}{F_m}$	Contributes to understanding the balance between photochemical utilization and energy dissipation	[35]
qP	Coefficient of photochemical quenching	$\frac{F_m{}' - F_s}{F_m{}' - F_0}$	Reflects the proportion of open PSII reaction centers and is used to assess the efficiency of the photochemical phase	[29]

However, this technique has several limitations. The measurement protocol requires exposing plants to continuous actinic red light (617 nm; 600 µmol m^−2 s^−1) and a saturating pulse (cool white; 2000 µmol m^−2 s^−1) following 25 minutes of dark adaptation [34]. Which means the imaging process imaging more suitable for indoor or laboratory proximal sensing applications and the throughput is lower compared with other phenotyping technology like hyperspectral. The equipment required for high-resolution fluorescence imaging can be costly, potentially limiting accessibility for some researchers [34]. Despite these challenges, fluorescence imaging remains a powerful tool for researchers in breeding programs and herbicide development groups, where controlled conditions facilitate precise measurements and analyses.

2.4. Thermal Imaging

Thermal imaging technology has been used because of its capacity to detect differences in temperature between herbicide-resistant and susceptible weed populations. This method hinges on the observation that susceptible plants, particularly when treated with herbicides like glyphosate [20,40], exhibit a reduction in photosynthesis and stomatal conductance, leading to decreased transpiration and a subsequent increase in leaf temperature. Conversely, resistant plants do not undergo similar physiological changes, thus maintaining lower temperatures.

Thermal imaging employs long wave infrared (LWIR) cameras to capture the radiation emitted from plant canopies, converting it into temperature readings. These cameras can be deployed on unmanned aerial vehicles (UAVs) for expansive field studies or utilized in more controlled environments such as greenhouses. The subsequent steps involve extracting temperature data from

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2025 doi:10.20944/preprints202501.2113.v1

the images, focusing primarily on the plant canopy, and using this data to discern temperature differences between resistant and susceptible plant biotypes.

However, the application of thermal imaging in field environments presents challenges. Although greenhouse studies have shown promising results, such as classifying glyphosate-resistant horseweed with up to 89% accuracy, field studies have yielded inconsistent outcomes [41]. Factors such as uneven solar radiation, spatial variability within weed populations, and varying degrees of resistance impact the reliability of thermal imaging as a predictive tool for herbicide resistance. Additionally, the presence of crops and other vegetation can skew thermal readings, further complicating data interpretation.

Given these limitations, research suggests that alternative sensing technologies might provide more dependable results in field conditions [20,41]. For instance, multispectral imaging techniques that leverage indices like the Normalized Difference Vegetation Index (NDVI) or specific wavelength reflectance have demonstrated superior efficacy in some studies.

3. Applications of Imaging Techniques in Herbicide Challenges

3.1. Detection of Herbicide Damage on Crops

The following table summarizes key studies that have employed different phenotyping technologies, ranging from hyperspectral and multispectral imaging to chlorophyll fluorescence imaging. Each entry details the type of crop studied, the specific sensors used, the herbicides applied, and their respective action groups

Table 1. Application of image ophenotyping on detection of herbicide damage on crops.

Crop Type	Sensor Type	Herbicide Name	Herbicide Group Number	Herbicide Group Name	Reference
Wheat	Hyperspectral	Mesosulfuron-methyl	2	ALS inhibitors	[42]
Corn	Hyperspectral	Nicosulfuron	2		[43]
Maize	Hyperspectral	Nicosulfuron	2		[44]
Soybean	Hyperspectral	2,4-D	4	Synthetic auxins	[18]
Cotton	Hyperspectral	2,4-D	4		[45]
Soybean	Hyperspectral,RGB	Dicamba	4		[46]
Soybean	RGB	Dicamba	4		[47]
Soybean	Hyperspectral	Dicamba	4		[18]
Soybean	Hyperspectral	Dicamba	4		[36]
Wheat	Hyperspectral	MCPA-Na	4		[42]
Sugar Beet	Chlorophyll Fluorescence Imaging	Desmedipham	5	Photosystem II inhibitors	[39]
Sugar Beet	Chlorophyll Fluorescence Imaging	Phenmedipham	5		[39]
Wheat	Hyperspectral	Iso-proturon	7		[42]

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2025 doi:10.20944/preprints202501.2113.v1

Sugar Beet	Chlorophyll Fluorescence Imaging	Lenacil	7	Photosynthesis inhibitors	[39]
Soybean	Multispectral	Glyphosate	9		[48]
Soybean	Hyperspectral	Glyphosate	9		[49]
Black nightshade	Hyperspectral	Glyphosate	9	EPSP synthase inhibitors	[30]
Corn	Hyperspectral	Glyphosate	9		[50]
Maize	Hyperspectral	Glyphosate	9		[50]
Black nightshade	Hyperspectral	Glufosinate	10	Glutamine synthetase inhibitor	[30]
Soybean	Multispectral	Glufosinate	10		[51]
Sugar Beet	Chlorophyll Fluorescence Imaging	Ethofumesate	16	HRAC Group F3	[39]

According to the table, Dicamba, 2,4 – D, Glyphosate were analyzed a lot, as popular application of weed management for crops like corn and soybeans. The injury detection can be divided into two types, direct injury and drifting damage. The mean difference is the dosage of the herbicide applied in the study where drifting damage is caused by herbicide with much lower rate than the rate of field application. That lead to the difference level of strong of the stress.

