



Scientific Article

Detection of *Closterovirus tristeza* in Persian lime using machine learning and drones

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ABSTRACT

Background/Objective. Persian lime (*Citrus latifolia*) production in Mexico is threatened by various diseases. Among the most significant is the citrus tristeza virus (*Closterovirus tristeza*, CTV), which can infect plants asymptotically. This research aimed to evaluate the use of drone-acquired multispectral imagery and machine learning algorithms to identify diseased plants as a tool for early, non-destructive detection.

Materials and Methods. The study was conducted in three agricultural fields in Campeche, México: Champotón and Castamay (sections 1 and 2). Multispectral images (green, red, red-edge, and near-infrared) were acquired, and spectral signatures, vegetation indices (NDVI, GNDVI, NDRE, SIPI), and principal components (PCA) were extracted. Supervised classifiers—Random Forest (RF), Maximum Likelihood (MAXLIKE), Support Vector Machine (SVM), and Multilayer Perceptron (MLP)—were applied using hard and soft classification schemes.

Results. RF performed the best. In hard classification, it achieved overall accuracies of 64% in Champotón and Castamay Section 1, and 65% in Section 2, with diseased plant detection rates between 65 and 75%. It yielded better results in soft classification. In Champotón, it achieved 94% overall accuracy and detected 79% of diseased plants. In Castamay Section 1, the figures were 91 and 92%, respectively; and in Section 2, it achieved 99% overall accuracy and 100% disease detection.

Conclusion. Random Forest demonstrated the most robust and stable performance across the various scenarios and fields analyzed—in both hard classification (65%) and soft classification (94%)—confirming its ability to handle non-linear relationships and spectral variability in real agricultural environments. The use of multispectral imagery combined with machine learning algorithms—specifically Random Forest (RF) for soft classification—represents an effective tool for early diagnosis of CTV in Persian lime.

Keywords: *Citrus latifolia*, CTV, Machine learning, Early detection



INTRODUCTION

The persian lime (*Citrus latifolia*) is an export crop in Mexico (SADER, 2018). By the end of 2023, Mexico ranked as the world's second-largest producer and exporter of lemons and limes (SADER, 2024). This crop is threatened by various pathogens, including *Closterovirus tristeza* (CTV), the causative agent of citrus tristeza disease. Since 2017, this virus has caused the death of more than 300 000 trees in states such as Veracruz, Campeche, and Yucatán (INIFAP, 2024).

CTV is an RNA-genome virus that infects the host's phloem (Ramírez-Pool *et al.*, 2022) and causes a variety of symptoms modulated by the pathogen genotype, the host genotype, and the environment (Garnsey *et al.*, 2005). Common symptoms include stem pitting, stunting, chlorosis, leaf deformation, graft collapse, and even tree death (Rivas-Valencia *et al.*, 2020). A critical factor is the asymptomatic behavior of certain genotypes in the early stages, which hinders timely detection through visual inspection and delays management decisions. Furthermore, genetic variability among genotypes complicates field diagnosis and limits the effectiveness of containment measures (Futch and Brlansky, 2005). Diagnostic methods for CTV include biological indexing (grafting onto indicator plants), serology (DAS-ELISA, tissue-print, and immunostrips), and molecular techniques (IC-RT-PCR, nested RT-PCR, and RT-qPCR); however, these techniques are slow, labor-intensive, and require demanding methodological requirements and incur high costs (IPPC, 2016).

In response to this challenge, farmers have begun adopting digital technologies to transform traditional practices into more sustainable and efficient systems. Key tools include remote sensors, multispectral cameras, and machine learning algorithms; together, these enable monitoring of crop physiological status, detection of anomalies before they become visible to the human eye, and optimization of resource use (EOS Data Analytics, 2025). This analysis relies on remote sensing, defined as the measurement of electromagnetic radiation from a distance. This radiation is recorded by sensors (mounted on remotely piloted aircraft) after interacting with the surface and the atmosphere. Based on this information, variables or characteristics of the object under study are estimated (Boyd, 2005; Campbell and Wynne, 2011).

