Weed Technology

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Rapid detection of acetolactate synthase inhibitor–resistant weeds using novel full-spectrum imaging and a hyperparameter-tuned convolutional neural network

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Research Article

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common chickweed, *Stellaria media* (L.) Vill.

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Abstract

Herbicide-resistant weeds are fast becoming a substantial global problem, causing significant crop losses and food insecurity. Late detection of resistant weeds leads to increasing economic losses. Traditionally, genetic sequencing and herbicide dose-response studies are used to detect herbicide-resistant weeds, but these are expensive and slow processes. To address this problem, an artificial intelligence (AI)-based herbicide-resistant weed identifier program (HRIP) was developed to quickly and accurately distinguish common chickweed plants that are resistant to acetolactate synthase (ALS) inhibitor herbicides and those that are susceptible to ALS inhibitors. A regular camera was converted to capture light wavelengths from 300 to 1,100 nm. Full-spectrum images from a 2-yr experiment were used to develop a hyperparameter-tuned convolutional neural network model using a "train from scratch" approach. This novel approach exploits the subtle differences in the spectral signature of ALS inhibitor-resistant and ALS inhibitor-susceptible common chickweed plants as they react differently to the ALS-inhibiting herbicide treatments. The HRIP was able to identify ALS-inhibitor–resistant common chickweed as early as 72 h after treatment at an accuracy of 88%. It has broad applicability due to its ability to distinguish common chickweed plants that are resistant to ALS-inhibitor herbicides from those that are susceptible to becoming resistant to them regardless of the type of ALS herbicide or dose used. Using tools such as the HRIP will allow farmers to make timely interventions to prevent the herbicide-escape plants from completing their life cycle and adding to the weed seedbank.

Introduction

Use of synthetic herbicides to control weeds in agricultural and non-agricultural systems is a common practice in many parts of the world (Gianessi 2013). However, reliance on continuous use of herbicides with the same mode of action to control weeds can lead to rapid evolution of herbicide-resistant weed populations (Ofosu et al. 2023). Recent data show that the number of unique cases (species × site of action) of herbicide-resistant weeds has significantly increased from zero to about 530 worldwide in just a span of 45 yr (Heap 2024).

Globally, farmers lose an estimated US$95 billion annually from yield reduction due to uncontrolled weed infestation (ISAAA 2009). Herbicide-resistant weeds may exacerbate this problem (Clay 2021) and further pose a significant threat to crop production and food security (Heap 2014; Lonhienne et al. 2018). Herbicide-resistant weeds require herbicides with an alternative mode of action or more expensive control methods to achieve effective control (Clay 2021), which further increases crop production costs.

Herbicide resistance is becoming a big problem in the San Joaquin Valley of California, which is considered the food basket of the world (Shrestha et al. 2010). There are now 161 different unique cases of herbicide resistance documented in the United States, 32 of which are in California (Heap 2024), and these numbers are expected to increase. Most of these cases are of evolved resistance to herbicides that inhibit acetolactate synthase (ALS) and 5-enolpyruvylshikimate 3-phosphate (EPSPS) (Dias et al. 2021). The occurrence of ALS-inhibitor herbicide resistance in more weed species is continuously rising at an alarming rate, posing a greater challenge for weed management, food production, and environmental health (Hanson et al. 2014). Furthermore, documented cases of ALS inhibitor resistance in different weed species are significantly higher than for any other class of agricultural herbicides (Heap 2024).

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Common chickweed is a broadleaf annual weed species commonly found in agricultural fields infesting wheat (*Triticum aestivum* L.), triticale (×Triticosecale Wittmack), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), and several other annual and perennial crops in the Central Valley of California. Overuse and reliance on a single herbicide or a similar mode of action to control common chickweed for extended duration has led to the evolution of herbicide-resistant populations. Over time, the common chickweed has developed resistance to ALS-inhibiting herbicides (Saari et al. 1992). ALS inhibitors are Group 2 herbicides (as categorized to the Weed Science Society of America) that prevent the ALS enzyme from biosynthesizing the essential branch-chain amino acids (isoleucine, leucine, and valine), thus impairing plants from functioning properly (Whitcomb 1999; Zhou et al. 2007). This is due to mutations in the ALS gene leading to altered herbicide binding sites, which make the enzyme less sensitive to inhibition by the herbicide (Heap 2024).

The first common chickweed found to be resistant to three different ALS-inhibiting herbicides in California was reported in 2022, in small grain crop fields (Heap 2024). Target-site resistance is the most common type of herbicide resistance in common chickweed and has been reported in all major herbicide classes, including glyphosate, ALS inhibitors, and triazines. Sulfonylurea and imidazolinone herbicides are both linked to target-site resistance in common chickweed (Roberson 2009). Over the past three decades, resistance to these two ALS-inhibiting herbicides has been observed in common chickweed biotypes (Saari et al. 1992). However, it has not yet been confirmed whether the ALS inhibitor-resistant common chickweed has a target site resistance mechanism.