These study usually can achieve over 80% accuracy to detect the specific herbicide damage on crop within 72 hours which is much earlier than human eyes. That prove the advantage of plant phenotyping in high-throughput and sensors like hyperspectral cameras can provide more information than visual observation.

In herbicide research, HSI has been instrumental in monitoring and detecting herbicide drift. It identifies spectral changes in plants resulting from unintended herbicide exposure, enabling the detection of herbicide drift [18,52]. Herbicide drift can cause physiological and biochemical alterations in non-target plants, affecting their spectral reflectance properties. By detecting these subtle spectral variations, HSI allows for early and non-destructive detection of herbicide drift, facilitating timely interventions to minimize crop damage. For instance, researchers used a field spectrometer and a hyperspectral imaging camera to measure grass sods treated with glyphosate, drought-stressed, and control plants [29]. They found that certain spectral indices, such as the normalized difference lignin index (NDLI) and indices related to photosynthetic pigments like the carotenoid reflectance index (CRI-1) and the photochemical reflectance index (PRI), were sensitive to glyphosate treatment as early as two days after application. Another study utilized visible/near-infrared hyperspectral imaging (Vis/NIR HSI) to distinguish glyphosate types and stress levels in wheat seedlings at different stress durations, observing spectral reflectance differences at specific wavelengths [25].

The analysis, while comprehensive, reveals the necessity for broader environmental validation of sensor technologies to ensure reliability under varied field conditions. Future research should aim to establish standardized protocols for deploying these technologies in different climates and across a wider range of soil types.

Moreover, expanding the database to include underrepresented herbicides and crops could provide a more complete picture of the global state of herbicide impact. Integrating sensor feedback with automated application systems could also revolutionize real-time herbicide management, enhancing both efficacy and sustainability.

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2025 doi:10.20944/preprints202501.2113.v1

3.2. Weed Herbicide-Resistance Analysis

In the ongoing battle against herbicide resistance, leveraging advanced sensor technologies has become essential for characterizing and understanding resistance mechanisms in various weed species. The integration of sensor data with sophisticated analytical methods is pivotal for assessing the efficacy of herbicides and the adaptive resistance traits exhibited by these plants. Such studies are crucial for refining management strategies and mitigating the impacts of resistance across agricultural systems. Table 2 presents a compilation of research efforts that employ different sensor technologies to investigate herbicide resistance in diverse weed species.

Table 2. Herbicide resistance.

Weed Scientific Name	Sensor Type	Herbicide Group Number	Herbicide Group Name	Reference
Alopecurus myosuroides	Chlorophyll Fluorescence Imaging	1	ACCase inhibitors	[53]
Alopecurus myosuroides	Chlorophyll Fluorescence Imaging	2	ALS inhibitors	[16]
Papaver rhoeas	Chlorophyll Fluorescence Imaging	2	ALS inhibitors	[54]
Stellaria media	Chlorophyll Fluorescence Imaging	2	ALS inhibitors	[54]
Kochia scoparia, marestail, Conyza canadensis, Chenopodium album	Hyperspectral	4	Synthetic auxins	[55]
Kochia scoparia	Hyperspectral	4	Synthetic auxins	[15]
Echinochloa crus-galli	Multispectral, RGB	5	Photosystem II inhibitors	[56]
Abutilon theophrasti	Multispectral, RGB	5	Photosystem II inhibitors	[56]
Amaranthus palmeri	Hyperspectral	9	Glyphosate (EPSP synthase inhibitors)	[8]

 doi:10.20944/preprints202501.2113.v1

Kochia, Conyza canadensis, Chenopodium album	Hyperspectral	9	Glyphosate (EPSP synthase inhibitors)	[55]
Kochia scoparia	Hyperspectral	9	Glyphosate (EPSP synthase inhibitors)	[15]
Amaranthus rudis	Thermal	9	Glyphosate (EPSP synthase inhibitors)	[41]
Conyza canadensis	Thermal	9	Glyphosate (EPSP synthase inhibitors)	[41]
Amaranthus rudis, Kochia scoparia, Ambrosia artemisiifolia	Multispectral	9	Glyphosate (EPSP synthase inhibitors)	[57]
Kochia scoparia	Thermal, Multispectral	9	Glyphosate (EPSP synthase inhibitors)	[20]
Amaranthus retroflexus	Multispectral	10	Glutamine synthetase inhibitors	[51]
Amaranthus retroflexus	Multispectral	14	PPO inhibitors	[58]

Herbicide resistance, an escalating issue in agricultural management, profoundly affects crop yield and economics due to the resilience that weeds develop against various herbicide actions. This resistance stems from several complex mechanisms, such as target-site mutations that alter the protein targeted by the herbicide, non-target-site resistance mechanisms including enhanced metabolism or reduced translocation, and gene amplification. Notably, resistance is not just widespread but also varied—glyphosate resistance is notably pervasive, and ALS inhibitors are frequently rendered ineffective across numerous farming regions. The implication of such widespread resistance extends beyond increased management costs; it fundamentally alters crop management strategies and necessitates the adoption of integrated weed management systems.