Although CTV can remain latent without visible symptoms, recent research has shown that remote sensing technologies can identify infected trees at an early stage. Argyriou *et al.* (2024) applied the Random Forest algorithm to multispectral imagery to detect CTV in Valencia oranges (*Citrus sinensis*) and achieved 89.7% accuracy, suggesting real potential for early detection in citrus crops. Studies based on time series of leaf spectra have shown that it is possible to distinguish CTV-infected plants prior to the onset of visible symptoms through spectral analysis (Afonso *et al.*, 2017). Likewise, preliminary remote sensing research has identified differences in the spectral signatures of infected trees that allow for large-scale monitoring (using both proximal and remote sensing methods) (Gualano *et al.*, 2012). Various studies have demonstrated that supervised machine learning algorithms can assist in interpreting imagery and spectral data to assess crop health (Rodrigues *et al.*, 2021; Sánchez-Calle and Castillo-Armas, 2022). In this context, the objective of this study was to evaluate and compare the performance of supervised machine learning classifiers for diagnosing individual Persian lime trees (*Citrus latifolia*) that lacked visible CTV symptoms, using UAV-acquired imagery and field diagnoses via immunostrips (ImmunoStrip®) as the reference.

MATERIALS AND METHODS

The study was conducted in commercial Persian lime (*Citrus latifolia*) plantations with a history of *Citrus tristeza virus* (CTV) infection, located in Champotón and Castamay, Campeche, Mexico. Although the selected trees showed no visible symptoms, immunochromatographic tests (ImmunoStrip®) confirmed latent CTV infection. The coordinates for the evaluation sites were: Champotón (19.37407° N, 90.71537° W), Castamay Section 1 (19.81790° N, 90.44403° W), and Castamay Section 2 (19.81909° N, 90.44483° W), datum WGS84.

Image acquisition. During October 2024, images were acquired using two unmanned aerial vehicle (UAV) platforms: a Phantom 4 Pro equipped with an FC6310 RGB camera and a Matrice 300 RTK equipped with a Parrot Sequoia multispectral sensor. The RGB camera captured images in three channels (red, green, and blue) expressed in digital levels, while the multispectral sensor acquired data in spectral bands centered on green (530–570 nm), red (640–680 nm), red-edge (730–740 nm), and near-infrared (NIR; 770–810 nm). RGB and multispectral orthomosaics, as well as digital terrain models, were generated from these images using Pix4D® software.

Image processing. The RGB images had a spatial resolution of 0.76 to 0.83 cm cell⁻¹ and served as a reference for co-registering the multispectral images (2.77 to 3.21 cm cell⁻¹). Both datasets were resampled to a final resolution of 2.72–3.18 cm cell⁻¹. Co-registration was performed in QGIS (v3.28.14) using georeferencing with control points and bilinear interpolation; the resulting residual error in the image alignment was considered acceptable for the study's comparative purposes. Finally, both image sets were converted to 8-bit (Byte) format and projected to the WGS84/UTM zone 15N coordinate system (EPSG:32615). Due to the heterogeneous distribution of other vegetation types across the site, Persian lime tree crowns were manually delineated in QGIS using a vector layer that excluded areas of no interest (other vegetation types and bare soil).

Class assignment. Once delineated, the Persian lime tree canopies were labeled as healthy or diseased based on field results obtained using ImmunoStrip®. In Champotón, 203 healthy and 63 diseased canopies were identified; in Castamay Section 1, 65 healthy and 63 diseased; and in Section 2, 54 healthy and 32 diseased. Reflectance data recorded for the cells within these canopies were used for training and validation in the supervised classification.