Different approaches have been developed to confirm herbicide-resistant weed populations. The standard methods are the use of genetic sequencing (Jones et al. 2023), which detects and confirms a resistant gene or genes in weeds, and herbicide dose-response studies (Seefelt et al. 1995). These methods are accurate but cannot be used in the field, are time- and labor-intensive, and costly. A new technique developed in 2020 was the use of spectral weed indices (SWIs) to try to identify glyphosate-resistant weeds. This method uses reflectance values from eight relevant spectral wavelengths specific for weed species to develop the various SWIs (Shirzadifar et al. 2020). Shirzadifar et al. used discriminative wavebands of 490, 760, 520, 820, 850, 910, 880, and 790 nm to develop the SWI for waterhemp. The results were promising, but the procedure required the use of a proprietary expensive apparatus—an imaging spectrometer for hyperspectral imaging, labor-intensive pre-processing of data, and complex calculations. Other techniques include acquiring thermal and multispectral images with an unmanned aerial vehicle to detect the response of weeds. The unmanned aerial vehicles used ArcGIS for raster and spectral classification to differentiate herbicide-resistant weeds from herbicide-susceptible ones (Eide et al. 2021). The study, however, showed that the use of thermal data is not as reliable as the use of the Normalized Difference Vegetation Index (NDVI).

Previous studies by Hennessy et al. (2022) applied a convolutional neural network (CNN) to distinguish weeds from regular crops using red-green-blue (RGB) images, but they did not distinguish herbicide-resistant from herbicide-susceptible weeds. The use of CNNs to distinguish herbicide-resistant from -susceptible weeds within the same species using full-spectrum (ultraviolet [UV] + visible light + near-infrared [IR]) images acquired through a readily available, converted, low-cost, off-the-shelf cameras has never been done. Development of a hyperparameter-tuned CNN model for early, quick, and accurate detection and classification of resistant weeds even before observing the visible symptoms of herbicide injury is crucial in making timely interventions and establishing cost-effective, environmentally friendly management strategies (Weis and Sökefeld 2010).

The objective of this study was to develop an artificial intelligence (AI)-based herbicide-resistant chickweed classification model that could accurately and expeditiously identify herbicide-resistant weeds and more reliably using a low-cost, readily available consumer camera, converted to capture full-spectrum imagery.

Materials and Methods

Phase 1. Converting a Camera

A Fujifilm X-T200 camera (Fujifilm Corp., Valhalla, NY) was modified for use in this experiment (Figure 1). All standard photography cameras are equipped with a hot mirror filter that excludes the IR and UV light spectra from reaching the sensor. This is essential for producing high-quality photographs that cameras are built for. The camera was converted by disassembling it and removing the built-in hot mirror filter to allow the sensor to capture the full light spectrum, including UV, near-IR, and visible light. Aside from the full-spectrum images, NDVI and hot mirror filters were also applied on the modified full spectrum camera to capture NDVI and regular RGB images.

Phase 2. Obtaining Full-Spectrum Images

This study used data from an herbicide-resistance dose-response experiment conducted in 2023 and repeated in 2024. In both years, common chickweed plants were grown in a greenhouse at California State University–Fresno for a suspected ALS-inhibitor resistance dose-response study. The greenhouse temperature and relative humidity were set at 21 ± 2 C and 70%, respectively, with no supplementary lighting. In those studies, there were three populations of common chickweed plants that were suspected of being resistant to ALS inhibitors and a population of plants that were confirmed to be resistant to ALS inhibitors grown from seeds collected from an organic pistachio (*Pistacia vera* L.) orchard in the southern Central Valley of California. The common chickweed seeds were planted in plastic trays containing potting soil on February 19, 2023, for the first-year trial, and on December 15, 2023, for the second-year trial. Seedlings were transplanted on March 13, 2023, and January 5, 2024, in plastic pots that were 6.7 cm wide and 8.9 cm deep containing an OMRI-certified organic garden soil (Kellogg, Carson, CA). The plants were grown until the appropriate stage (approximately 7.5 cm tall with two true leaves) for herbicide treatments. All the plants were treated on March 23, 2023, and January 30, 2024, with five different ALS-inhibiting herbicides (imazamox, imazethapyr, mesosulfuron-methyl, pyroxsulam, and tribenuron-methyl), at 0× (control), 0.5×, 1×, 2×, 4×, and 8× dosage rates (where × = recommended label rate). The recommended label rates were 10.6 g ai ha^{-1}, 21.3 g ai ha^{-1}, 1.58 g ai ha^{-1}, 14.8 g ai ha^{-1}, and 17.5 g ha^{-1} for imazamox, imazethapyr, mesosulfuron-methyl, pyroxsulam, and tribenuron-methyl, respectively. Both experiments were laid out in a completely randomized design with five replications of each treatment. Each plant in a pot was an experimental unit. The herbicides were applied at a spray volume of 93.5 L ha^{-1} with a CO_2-pressurized backpack sprayer calibrated at a speed of 4.8 km h^{-1} with 0.21 MPa. The sprayer was equipped with 8002 flat fan

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Figure 1. A converted Fujifilm X-T200 camera.

```
[ ] train_ds = tf.keras.utils.image_dataset_from_directory(
      data_dir,
      validation_split=0.2,
      subset="training",
      seed=123,
      image_size=(img_height, img_width),
      batch_size=batch_size)

    Found 1500 files belonging to 2 classes.
    Using 1200 files for training.

[ ] val_ds = tf.keras.utils.image_dataset_from_directory(
      data_dir,
      validation_split=0.2,
      subset="validation",
      seed=123,
      image_size=(img_height, img_width),
      batch_size=batch_size)

    Found 1500 files belonging to 2 classes.
    Using 300 files for validation.
```