Thermal imaging provides a powerful tool for differentiating between herbicide-resistant and susceptible plants. Herbicide-resistant plants may exhibit distinct temperature patterns compared to susceptible plants in response to herbicide treatment. This difference in thermal response can be attributed to variations in physiological processes affected by herbicide. For example, a resistant plant might maintain a lower leaf temperature due to sustained transpiration, while a susceptible plant might exhibit a higher leaf temperature due to reduced transpiration resulting from herbicide-induced stress. This method offers a rapid and non-destructive way to screen for herbicide resistance, facilitating efficient high-throughput screening of large plant populations [20]. This high-throughput screening capability is especially valuable in breeding programs where large numbers of plants need to be evaluated for herbicide tolerance.

 doi:10.20944/preprints202501.2113.v1

Fluorescence imaging has been instrumental in analyzing herbicide resistance in weeds [54,59]. Resistant plants often exhibit less pronounced changes in fluorescence parameters following herbicide application, reflecting their ability to maintain photosynthetic function despite the presence of herbicides [54]. By comparing fluorescence responses between resistant and susceptible weed biotypes, the mechanisms of resistance can be better understood [59].

3.3 Discovery of herbicide mode of actions.

Herbicide Name	Herbicide Group Number	Herbicide Group Name	Sensor Type	Reference
Pinoxaden	1	ACCase inhibitors	Chlorophyll Fluorescence Imaging	[59]
U-46 Combi Fluid			Chlorophyll Fluorescence Imaging	[13]
Penoxsulam	2	ALS inhibitors	RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Chlorimuron			Hyperspectral	[21]
Amidosulfuron			Raman spectroscopy, chlorophyll fluorescence imaging	[60]
Cruz	4	Synthetic auxins	Chlorophyll Fluorescence Imaging	[13]
2,4-D			Hyperspectral	[61]
Atrazine	5		Hyperspectral	[21]
Bentazon	6	Photosystem II inhibitors	Chlorophyll Fluorescence Imaging	[19]
Basagran			Chlorophyll Fluorescence Imaging	[13]
Bromicide			Chlorophyll Fluorescence Imaging	[13]
Dinoseb			Hyperspectral	[21]
Glyphosate	9	EPSP synthase inhibitors	RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Glyphosate			Hyperspectral	[30]
Glyphosate			Hyperspectral	[21]
Glufosinate	10	Glutamine synthetase inhibitors	RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Glufosinate			Hyperspectral	[30]
Glufosinate			Hyperspectral	[21]
Diflufenican	12	Carotenoid biosynthesis inhibitors	Raman spectroscopy, chlorophyll fluorescence imaging	[60]
Clomazone	13	Long-Chain Fatty Acid Inhibitors	Raman spectroscopy, chlorophyll fluorescence imaging	[60]
Tiafenacil	14	PPO inhibitors	RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Flumioxazin			Hyperspectral	[21]

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 January 2025 doi:10.20944/preprints202501.2113.v1

Carfentrazone-ethyl			Raman spectroscopy, chlorophyll fluorescence Imaging	[60]
Gramoxone	22	Bipyridylium	Chlorophyll Fluorescence Imaging	[13]
Paraquat	22		RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Paraquat	22		Hyperspectral	[21]
Isoxaflutole	27	HPPD inhibitors	RGB, Thermal, Chlorophyll Fluorescence Imaging	[35]
Mesotrione	27	HPPD inhibitors	Raman spectroscopy, chlorophyll fluorescence Imaging	[60]
Indaziflam	29	Cellulose biosynthesis inhibitors (CBIs)	Hyperspectral	[21]
Pyroxsulam + Florasulam	2	ALS inhibitors	Chlorophyll Fluorescence Imaging	[59]
Lumax (S-metolachlor+Mesotrione +Terbuthylazine)	5	Photosystem II inhibitors	Chlorophyll Fluorescence Imaging	[13]
	15	Seedling Growth Inhibitors	Chlorophyll Fluorescence Imaging	[13]
	27	HPPD Inhibitors	Chlorophyll Fluorescence Imaging	[13]

HSI also contributes to the development of new herbicides with novel modes of action by providing insights into the biochemical responses of plants to experimental compounds. By analyzing spectral changes over time, researchers can understand a herbicide's mode of action, target sites, and impact on plant physiological processes. This information is crucial for screening and refining new herbicide candidates to enhance efficacy and minimize off-target effects. A study employing HSI to classify eight herbicides with different sites of action achieved an overall accuracy of 81.5% one day after treatment using support vector machine (SVM) models [21]. The research identified distinct spectral feature bands associated with specific herbicide modes of action, demonstrating the feasibility of using HSI for high-throughput herbicide screening and aiding in the discovery of new herbicide targets.