Spectral analysis. Seven images were obtained via resampling: three RGB images (RGB-A, RGB-V, RGB-R) and four multispectral images (MS-G, MS-R, MS-RE, MS-N). Spectral signatures were analyzed using TerrSet LiberaGIS software (v20; Eastman, 2024), and vegetation indices—NDVI, GNDVI, NDRE, SIPI, and principal components—were calculated (Rouse *et al.*, 1974; Gitelson *et al.*, 1996; Barnes *et al.*, 2000; Peñuelas *et al.*, 1995; Jolliffe, 2002). The distribution of cell values for healthy and diseased plants was analyzed using R software (v4.3.2; R Core Team, 2023). Mean, standard deviation, minimum and maximum values, mode, skewness, and kurtosis were also calculated.

Supervised classification. Using TerrSet LiberaGIS software, the following classifiers were applied: Maximum Likelihood (MAXLIKE), Decision Forest (Random Forest, RF), Multilayer Perceptron (MLP), and Support Vector Machines (SVM) with radial (SVM-RBF), sigmoidal (SVM-SIG), linear (SVM-LIN), and polynomial (SVM-POLY) kernels.

For the MLP, three activation functions were employed—Softmax (MLP-SMX), linear (MLP-LIN), and sigmoidal (MLP-SIG)—with two hidden layers, four nodes per layer, and up to 10,000 iterations. In hard classification, each image cell was assigned to one of the two classes. In the soft classification, various membership thresholds were evaluated incrementally. For each threshold, overall accuracy, total error, and detection rate per class were calculated. The values reported in the results section correspond to the thresholds that maximized each classifier's performance, to analyze their sensitivity and stability to changes in the decision criterion.

RESULTS AND DISCUSSION

Spectral signatures. Across the three fields (Champotón, Castamay-Section 1, and Castamay-Section 2), the spectral averages for healthy and diseased plants showed a high degree of overlap. Subtle differences were observed in the original bands—particularly MS-RE and MS-N—whereas differences between classes were even smaller regarding vegetation indices. Similarly, the principal components showed marked overlap between the classes. The distribution of spectral values from the original images (Figures 1, 2, and 3) demonstrates this overlap in the visible region, with changes in skewness and kurtosis that do not yield clear separation between the classes.

In contrast, a relative shift in distributions is observed in the near-infrared (MS-N)—with higher values for healthy plants and lower values for diseased plants—suggesting greater discriminative potential compared to the other bands. The corresponding averages and descriptive statistics are presented in Tables 1 and 2; these summarize the spectral values used as input variables for the classifiers and are interpreted in relative terms across the classes.

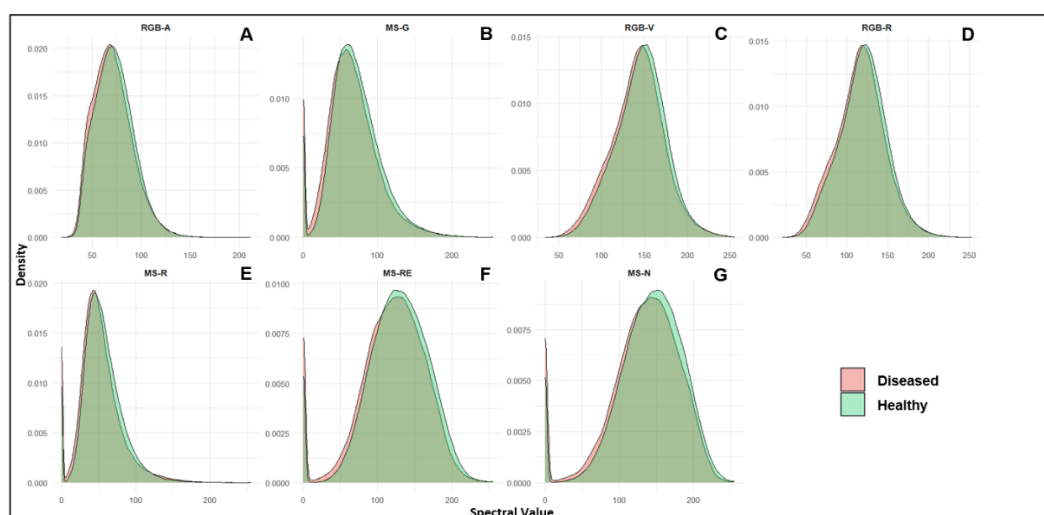


Figure 1. Spectral density curves by band: comparison between healthy plants and those infected with *Closterovirus tristezae* (CTV) in the Champotón, Campeche field.