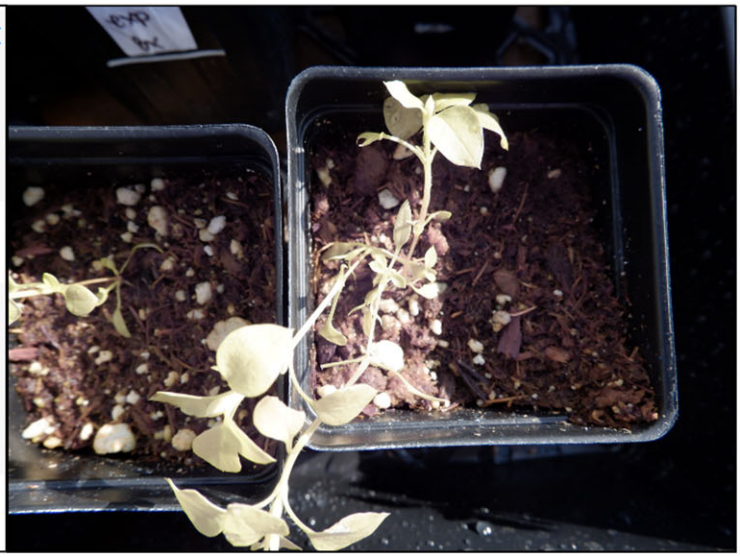


Figure 2. Program used to successfully import the full-spectrum images.

nozzles (TeeJet Technologies, Glendale Heights, IL) at a spray height of approximately 45 cm above the plants.

High-resolution full-spectrum images of common chickweed plants treated with different ALS-inhibiting herbicides at different dosage rates were obtained using the converted camera in year 1 and year 2. The captured images of ALS-inhibitor resistant and susceptible common chickweed plants grown in the 2 yr were used for the development of an AI-based herbicide-resistant weed classification model. Full-spectrum, NDVI, and RGB images were obtained after the various herbicides were applied. The converted camera was used to capture the full-spectrum images. NDVI and hot mirror filters were used to capture NDVI and RGB images. A total of 5,000 full-spectrum, NDVI, and RGB (regular photos) images of herbicide-resistant and -susceptible common chickweed plants were obtained at 1, 2, and 3 d after herbicide treatment in both years. After preliminary analysis, full-spectrum images captured 3 d after herbicide application were determined to be best suited for the development of an AI-based herbicide-resistant weed classification model. The training and validation accuracy of the models developed using full-spectrum images obtained 1 and 2 d after herbicide treatments during preliminary analysis were significantly lower than those obtained at 3 d. This is likely due to poor detection of any appreciable changes in light reflectance from the slower development of injury symptoms due to ALS inhibitor

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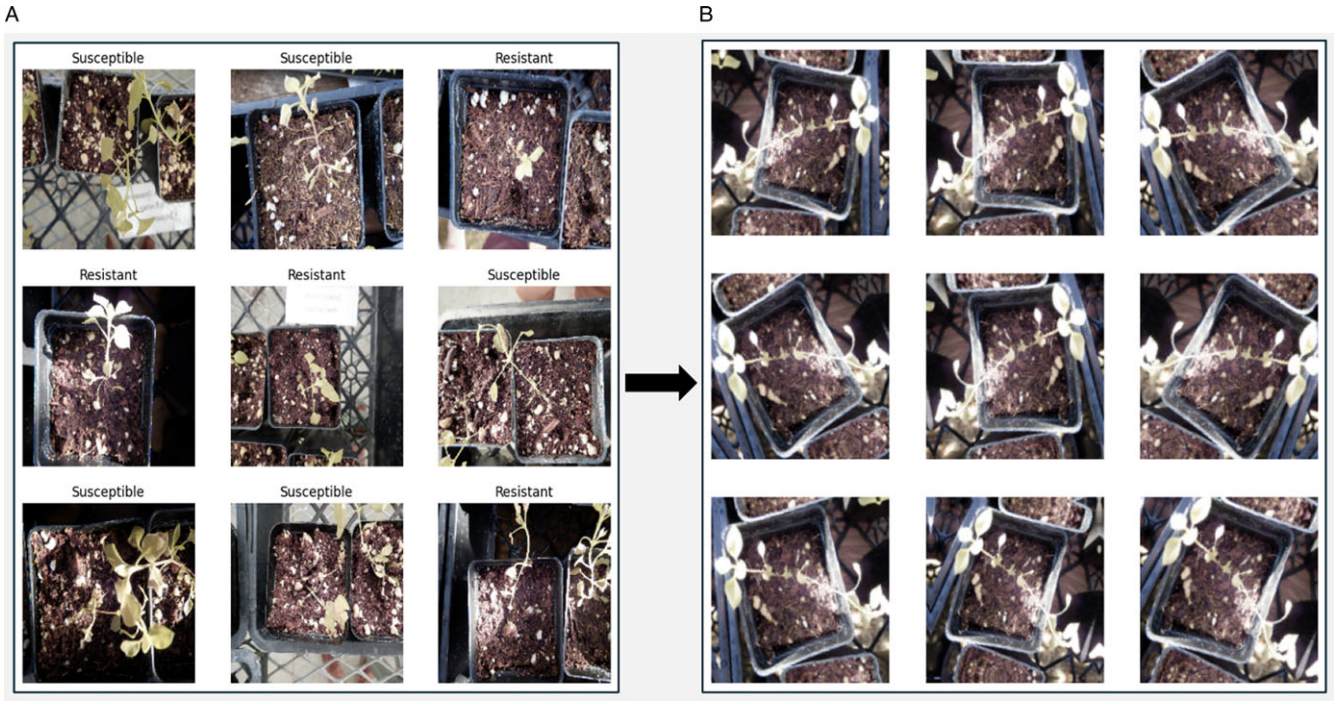


Figure 3. Full-spectrum images before the Keras data augmentation program was employed (A). Rotated and flipped full spectrum images improved by Keras data augmentation program (B).

herbicides. This result is consistent with the study by Shirzadifar et al. (2020), which found the earliest detectable symptoms appeared 3 d after treatment.