In the development of new herbicides with novel modes of action, fluorescence imaging contributes by revealing how experimental compounds affect plant physiology at the molecular level [34,37]. Studies have demonstrated that PSII-inhibiting herbicides cause measurable increases in chlorophyll fluorescence due to inhibited electron flow [19,33]. Early detection of herbicide stress has been achieved by monitoring decreases in Fv/Fm and ΦPSII parameters, enabling interventions before irreversible damage occurs [38,39].

The variability in plant responses to herbicides, influenced by species-specific physiology, developmental stages, and environmental conditions, introduces inconsistencies in research outcomes and complicates the generalization of findings. Addressing this requires rigorous experimental designs that encompass diverse developmental stages and environmental settings. Additionally, the limited spatial resolution of certain phenotyping techniques, like those using fiber-optic probes, restricts data comprehensiveness, potentially missing significant intra- and inter-plant variations. Enhanced imaging techniques with higher spatial resolution could improve data granularity and utility. The field also suffers from a lack of standardized methods for sensor-based measurements, hindering result comparability across studies. Establishing universally accepted protocols and calibration standards is essential for advancing plant phenotyping. Moreover, the prohibitive cost of advanced imaging systems, especially hyperspectral cameras, limits their

 doi:10.20944/preprints202501.2113.v1

accessibility, particularly in resource-constrained environments. Investments in more cost-effective technologies or the development of shared resources could mitigate these financial barriers. Lastly, while hyperspectral imaging effectively detects plant stress and damage, accurately linking these spectral signatures to specific stressors or damage mechanisms remains a significant challenge, necessitating further detailed physiological and biochemical studies.

4. Conclusions, Future Perspectives and Challenges

In the realm of plant phenotyping for herbicide analysis, several pervasive challenges impact the reliability and consistency of results. These include the influences of environmental factors, variations in herbicide dosage, and genetic diversity among crop genotypes. Due to the high sensitivity of the sensors used in phenotyping technologies, maintaining strict control over imaging environments is crucial.

To mitigate the impact of environmental variables, research can be conducted within controlled environments such as greenhouses or growth chambers where conditions such as temperature, humidity, and lighting are carefully regulated. Additionally, improvements in imaging hardware can also help reduce environmental effects; for example, incorporating self-lighting sources within imaging setups can eliminate the variability introduced by external light sources like sunlight or artificial greenhouse lights. This approach ensures a consistent imaging environment that is critical for acquiring reproducible phenotypic data.

The complexity and variability of plant responses to herbicide application, reflected in the spectral data, present a major challenge. The field currently lacks standardized data collection protocols, and there is a pressing need for a publicly accessible standard herbicide dataset. The inherently high throughput of phenotyping technologies complements the capabilities of machine learning, which can efficiently process and analyze large datasets to detect subtle patterns that may not be evident through traditional analysis methods.

Exploring the potential of Large Language Models (LLMs), such as vision transformers, could revolutionize herbicide analysis through imaging phenotyping. Traditional machine learning models, while highly explanatory, often lack stability; in contrast, deep learning models typically offer greater stability but at the cost of reduced explainability. Vision transformers provide a promising middle ground by allowing for the integration of diverse inputs, including prior knowledge and image data, into a comprehensive analytical model. These models, trained with extensive resources, can be fine-tuned with relatively modest herbicide-specific data sets to address the challenges posed by limited data availability in the field.

Addressing these limitations requires ongoing research in advanced data processing methods, integration with other sensing technologies, development of robust spectral indices tailored to detect herbicide effects, and advancements in sensor technology to improve spatial and spectral resolution while reducing costs. For instance, combining HSI with complementary technologies like chlorophyll fluorescence imaging can provide a more comprehensive understanding of herbicide effects on plants [1,16,17]. Developing and validating spectral indices that minimize sensitivity to environmental factors can enhance the robustness of assessments.