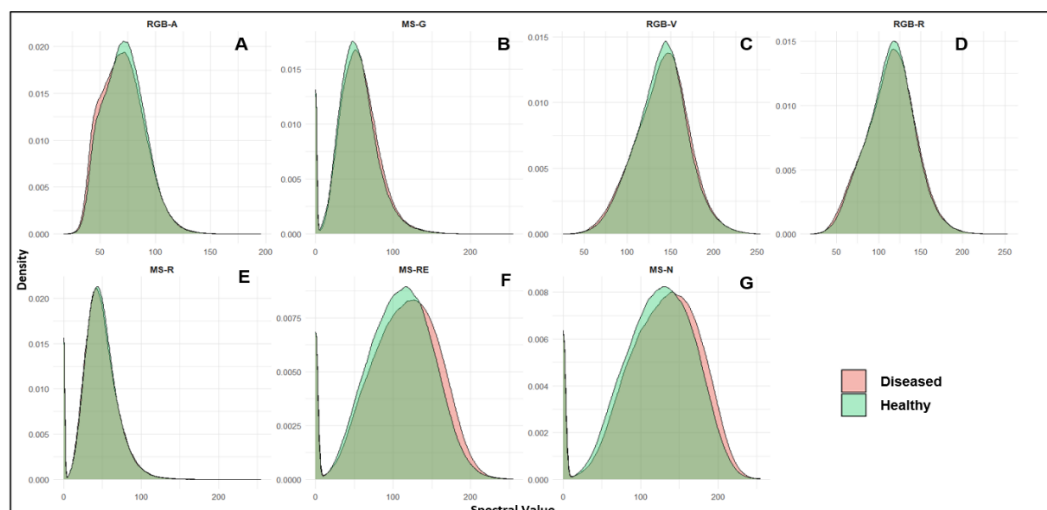


Figure 2. Spectral density curves by band: comparison between healthy plants and those infected with *Closterovirus tristeza* (CTV) in the Castamay Section 1 field, Campeche.

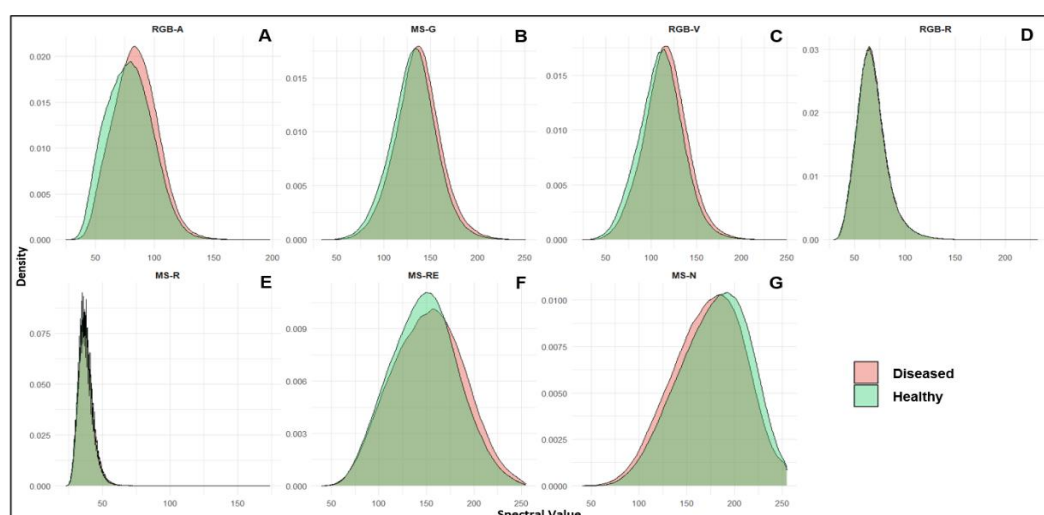


Figure 3. Spectral density curves by band: comparison between healthy plants and those infected with *Closterovirus tristeza* (CTV) in the Castamay Section 2 field, Campeche.