Resistant or susceptible classification of common chickweed plants were based on the survival evaluation 28 d after ALS-inhibitor herbicides were applied. Common chickweed plants that died were classified as susceptible to ALS inhibitor resistance, whereas plants with any green tissue remaining and growing were classified as being resistant to ALS inhibitors based on visual observation. Results of the completed dose-response study indicated that the three common chickweed populations are resistant to ALS-inhibitor herbicides (Herrera et al. 2024).

Phase 3. Development of an Herbicide-Resistant Weed Classification Model

Full-spectrum JPEG images taken directly from the camera 3 d after herbicide application in both years were classified and separated into two groups: common chickweed plants that were resistant to ALS-inhibiting herbicides, and those that were susceptible to them, based on final results of the concomitant dose-response studies. The images of resistant and susceptible plants from both years were uploaded to the program and used to develop an AI classification CNN model (herbicide-resistant classification model).

The herbicide-resistant weed classification model was programmed on Colab (Google, LLC, Mountain View, CA), using Python 3.10 programming language, running TensorFlow 2.17 (Google), with the Keras API. A "sequential" model with four convolutional 2-D layers and 10 dense neural net layers was constructed using ADAM as the optimizer, *relu*, *tanh*, and *sigmoid* as the activation functions, and sparse categorical cross-entropy loss as the loss function. Metrics that were used in the model included training and validation accuracy, and training and validation loss.

A hyperparameter tuner algorithm was then incorporated into the program to create the best CNN model that could yield optimal accuracy and reliability. An early stopping protocol through best epoch detection was then used to prevent model overfitting.

Full-spectrum images of common chickweed plants that were both resistant and susceptible to ALS inhibitors were captured using the converted camera. A total of 1,500 images were successfully imported and used by the program to develop and train the herbicide-resistant classification model. The full-spectrum photograph displayed in Figure 2 is an example of the successful importation of all the 1,500 images from the data directory. Keras data augmentation was used to improve the training process by rotating and flipping the images before the construction of the CNN model. Figure 3A shows the images in their original orientation while Figure 3B shows the augmented and rotated images arranged on the same orientation. The augmented images were used to construct, train, and validate the CNN model.

The neural network was designed as a sequential algorithm, and it was trained from scratch. Eighty percent of the 1,500 collected images was used as a training data set and the remaining 20% was used as the validation data set. The program was deployed, and 10 trials of 100 epochs were completed with the best model being chosen by the program based on the validation accuracy. The best performing herbicide-resistant weed classification model was then trained and validated on the entire 1,500-image dataset. A hyperparameter tuning library (KerasTuner) was used to enhance the model's learning process by incorporating optimal hyperparameter combinations to arrive at the best possible herbicide-resistant classification model. An early stopping function was also included to prevent overfitting. The resistant-weed classification model demonstrated superior performance at 36-epochs without overfitting (Figure 4).