In conclusion, hyperspectral imaging represents a powerful tool in herbicide research within weed science, offering detailed spectral information that enhances the detection and analysis of herbicide effects. Its applications in monitoring herbicide drift, analyzing herbicide resistance, and aiding the development of new herbicides with novel modes of action contribute significantly to sustainable and effective weed management strategies. By integrating HSI with advanced analytical methods and machine learning algorithms, its potential can be further expanded, supporting the agricultural sector in overcoming existing herbicide challenges.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Horvath, D.P.; Clay, S.A.; Swanton, C.J.; Anderson, J.V.; Chao, W.S. Weed-Induced Crop Yield Loss: A New Paradigm and New Challenges. *Trends in Plant Science* **2023**, *28*, 567–582, doi:10.1016/j.tplants.2022.12.014.
2. Sapkota, R.; Stenger, J.; Ostlie, M.; Flores, P. Towards Reducing Chemical Usage for Weed Control in Agriculture Using UAS Imagery Analysis and Computer Vision Techniques. *Sci Rep* **2023**, *13*, 6548, doi:10.1038/s41598-023-33042-0.
3. Paul, S.K.; Mazumder, S.; Naidu, R. Herbicidal Weed Management Practices: History and Future Prospects of Nanotechnology in an Eco-Friendly Crop Production System. *Heliyon* **2024**, *10*, e26527, doi:10.1016/j.heliyon.2024.e26527.
4. Duke, S.O. Why Have No New Herbicide Modes of Action Appeared in Recent Years? *Pest Manag Sci* **2012**, *68*, 505–512, doi:10.1002/ps.2333.
5. McCurdy, J.D.; Bowling, R.G.; de Castro, E.B.; Patton, A.J.; Kowalewski, A.R.; Mattox, C.M.; Brosnan, J.T.; Ervin, D.E.; Askew, S.D.; Goncalves, C.G.; et al. Developing and Implementing a Sustainable, Integrated Weed Management Program for Herbicide-Resistant Poa Annua in Turfgrass. *Crop, Forage & Turfgrass Management* **2023**, *9*, e20225, doi:10.1002/cft2.20225.
6. Gaines, T.A.; Duke, S.O.; Morran, S.; Rigon, C.A.G.; Tranel, P.J.; Küpper, A.; Dayan, F.E. Mechanisms of Evolved Herbicide Resistance. *J Biol Chem* **2020**, *295*, 10307–10330, doi:10.1074/jbc.REV120.013572.
7. Dayan, F.E. Current Status and Future Prospects in Herbicide Discovery. *Plants (Basel)* **2019**, *8*, 341, doi:10.3390/plants8090341.
8. Reddy, K.N.; Huang, Y.; Lee, M.A.; Nandula, V.K.; Fletcher, R.S.; Thomson, S.J.; Zhao, F. Glyphosate-Resistant and Glyphosate-Susceptible Palmer Amaranth (Amaranthus Palmeri S. Wats.): Hyperspectral Reflectance Properties of Plants and Potential for Classification. *Pest Management Science* **2014**, *70*, 1910–1917, doi:10.1002/ps.3755.
9. Tao, M.; He, Y.; Bai, X.; Chen, X.; Wei, Y.; Peng, C.; Feng, X. Combination of Spectral Index and Transfer Learning Strategy for Glyphosate-Resistant Cultivar Identification. *FRONTIERS IN PLANT SCIENCE* 2022, *13*.
10. Zhang, J.; Huang, Y.; Reddy, K.N.; Wang, B. Assessing Crop Damage from Dicamba on Non-Dicamba-Tolerant Soybean by Hyperspectral Imaging through Machine Learning. *Pest Management Science* **2019**, *75*, 3260–3272, doi:10.1002/ps.5448.
11. Jin, X.; Bagavathiannan, M.; Maity, A.; Chen, Y.; Yu, J. Deep Learning for Detecting Herbicide Weed Control Spectrum in Turfgrass. *Plant Methods* **2022**, *18*, 94, doi:10.1186/s13007-022-00929-4.
12. Huang, Y.; Lee, M.A.; Thomson, S.J.; Reddy, K.N. Ground-Based Hyperspectral Remote Sensing for Weed Management in Crop Production. *International Journal of Agricultural and Biological Engineering* **2016**, *9*, 98–109, doi:10.25165/ijabe.v9i2.2137.
13. Hassannejad, S.; Lotfi, R.; Ghafarbi, S.P.; Oukarroum, A.; Abbasi, A.; Kalaji, H.M.; Rastogi, A. Early Identification of Herbicide Modes of Action by the Use of Chlorophyll Fluorescence Measurements. *Plants* **2020**, *9*, 529, doi:10.3390/plants9040529.
14. Ghatrehsamani, S.; Jha, G.; Dutta, W.; Molaei, F.; Nazrul, F.; Fortin, M.; Bansal, S.; Debangshi, U.; Neupane, J. Artificial Intelligence Tools and Techniques to Combat Herbicide Resistant Weeds—A Review. *Sustainability* **2023**, *15*, 1843, doi:10.3390/su15031843.
15. Nugent, P.W.; Shaw, J.A.; Jha, P.; Scherrer, B.; Donelick, A.; Kumar, V. Discrimination of Herbicide-Resistant Kochia with Hyperspectral Imaging. *JARS* **2018**, *12*, 016037, doi:10.1117/1.JRS.12.016037.
16. Wang, P.; Peteinatos, G.; Li, H.; Brändle, F.; Pfündel, E.; Drobny, H.G.; Gerhards, R. Rapid Monitoring of Herbicide-Resistant Alopecurus Myosuroides Huds. Using Chlorophyll Fluorescence Imaging Technology. *J Plant Dis Prot* **2018**, *125*, 187–195, doi:10.1007/s41348-017-0131-7.