The limited differentiation between healthy and diseased plants observed in the spectral patterns of the original images suggests that reflectance variations associated with citrus tristeza are generally subtle at the canopy level. This behavior is consistent with CTV infection being restricted to the phloem—located within the inner parts of the stem and branches—which hinders the immediate emergence of visible foliage symptoms, particularly during the early stages of infection (Loebenstein and Katis, 2014). Furthermore, the virus's genetic diversity, involving multiple strains or races, can elicit heterogeneous physiological responses in hosts, resulting in variable symptom expression. Collectively, these conditions explain why discriminating between plants based solely on average spectral signatures is limited in asymptomatic infections or in cases of mild symptom manifestation, as previously reported (Hilf *et al.*, 2005; Rivas-Valencia *et al.*, 2020). The quantitative assessment of this separability is subsequently addressed using supervised classification models.

Table 1. Statistical summary of spectral values by band and class across the three fields evaluated in commercial Persian lime plantations.

Field	Band	Class	Mean	SD ^z	Min	Max	Mode	Skewness	Kurtosis
CHAMPOTÓN FIELD	RGB-A	Diseased	83.9	19.5	29	198	82.9	0.253	0.0295
		Healthy	78.9	19.3	25	189	78.5	0.282	-0.166
	MS-G	Diseased	136.2	24.2	46.2	248	137	0.1	0.399
		Healthy	132.2	24.3	34	252	133	0.0359	0.338
	RGB-V	Diseased	116	24	33	241	116	0.124	0.335
		Healthy	113	24.4	24	250	113	0.117	0.28
	RGB-R	Diseased	67.7	15.7	22	221	64.6	1.01	2.46
		Healthy	67.4	15.6	29	233	63.8	1.08	3.07
	MS-R	Diseased	35.1	6.65	26	120	26.4	0.342	3.26
		Healthy	37.6	5.57	23	174	37.1	1.26	6.59
	MS-RE	Diseased	152	30.5	40	255	158	0.0142	-0.469
		Healthy	168	32.2	41	255	152	0.0725	-0.345
	MS-N	Diseased	173	35.9	41	255	185	-0.225	-0.406
		Healthy	179	36.3	53	255	193	-0.302	-0.442
CASTAMAY SECC 1 FIELD	RGB-A	Diseased	70.5	19.5	17	196	71.3	0.447	0.143
		Healthy	71.8	18.8	19	190	70.9	0.364	0.0575
	MS-G	Diseased	56.8	32.8	2.01E-06	254	50.8	0.501	1.52
		Healthy	53.3	26.2	1.14E-06	234	48	0.515	1.28
	RGB-V	Diseased	140	30.8	26	254	148	-0.149	0.0145
		Healthy	140	28.5	29	254	148	-0.0793	0.112
	RGB-R	Diseased	114	29.3	26	254	117	-0.0327	-0.0544
		Healthy	114.4	28.3	19	249	118	-0.00817	0.00512
	MS-R	Diseased	46.4	17.2	3.93E-06	255	42.5	0.56	1.72
		Healthy	46.6	22	3.93E-06	239	43.1	0.432	1.26
	MS-RE	Diseased	106	45.2	4.84E-06	255	106	-0.4	-0.118
		Healthy	106	47.2	4.84E-06	255	106	-0.318	-0.0592
	MS-N	Diseased	126	50.7	4.53E-06	255	141	-0.574	0.101
		Healthy	120	48.9	1.85E-06	255	130	-0.486	0.0315
CASTAMAY SECC 2 FIELD	RGB-A	Diseased	71.2	19.9	22	212	68.4	0.644	0.59
		Healthy	73.1	19.5	19	200	70.7	0.538	0.433
	MS-G	Diseased	65.4	34.1	0.95	255	58.1	0.664	1.29
		Healthy	70.8	33.7	-9	255	61.2	0.679	1.42
	RGB-V	Diseased	145	29.9	34	255	147	-0.0335	0.153
		Healthy	145	29.5	34	255	151	-0.0841	0.119
	RGB-R	Diseased	116	29.2	21	253	119	0.104	0.158
		Healthy	116	29.1	21	253	123	0.0586	0.129
	MS-R	Diseased	51.8	28.7	0.027	255	44.5	1.15	3.38
		Healthy	55.4	26.6	0.027	255	45.9	0.945	2.83
	MS-RE	Diseased	119	46.3	1.3	255	130	-0.629	0.543
		Healthy	125	46.1	1.3	255	124	-0.589	0.847
	MS-N	Diseased	133	49.6	6	255	142	-0.847	0.805
		Healthy	141	45.7	1	255	152	-0.84	1.27