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```
Epoch 22/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7026 - loss: 0.5476 - val_accuracy: 0.7100 - val_loss: 0.5536
Epoch 23/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7617 - loss: 0.4970 - val_accuracy: 0.7033 - val_loss: 0.5497
Epoch 24/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7528 - loss: 0.4955 - val_accuracy: 0.7100 - val_loss: 0.5412
Epoch 25/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7585 - loss: 0.4999 - val_accuracy: 0.7100 - val_loss: 0.5515
Epoch 26/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7240 - loss: 0.5190 - val_accuracy: 0.6967 - val_loss: 0.5828
Epoch 27/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7436 - loss: 0.4974 - val_accuracy: 0.7233 - val_loss: 0.5118
Epoch 28/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7555 - loss: 0.4732 - val_accuracy: 0.6833 - val_loss: 0.5678
Epoch 29/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7679 - loss: 0.4792 - val_accuracy: 0.7033 - val_loss: 0.5699
Epoch 30/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 94ms/step - accuracy: 0.7842 - loss: 0.4573 - val_accuracy: 0.6900 - val_loss: 0.5635
Epoch 31/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7818 - loss: 0.4698 - val_accuracy: 0.6733 - val_loss: 0.5938
Epoch 32/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7759 - loss: 0.4559 - val_accuracy: 0.7100 - val_loss: 0.5445
Epoch 33/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7678 - loss: 0.4489 - val_accuracy: 0.7500 - val_loss: 0.4968
Epoch 34/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7971 - loss: 0.4189 - val_accuracy: 0.6900 - val_loss: 0.5511
Epoch 35/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7678 - loss: 0.4520 - val_accuracy: 0.7233 - val_loss: 0.5218
Epoch 36/50
38/38 ━━━━━━━━━━━━━━━━━━━━ 4s 95ms/step - accuracy: 0.7988 - loss: 0.4224 - val_accuracy: 0.7400 - val_loss: 0.5356
Last epoch: 36
```

Figure 4. Output showing 36 epochs needed to generate the most optimized/best convolutional neural network (CNN) model.

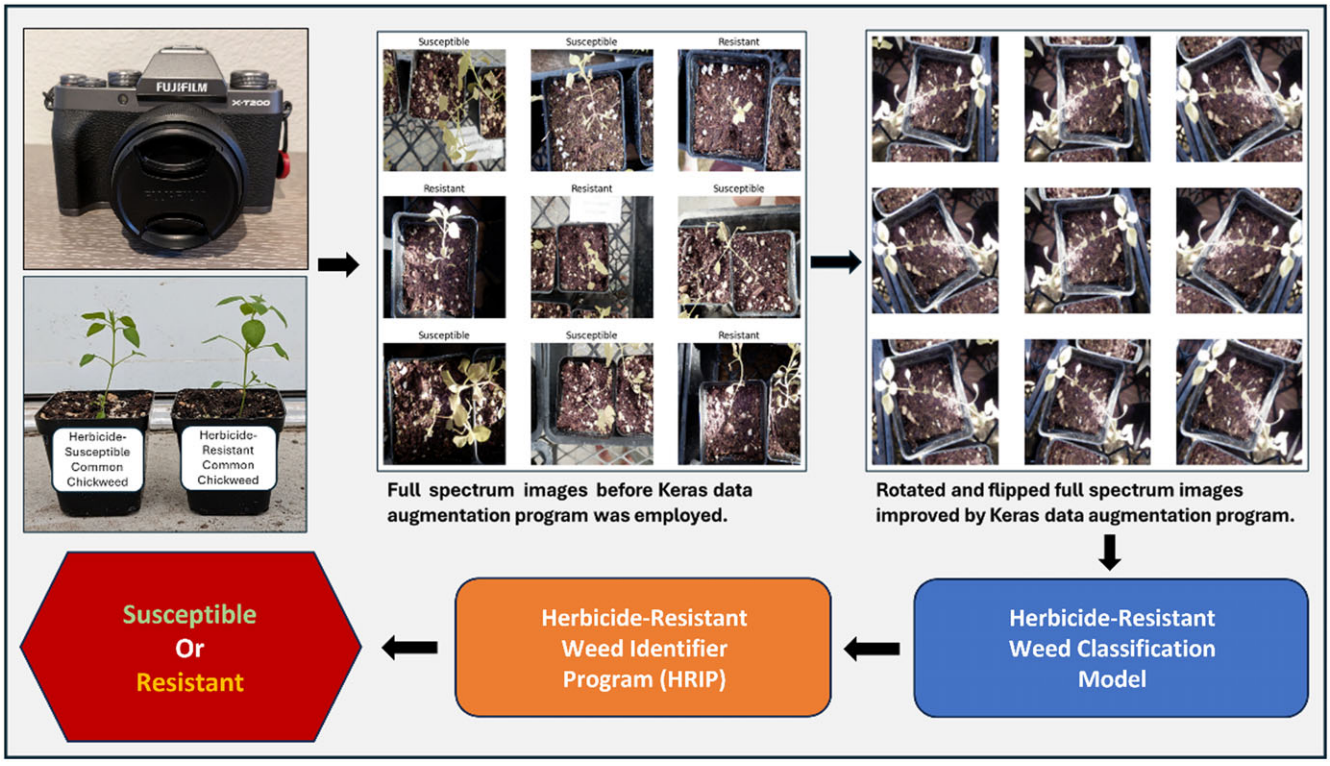


Figure 5. Step-by-step identification of herbicide-resistant weeds using the herbicide-resistant weed identifier program (HRIP).

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Model: "sequential_14"

Layer (type)	Output Shape	Param #
sequential (Sequential)	(None, 180, 180, 3)	0
rescaling_14 (Rescaling)	(None, 180, 180, 3)	0
conv2d_56 (Conv2D)	(None, 178, 178, 112)	3,136
max_pooling2d_56 (MaxPooling2D)	(None, 89, 89, 112)	0
conv2d_57 (Conv2D)	(None, 87, 87, 112)	113,008
max_pooling2d_57 (MaxPooling2D)	(None, 43, 43, 112)	0
conv2d_58 (Conv2D)	(None, 41, 41, 112)	113,008
max_pooling2d_58 (MaxPooling2D)	(None, 20, 20, 112)	0
conv2d_59 (Conv2D)	(None, 18, 18, 112)	113,008
max_pooling2d_59 (MaxPooling2D)	(None, 9, 9, 112)	0
flatten_14 (Flatten)	(None, 9072)	0
dense_140 (Dense)	(None, 112)	1,016,176
dense_141 (Dense)	(None, 16)	1,808
dense_142 (Dense)	(None, 32)	544
dense_143 (Dense)	(None, 32)	1,056
dense_144 (Dense)	(None, 96)	3,168
dense_145 (Dense)	(None, 32)	3,104
dense_146 (Dense)	(None, 112)	3,696
dense_147 (Dense)	(None, 128)	14,464
dense_148 (Dense)	(None, 112)	14,448
dense_149 (Dense)	(None, 112)	12,656
outputs (Dense)	(None, 2)	226

Total params: 1,413,506 (5.39 MB)
Trainable params: 1,413,506 (5.39 MB)
Non-trainable params: 0 (0.00 B)

Figure 6. Output describing the characteristics of the herbicide-resistant weed classification model.

Phase 4. Coding the Herbicide-Resistant Weed Identifier Program

The herbicide-resistant weed identifier program (HRIP) was also programmed with Colab (Google) using Python 3.10, running TensorFlow 2.17 (Google) with the Keras API. To check the classifying ability of the newly developed CNN model and its accuracy, 25 images of common chickweed plants that were both resistant and susceptible to ALS-inhibitor herbicides were tested in the HRIP using the herbicide-resistant weed classification model. These images of susceptible and resistant common chickweed plants used were not included in the original training and validation datasets. The complete step-by-step process used in the identification of ALS inhibitor-resistant common chickweed plants using the novel full-spectrum imaging and a hyperparameter-tuned CNN (herbicide-resistant classification model) is summarized in Figure 5.

Results and Discussion

The herbicide-resistant classification model with optimal hyperparameter combinations performed best at 36 epochs (Figure 4). It has four convolutional 2-D layers, 10 dense neural layers, and an output layer. The herbicide-resistant weed classification model has a total of 1,413,506 parameters, all of which are considered trainable. The characteristics and properties of the newly built CNN model are shown in Figure 6.

The training and validation accuracy curves for the herbicide-resistant weed classification model exhibited a steady increase over 36 epochs (Figure 7). The set of hyperparameters used in the model rendered a remarkably high training accuracy and at the same time high validation accuracy, indicating that the model was very accurate in differentiating and classifying between common chickweed plants that were resistant or susceptible to ALS inhibitors. In addition, both training loss and validation loss

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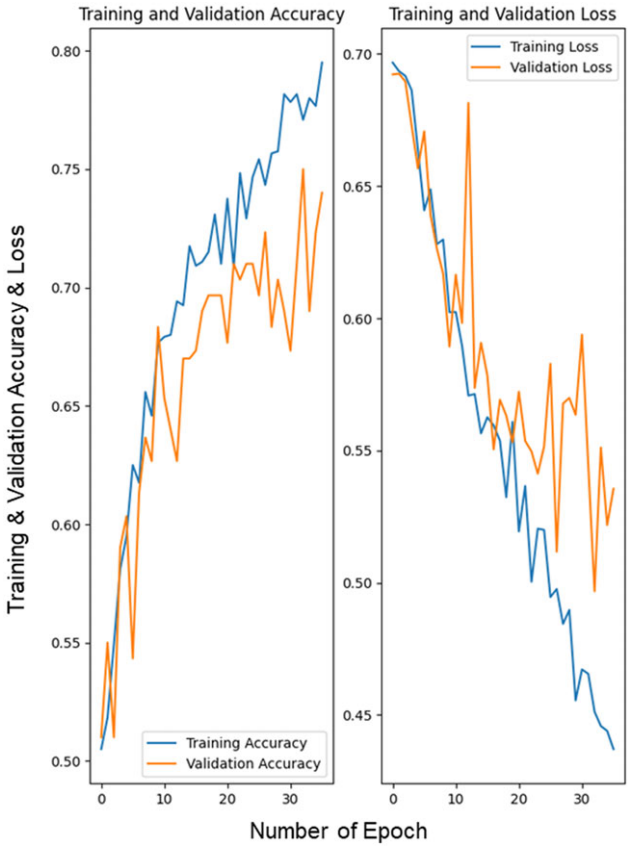


Figure 7. Training and validation loss curves showing steadily decreasing values and the accuracy curves show steadily increasing values with optimal gaps between them indicating optimal learning without overfitting.