17. Robinson, A.P.; Davis, V.M.; Simpson, D.M.; Johnson, W.G. Response of Soybean Yield Components to 2,4-D. *Weed Science* **2013**, *61*, 68–76.
18. Niu, Z.; Young, J.; Johnson, W.G.; Young, B.; Wei, X.; Jin, J. Early Detection of Dicamba and 2, 4-D Herbicide Drifting Injuries on Soybean with a New Spatial–Spectral Algorithm Based on LeafSpec, an Accurate Touch-Based Hyperspectral Leaf Scanner. *Remote Sensing* **2023**, *15*, 5771.
19. Bazhenov, M.; Litvinov, D.; Kocheshkova, A.; Karlov, G.; Divashuk, M. Chlorophyll Fluorescence Imaging Reveals the Dynamics of Bentazon Action on Sunflower (*Helianthus Annuus* L.) Plants. *AGRONOMY-BASEL* 2024, *14*.
20. Eide, A.; Koparan, C.; Zhang, Y.; Ostlie, M.; Howatt, K.; Sun, X. UAV-Assisted Thermal Infrared and Multispectral Imaging of Weed Canopies for Glyphosate Resistance Detection. *REMOTE SENSING* 2021, *13*.
21. Niu, Z.; Rehman, T.; Young, J.; Johnson, W.G.; Yokoo, T.; Young, B.; Jin, J. Hyperspectral Analysis for Discriminating Herbicide Site of Action: A Novel Approach for Accelerating Herbicide Research. *Sensors* **2023**, *23*, 9300, doi:10.3390/s23239300.
22. Chen, Z. PhenoBee: Drone-Based Robot for Advanced Field Proximal Phenotyping in Agriculture. PhD Thesis, Purdue University Graduate School, 2023.
23. Ma, D.; Amatya, S.; Wang, L.; Carpenter, N.; Maki, H.; Zhang, L.; Neeno, S.; Tuinstra, M.R.; Jin, J. Removal of Greenhouse Microclimate Heterogeneity with Conveyor System for Indoor Phenotyping High-Throughput Field Gantry Hyperspectral Platform View Project High Throughput Phenotyping Systems View Project Removal of Greenhouse Microclimate Heterogeneity. *Computers and Electronics in Agriculture* **2019**, *166*, 104979, doi:10.1016/j.compag.2019.104979.
24. Song, Z.; Zhao, T.; Jin, J. Early Identification of Root Damages Caused by Western Corn Rootworms Using a Minimally Invasive Root Phenotyping Robot—MISIRoot. *Sensors* **2023**, *23*, 5995, doi:10.3390/s23135995.
25. Huang, Y.; Zhao, X.; Pan, Z.; Reddy, K.N.; Zhang, J. Hyperspectral Plant Sensing for Differentiating Glyphosate-Resistant and Glyphosate-Susceptible Johnsongrass through Machine Learning Algorithms. *Pest Management Science* **2022**, *78*, 2370–2377, doi:10.1002/ps.6864.
26. Zhang, C.; Lane, B.; Fernandez-Campos, M.; Cruz-Sancan, A.; Lee, D.-Y.; Gongora-Canul, C.; Ross, T.; Silva, C.D.; Telenko, D.; Goodwin, S.; et al. Monitoring Tar Spot Disease at Different Temporal and Canopy Levels Using Aerially-Based Multispectral Imaging and Machine Learning.; ASA-CSSA-SSSA, November 9 2022.
27. Ramirez-Rojas, C.; Peña-Valdivia, C.; García-Esteva, A.; Padilla-Chacón, D. Phenotyping of Corn Plants with Effect of Mesotrione Herbicide.; 2022.
28. Duddu, H.S.N.; Johnson, E.N.; Willenborg, C.J.; Shirtliffe, S.J. High-Throughput UAV Image-Based Method Is More Precise Than Manual Rating of Herbicide Tolerance. *Plant Phenomics* **2019**, *2019*, 6036453, doi:10.34133/2019/6036453.
29. Feng, X.; Yu, C.; Chen, Y.; Peng, J.; Ye, L.; Shen, T.; Wen, H.; He, Y. Non-Destructive Determination of Shikimic Acid Concentration in Transgenic Maize Exhibiting Glyphosate Tolerance Using Chlorophyll Fluorescence and Hyperspectral Imaging. *FRONTIERS IN PLANT SCIENCE* 2018, *9*.
30. Nehurai, O.; Atsmon, G.; Kizel, F.; Kamber, E.; Bar, N.; Eizenberg, H.; Lati, R.N. Early Detection of the Herbicidal Effect of Glyphosate and Glufosinate by Using Hyperspectral Imaging. *Agronomy Journal* **2023**, *115*, 2558–2569, doi:10.1002/agj2.21415.
31. Sanaeifar, A.; Yang, C.; Guardia, M. de la; Zhang, W.; Li, X.; He, Y. Proximal Hyperspectral Sensing of Abiotic Stresses in Plants. *SCIENCE OF THE TOTAL ENVIRONMENT* 2023, *861*.
32. Wang, J.; Zhang, C.; Shi, Y.; Long, M.; Islam, F.; Yang, C.; Yang, S.; He, Y.; Zhou, W. Evaluation of Quinclorac Toxicity and Alleviation by Salicylic Acid in Rice Seedlings Using Ground-Based Visible/near-Infrared Hyperspectral Imaging. *PLANT METHODS* 2020, *16*.
33. Legendre, R.; Basinger, N.T.; van Iersel, M.W. Low-Cost Chlorophyll Fluorescence Imaging for Stress Detection. *SENSORS* 2021, *21*.