^z SD: Standard deviation.

Table 2. Mean values of variables derived from the images (original bands, principal components, and vegetation indices) in the three fields analyzed in commercial Persian lime plantations.

Field	Original bands	Class	Spectral average	PCA ^z	Class	Spectral average	Indexes	Class	Spectral average
CHAMPOTÓN FIELD	RGB-A	Diseased	80.61	PCA 1	Diseased	289.92	NDVI	Diseased	0.6
		Healthy	75.5		Healthy	282.92		Healthy	0.61
	MS-G	Diseased	65.01	PCA 2	Diseased	40.39	GNDVI	Diseased	0.41
		Healthy	64.49		Healthy	48.65		Healthy	0.43
	RGB-V	Diseased	131.18	PCA 3	Diseased	-56.47	NDRE	Diseased	0.071
		Healthy	126.37		Healthy	-59		Healthy	0.099
	RGB-R	Diseased	111.44	PCA 4	Diseased	-1.87	SIPI	Diseased	0.78
		Healthy	106.21		Healthy	-0.137		Healthy	0.78
	MS-R	Diseased	36.65	PCA 5	Diseased	8.051			
		Healthy	36.01		Healthy	7.53			
	MS-RE	Diseased	146.06	PCA 6	Diseased	4.88			
		Healthy	141.48		Healthy	4.66			
	MS-N	Diseased	167.26	PCA 7	Diseased	-1.423			
		Healthy	172.19		Healthy	-14.545			
CASTAMAY SECC 1 FIELD	RGB-A	Diseased	68	PCA 1	Diseased	240.092	NDVI	Diseased	0.444
		Healthy	69.32		Healthy	232.590		Healthy	0.424
	MS-G	Diseased	56.2	PCA 2	Diseased	-104.889	GNDVI	Diseased	0.384
		Healthy	54.29		Healthy	-108.368		Healthy	0.379
	RGB-V	Diseased	134.76	PCA 3	Diseased	6.559	NDRE	Diseased	0.064
		Healthy	134.63		Healthy	4.554		Healthy	0.065
	RGB-R	Diseased	110.08	PCA 4	Diseased	6.546	SIPI	Diseased	0.743
		Healthy	110.21		Healthy	7.937		Healthy	0.583
	MS-R	Diseased	47.39	PCA 5	Diseased	-4.623			
		Healthy	47.5		Healthy	-4.177			
	MS-RE	Diseased	113.75	PCA 6	Diseased	6.983			
		Healthy	107.93		Healthy	7.113			
	MS-N	Diseased	128.46	PCA 7	Diseased	-8.070			
		Healthy	122.51		Healthy	-8.236			
CASTAMAY SECC 2 FIELD	RGB-A	Diseased	69.50	PCA 1	Diseased	256.739	NDVI	Diseased	0.437
		Healthy	71.56		Healthy	267.825		Healthy	0.434
	MS-G	Diseased	67.40	PCA 2	Diseased	-96.202	GNDVI	Diseased	0.350
		Healthy	71.62		Healthy	-97.860		Healthy	0.341
	RGB-V	Diseased	138.14	PCA 3	Diseased	47.385	NDRE	Diseased	0.056
		Healthy	142.20		Healthy	48.188		Healthy	0.055
	RGB-R	Diseased	113.50	PCA 4	Diseased	13.126	SIPI	Diseased	0.545
		Healthy	117.08		Healthy	13.359		Healthy	0.542
	MS-R	Diseased	53.37	PCA 5	Diseased	13.064			
		Healthy	56.08		Healthy	13.067			
	MS-RE	Diseased	122.41	PCA 6	Diseased	-4.603			
		Healthy	128.21		Healthy	-4.661			
	MS-N	Diseased	136.89	PCA 7	Diseased	-11.164			
		Healthy	142.72		Healthy	-11.311			