curves for the herbicide-resistant weed classification model followed decreasing trends with an optimal gap between them, suggesting optimal learning without overfitting. A steadily decreasing trend in the training loss curve suggested that the herbicide-resistant weed classification model was improving its learning from the data it was trained with (Figure 7). The very low validation value also indicated that the model had achieved "optimal learning". The model performance on the unseen data and images was evaluated using the validation loss. A very low validation loss value achieved in the model indicated that its error on unseen images was very low, and the model was accurate in distinguishing ALS-inhibitor resistant common chickweed plants from those that were susceptible to it.

The HRIP was developed and appended to the newly developed herbicide-resistant weed classification model. Twenty-five ALS inhibitor-susceptible and -resistant full-spectrum images of common chickweed plants that the model had not analyzed before, independent of the 300-image validation set, were used for secondary verification of accuracy using the HRIP running the herbicide resistance classification model. The HRIP was able to independently classify the full-spectrum images correctly at 88% accuracy. The performance of HRIP in identifying both herbicide-resistant and -susceptible common chickweed plants were equally high with classification accuracies of 87.5% and 88.2%, respectively (Table 1). An example of a full-spectrum image that was accurately identified by the model as a "resistant" common chickweed plant is shown in Figure 8. The results of this study were also matched with the actual plant mortality evaluation, which corresponded well (data not shown).

Table 1. Discriminative classification of common chickweed plants.[a]

Classification	Total no. of images tested	Right classification	Wrong classification	Accuracy
Resistant	8	7	1	87.5%
Susceptible	17	15	2	88.2%
Total	25	22	3	88.0%

[a]Discriminative classification is based on the herbicide-resistant weed identifier program, developed with artificial intelligence, running the herbicide-resistant weed classification model.

The confusion matrix for resistant and susceptible chickweed image identification using the HRIP is summarized in Figure 9. It shows that out of eight images that are resistant, the herbicide-resistant weed classification model predicted that one image is susceptible chickweed, and of the 17 susceptible chickweed images, it predicted that two images were resistant chickweed. The diagonal values indicate the correct predictions by the HRIP, whereas values outside of it are prediction errors.

The development of the herbicide-resistant classification model in this study involved training it from scratch. This was to ensure that the model would learn effectively from a dataset that had been fully vetted via a completed dose-response study (Herrera et al. 2024). With this approach the model eliminated any biases from preexisting knowledge bases and pretrained weights, which are commonly encountered when using publicly available image datasets for transfer-learning.

The use of the hyperparameter tuner was very helpful in determining the best hyperparameter combinations that led to the development of the best performing herbicide resistance classification model. It enhanced the model's performance and improved its accuracy, precision, and recall (Bartz-Beielstein et al. 2023). Although hyperparameters can be manually determined, it is a time-consuming endeavor, and it is uncertain whether the optimal model had been reached with this trial-and-error approach.

The effect of ALS inhibition in susceptible weeds includes disruption of the photosynthesis transport and respiration system, which leads to chlorophyll degradation (Zhou et al. 2007). Stress or disruption in the transport chain due to the application of an ALS-inhibiting herbicide can be detected by measuring changes in the chlorophyll light absorption, reflectance patterns, and chlorophyll fluorescence (Kaiser et al. 2013). All vegetation, including weeds, have distinct light reflectance patterns that can be measured and graphed using a spectrometer (Figure 10). This light reflectance pattern is known as its *spectral signature*. Healthy, thriving plants have a noticeably different spectral signature than stressed, diseased, or unhealthy plants (Govender et al. 2007). It is this variation in the spectral reflectance between susceptible and resistant weeds when subjected to herbicide treatment, even when very subtle at 72 h after application, which is exploited by the herbicide resistance classification model to rapidly identify resistant from susceptible strains.

Different weed species have unique spectral signature changes depending on their resistance or susceptibility to herbicides, which may fall within or outside the visible light spectrum. The spectral signature discriminative wavebands of Kochia (*Bassia scoparia*) and common waterhemp (*Amaranthus tuberculatus*) are in the near-IR range (>750 nm), whereas those for common ragweed (*Ambrosia artemisiifolia*) are in the visible light wavelength of 450 to 630 nm. (Shirzadifar et al. 2020).

Converted full spectrum camera allows it to capture the subtle differences in the spectral signature of herbicide-treated plants

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```
[ ] class_names = ['Resistant', 'Susceptible']
    print(class_names)

    ['Resistant', 'Susceptible']

[ ] img_path = '/content/drive/MyDrive/Colab Notebooks/For Testing/Resistant/DSCF6490.JPG'

    img = tf.keras.utils.load_img(
        img_path, target_size=(img_height, img_width)
    )
    img_array = tf.keras.utils.img_to_array(img)
    img_array = tf.expand_dims(img_array, 0) # Create a batch

    predictions = model.predict(img_array)
    score = tf.nn.relu(predictions[0])

    print(
        "This image most likely belongs to {} with a {:.2f} percent confidence."
        .format(class_names[np.argmax(score)], 100 * np.max(score))
    )

    1/1 ━━━━━━━━━━━━━━━━━━━━ 0s 20ms/step
    This image most likely belongs to Resistant with a 85.64 percent confidence.
```