 doi:10.20944/preprints202501.2113.v1

34. Vitek, P.; Vesela, B.; Klem, K. Spatial and Temporal Variability of Plant Leaf Responses Cascade after PSII Inhibition: Raman, Chlorophyll Fluorescence and Infrared Thermal Imaging. *SENSORS* 2020, *20*.
35. Jeong, S.-M.; Noh, T.-K.; Kim, D.-S. Herbicide Bioassay Using a Multi-Well Plate and Plant Spectral Image Analysis. *Sensors* **2024**, *24*, 919, doi:10.3390/s24030919.
36. Jiang, Y.; Snider, J.L.; Li, C.; Rains, G.C.; Paterson, A.H. Ground Based Hyperspectral Imaging to Characterize Canopy-Level Photosynthetic Activities. *REMOTE SENSING* 2020, *12*.
37. Noble, E.; Kumar, S.; Gorlitz, F.G.; Stain, C.; Dunsby, C.; French, P.M.W. In Vivo Label-Free Mapping of the Effect of a Photosystem II Inhibiting Herbicide in Plants Using Chlorophyll Fluorescence Lifetime. *PLANT METHODS* 2017, *13*.
38. Li, H.; Wang, P.; Weber, J.F.; Gerhards, R. Early Identification of Herbicide Stress in Soybean (*Glycine Max* (L.) Merr.) Using Chlorophyll Fluorescence Imaging Technology. *SENSORS* 2018, *18*.
39. Weber, J.F.; Kunz, C.; Peteinatos, G.G.; Santel, H.-J.; Gerhards, R. Utilization of Chlorophyll Fluorescence Imaging Technology to Detect Plant Injury by Herbicides in Sugar Beet and Soybean. *WEED TECHNOLOGY* 2017, *31*, 523–535.
40. Huang, Y.; Thomson, S.J. Airborne Multispectral and Thermal Remote Sensing for Detecting the Onset of Crop Stress Caused by Multiple Factors. In Proceedings of the REMOTE SENSING FOR AGRICULTURE, ECOSYSTEMS, AND HYDROLOGY XII; Neale, C.M.U., Maltese, A., Eds.; Spie-Int Soc Optical Engineering: Bellingham, 2010; Vol. 7824, p. 78240E.
41. Eide, A.; Zhang, Y.; Koparan, C.; Stenger, J.; Ostlie, M.; Howatt, K.; Bajwa, S.; Sun, X. Image Based Thermal Sensing for Glyphosate Resistant Weed Identification in Greenhouse Conditions. *Comput. Electron. Agric.* **2021**, *188*, 106348, doi:10.1016/j.compag.2021.106348.
42. Chu, H.; Zhang, C.; Wang, M.; Gouda, M.; Wei, X.; He, Y.; Liu, Y. Hyperspectral Imaging with Shallow Convolutional Neural Networks (SCNN) Predicts the Early Herbicide Stress in Wheat Cultivars. *Journal of Hazardous Materials* **2022**, *421*, 126706, doi:10.1016/j.jhazmat.2021.126706.
43. Xiao, T.; Yang, L.; Zhang, D.; Cui, T.; Zhang, X.; Deng, Y.; Li, H.; Wang, H. Early Detection of Nicosulfuron Toxicity and Physiological Prediction in Maize Using Multi-Branch Deep Learning Models and Hyperspectral Imaging. *JOURNAL OF HAZARDOUS MATERIALS* 2024, *474*.
44. Xiao, T.; Yang, L.; Zhang, D.; Cui, T.; Wang, L.; Du, Z.; Xie, C.; Li, Z.; Gong, C.; Li, H.; et al. Early Prediction of Maize Resistance to Nicosulfuron Using Hyperspectral Imaging and Deep Learning: Method and Mechanism. *Computers and Electronics in Agriculture* **2024**, *227*, 109511, doi:10.1016/j.compag.2024.109511.
45. Suarez, L.A.; Apan, A.; Werth, J. Hyperspectral Sensing to Detect the Impact of Herbicide Drift on Cotton Growth and Yield. *ISPRS Journal of Photogrammetry and Remote Sensing* **2016**, *120*, 65–76, doi:10.1016/j.isprsjprs.2016.08.004.
46. Marques, M.G.; da Cunha, J.P.A.R.; Lemes, E.M. Dicamba Injury on Soybean Assessed Visually and with Spectral Vegetation Index. *AgriEngineering* **2021**, *3*, 240–250, doi:10.3390/agriengineering3020016.
47. Farooque, A.; Vieira, C.C.; Sarkar, S.; Tian, F.; Zhou, J.; Jarquin, D.; Nguyen, H.T.; Zhou, J.; Chen, P. Differentiate Soybean Response to Off-Target Dicamba Damage Based on UAV Imagery and Machine Learning. *mdpi.com* **2022**, doi:10.3390/rs14071618.
48. Huang, Y.; Reddy, K.N.; Thomson, S.J.; Yao, H. Assessment of Soybean Injury from Glyphosate Using Airborne Multispectral Remote Sensing. *Pest Management Science* **2015**, *71*, 545–552, doi:10.1002/PS.3839.
49. Zhao, F.; Guo, Y.; Huang, Y.; Reddy, K.N.; Lee, M.A.; Fletcher, R.S.; Thomson, S.J. Early Detection of Crop Injury from Herbicide Glyphosate by Leaf Biochemical Parameter Inversion. *International Journal of Applied Earth Observation and Geoinformation* **2014**, *31*, 78–85, doi:10.1016/j.jag.2014.03.010.