The model classified the image accurately

Figure 8. The herbicide-resistant weed identifier program (HRIP), which indicates a weed classification of "Resistant".

		Predicted Condition		
	Total (8 + 17)	Resistant 9	Susceptible 16	Accuracy
Actual Condition	Positive 8	True Positive 7	False Negative 1	87.5% 12.5%
	Negative 17	False Positive 2	True Negative 15	88.2% 11.8%

Figure 9. Confusion matrix showing the performance of herbicide-resistant weed identifier program (HRIP) running the herbicide-resistant classification model. (The resistant chickweed image is classified as positive, while the susceptible chickweed image is classified negative.)

wherever it may be in the light spectrum wavelengths between 300 to 1100 nm (Melentijevic 2015) – which also includes the chlorophyll fluorescence range of 620–750 nm. Chlorophyll fluorescence is the light emitted by the leaves on the red to far-red light (620–750 nm) when exposed to about 400–700 nm (Kalaji et al. 2017). This emitted light can be used as an indicator of a plant's photosynthetic activity and is also useful for the identification of herbicide-resistant weeds (Kaiser et al. 2013). Converting the regular consumer camera to capture full light spectrum (300–1,100 nm) allows the inclusion of all possible areas of differences in spectral signatures, including the chlorophyll fluorescence wavelengths as well as the near IR reflectance values in the full spectrum images of common chickweed. This is the key in the herbicide resistance classification model's accurate differential analysis.

Use of full-spectrum images to develop resistance to the ALS inhibitor herbicide classification model proved to be more robust and more accurate compared to using RGB or NDVI images. The validation accuracies of the CNN model developed using full-spectrum, NDVI, and RGB images were 0.80, 0.53 and 0.65, respectively. Higher training and validation accuracy were observed with the CNN model that was trained using full-spectrum images compared to the model that was rendered using NDVI or RGB images. This is likely due to the inclusion of wavelengths in the visible light spectrum (380–750 nm) as well as the UV (<380 nm) and near-IR (750–1,000 nm) in the full-spectrum images which reveals subtle differences and details that are invisible to the human eye or even standard camera image sensors (Zhen et al. 2021). Although near-IR wavelength can indicate signs of stress in a plant even before it becomes visible to the naked eye as an unhealthy leaf starts to absorb more photons, the visible light spectrum, including the far-red spectrum, can also reveal minute changes in the level of photosynthetic activity (Zhen and van Iersel 2017). RGB wavelength reflectance in plants indicates the amount of red and blue light being absorbed as utilized through photosynthesis and the degree of green light spectrum reflected correlating with the concentration of chlorophyll. As the plant sustains injury and stress from the herbicide, it is unable to absorb as many red and blue light wavelengths. This results in a flattening of the injured plant's spectral signature in the visible light range, as opposed to the usual peaked curve observed in healthy plants with the crest in the green wavelength and troughs in both the red and blue spectra (Figure 10).

The symptoms of ALS inhibitor injury, which include chlorosis, stunting, red leaf veins, and tissue necrosis, are supposed to be evident in 1 to 4 wk after herbicide treatment depending on the dosage used and environmental conditions at the time of application (Guo et al. 2015). However, the effect of the ALS inhibitor herbicide on common chickweed plants was detected 3 d after herbicide treatment. The model's ability to detect resistant chickweed plants will be consistent for both target site and non-target–site resistance because it was trained using full-spectrum images of all resistant plants, regardless of the type of resistance. Herbicide-induced injury symptoms were detected by the program 72 h after herbicide treatment. This is primarily due to the ability of the converted camera to obtain full-spectrum images and capture the spectral signature of common chickweed plants, in varying

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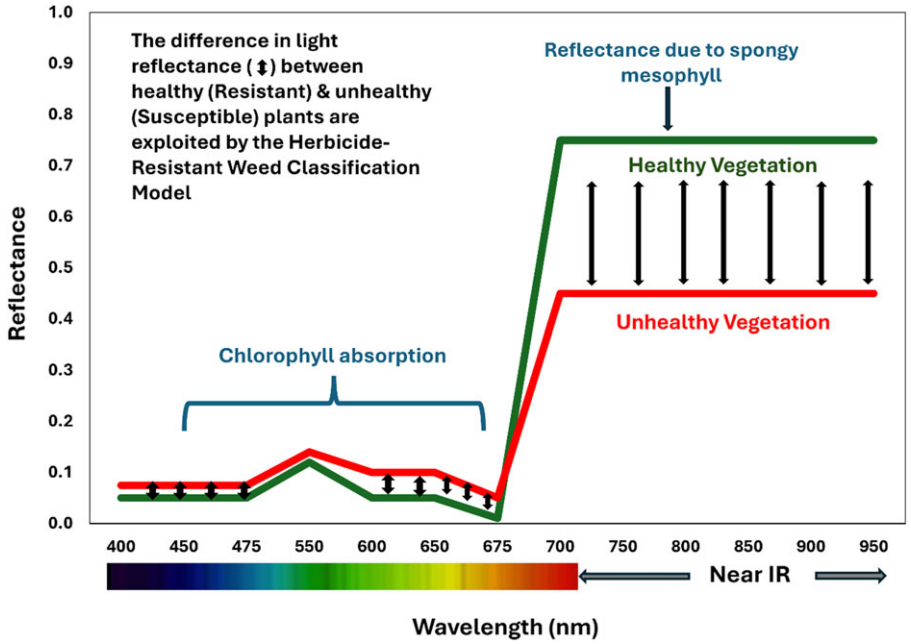


Figure 10. Illustration of comparison of spectral signatures of healthy and unhealthy plants.

degrees of ALS injury symptoms, even before they were visible to the naked eye. This study's findings are similar to those observed by Shirzadifar et al. (2020), which established that light reflectance and spectral signature can effectively detect herbicide injury symptoms such as chlorosis within 72 h. In contrast, dose-response studies take at least 2 mo to be completed and the fastest commercial genetic sequencing analysis can take a minimum of 2 wk to obtain results. Even newer bulky and expensive chlorophyll fluorescence imaging takes at least 96 h to detect and classify herbicide-resistant weeds after complex and potentially error prone calibration and calculations are carried out.

The use of different ALS-inhibiting herbicides and their varying dosage treatments in this research allowed the model to better distinguish herbicide-resistant from herbicide-susceptible plants. This is because the images used to train the model include a wide array or spectrum of injury symptoms from the application of different ALS-inhibiting herbicides and various dosage rates of –0.5× to 8×, where × = the recommended label rate.

The herbicide group numbering system was developed to help farmers select herbicides belonging to different groups to avoid repeatedly using the same mode of action (Hulme 2022). However, Neve (2007) suggested that the rotation of herbicide modes of action may increase the prevalence of herbicide-resistant weeds because such a practice could select for non-target–site resistance mechanisms. Therefore, the problem of herbicide-resistant weeds seems to be an ongoing problem in the future unless non-herbicidal alternative technologies are developed. Hence, any tools that help farmers recognize herbicide-resistant weeds and take proactive measures will be of great benefit, and tools such as this HRIP tool may play an important role in that.

Practical Implications

The results of this study support the research hypothesis that the use of full-spectrum images obtained from the modified consumer camera to develop a hyperparameter-tuned CNN model can quickly and accurately classify putative ALS inhibitor-resistant weeds. It also showed that the herbicide resistance classification model and HRIP developed using the full-spectrum JPEG images can quickly and accurately distinguish a common chickweed plant from one that is susceptible to ALS-inhibiting herbicides from a plant that is resistant to them.

The HRIP can identify ALS inhibitor-resistant chickweed plants as early as 72 h after herbicide application at an impressive accuracy of 88%. It also exhibited robustness, expanding its potential as a valuable tool for real-world and real-time application. It does not require preprocessing of the images or complex calculations to properly function or operate, which is the case with contemporary weed classifiers. The HRIP needs only JPEG images straight out of the camera from the converted full-spectrum consumer camera to be able to distinguish an ALS inhibitor-resistant from ALS inhibitor-susceptible common chickweed plant. Given this simplicity, even farmers who have no experience with this technology will be able to use the application with minimal training. It also has broad applicability due to its ability to accurately identify ALS inhibitor-resistant chickweed plants that were treated with different ALS-inhibiting herbicides, regardless of their chemical group or dosage rate.

Rapid and accurate detection of ALS inhibitor-resistant weeds using the innovative HRIP can serve as a powerful tool in the fight against the emerging agricultural resistant weed problem in California and around the world. This system can help farmers to establish more effective and safer weed management practices. The technology can also be expanded to use with other weed species such as Palmer amaranth and common waterhemp, which are resistant to ALS-inhibiting herbicides.

The HRIP running the herbicide resistance classification model can be integrated into a custom-built autonomous ground-based remotely operated vehicle or ROVER to detect and dispose of herbicide-resistant weeds in real time.

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