50. Zhang, T.; Huang, Y.; Reddy, K.N.; Yang, P.; Zhao, X.; Zhang, J. Using Machine Learning and Hyperspectral Images to Assess Damages to Corn Plant Caused by Glyphosate and to Evaluate Recoverability. *Agronomy* **2021**, *11*, 583.
51. Jones, E.A.L.; Austin, R.; Dunne, J.C.; Cahoon, C.W.; Jennings, K.M.; Leon, R.G.; Everman, W.J. Utilization of Image-Based Spectral Reflectance to Detect Herbicide Resistance in Glufosinate-Resistant and Glufosinate-Susceptible Plants: A Proof of Concept. *WEED SCIENCE* 2023, *71*, 11–21.
52. Henry, W.B.; Shaw, D.R.; Reddy, K.R.; Bruce, L.M.; Tamhankar, H.D. Remote Sensing to Detect Herbicide Drift on Crops. *Weed technol.* **2004**, *18*, 358–368, doi:10.1614/WT-03-098.
53. Wang, P.; Peteinatos, G.; Li, H.; Gerhards, R. Rapid In-Season Detection of Herbicide Resistant *Alopecurus Myosuroides* Using a Mobile Fluorescence Imaging Sensor. *Crop Protection* **2016**, *89*, 170–177, doi:10.1016/j.cropro.2016.07.022.
54. Linn, A., I.; Mink, R.; Peteinatos, G.G.; Gerhards, R. In-Field Classification of Herbicide-Resistant *Papaver Rhoeas* and *Stellaria Media* Using an Imaging Sensor of the Maximum Quantum Efficiency of Photosystem II. *WEED RESEARCH* 2019, *59*, 357–366.
55. Scherrer, B.; Sheppard, J.; Jha, P.; Shaw, J.A. Hyperspectral Imaging and Neural Networks to Classify Herbicide-Resistant Weeds. *JARS* **2019**, *13*, 044516, doi:10.1117/1.JRS.13.044516.
56. Xia, F.; Quan, L.; Lou, Z.; Sun, D.; Li, H.; Lv, X. Identification and Comprehensive Evaluation of Resistant Weeds Using Unmanned Aerial Vehicle-Based Multispectral Imagery. *Front. Plant Sci.* **2022**, *13*, doi:10.3389/fpls.2022.938604.
57. Shirzadifar, A.; Bajwa, S.; Nowatzki, J.; Bazrafkan, A. Field Identification of Weed Species and Glyphosate-Resistant Weeds Using High Resolution Imagery in Early Growing Season. *Biosystems Engineering* **2020**, *200*, 200–214, doi:10.1016/j.biosystemseng.2020.10.001.
58. Jones, E.A.L.; Austin, R.; Dunne, J.C.; Leon, R.G.; Everman, W.J. Discrimination between Protoporphyrinogen Oxidase-Inhibiting Herbicide-Resistant and Herbicide-Susceptible Redroot Pigweed (*Amaranthus Retroflexus*) with Spectral Reflectance. *WEED SCIENCE* 2023, *71*, 198–205.
59. Wang, P.; Li, H.; Jia, W.; Chen, Y.; Gerhards, R. A Fluorescence Sensor Capable of Real-Time Herbicide Effect Monitoring in Greenhouses and the Field. *SENSORS* 2018, *18*.
60. Vítek, P.; Novotná, K.; Hodaňová, P.; Rapantová, B.; Klem, K. Detection of Herbicide Effects on Pigment Composition and PSII Photochemistry in *Helianthus Annuus* by Raman Spectroscopy and Chlorophyll *a* Fluorescence. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2017**, *170*, 234–241, doi:10.1016/j.saa.2016.07.025.
61. Suarez, L.A.; Apan, A.; Werth, J. Detection of Phenoxy Herbicide Dosage in Cotton Crops through the Analysis of Hyperspectral Data. *https://doi.org/10.1080/01431161.2017.1362128* **2017**, *38*, 6528–6553, doi:10.1080/01431161.2017.1362128.