^z PCA: Principal components.

Guo *et al.* (2015) demonstrated that leaves infected with different CTV isolates exhibited reflectance differences—particularly in the near-infrared region (760–1,000 nm)—compared to healthy leaves. This finding supports the observation of slight spectral differentiation in that specific band, in contrast to what was recorded in other regions of the spectrum. Regarding vegetation indices, synthesizing information through mathematical ratios can dilute variations relevant to class discrimination. Reis-Pereira *et al.* (2024) noted that traditional indices do not always detect spectral differences associated with early-stage infections. Similarly, Principal Component Analysis (PCA)—by concentrating fractions of the total variance—does not necessarily highlight biological differences between classes; this point is raised by Jiang *et al.* (2018), who propose alternative approaches that are more sensitive to intraspecific spectral variability. In this study, these limitations justify using supervised classifiers that integrate multiple bands, indices, and principal components, rather than relying on a single spectral region for discrimination.

Analysis of reflectance distributions from the original images revealed that most bands showed similar behavior in healthy and diseased plants. In this context, approaches based on the direct inspection of individual variables are limited in their ability to distinguish healthy from diseased plants because they fail to account for interactions among spectral bands or the multivariate structure of the data (Camps-Valls *et al.*, 2013). It has been reported that, even with high-quality spectral information, infections caused by vascular pathogens may not yield clear class contrasts when bands are analyzed independently (Riefole *et al.*, 2021). In this study, the near-infrared band demonstrated more consistent class separation across all three fields, showing relatively higher values in healthy plants compared to the other bands. This behavior suggests that this spectral region holds greater discriminative potential for the early detection of stress or early-stage infections, a finding also documented by Afonso *et al.* (2017) and Lu *et al.* (2018). Similar observations have been reported in grapevine (*Vitis vinifera*) (Junges *et al.*, 2020) and tomato (*Solanum lycopersicum*) (Lu *et al.*, 2018), where spectral differences precede the visual manifestation of symptoms; this reinforces the value of using original bands as inputs for classification models in scenarios characterized by high heterogeneity.

Hard classification. At the Champotón site, the Random Forest (RF) model achieved an overall accuracy of 64%, correctly classifying 75% of the diseased plants (Figure 4A1-A2). The MAXLIKE classifier achieved an accuracy of 56% and detected 69% of the diseased trees. The SVM-RBF classifier achieved an overall accuracy of 71% but correctly identified only 1% of diseased plants, indicating limited capacity to differentiate between classes. The remaining classifiers evaluated failed to identify any diseased plants, classifying all tree crowns as healthy. Consequently, they were excluded from the subsequent stages of the analysis.

In Castamay Section 1, six classifiers were able to distinguish between healthy and diseased plants (Figure 4B1-B2). The RF classifier showed the best performance, with a 65% accuracy rate for the diseased class and 63% for the healthy class, achieving an overall accuracy of 64.1%. It was followed by SVM-RBF, which correctly classified 53% of the diseased plants and 64% of the healthy ones (overall accuracy of 58.5%). The SVM-SIG and SVM-LIN classifiers yielded similar results. SVM-SIG achieved 51% accuracy for diseased plants and 60% for healthy ones (overall accuracy of 56.5%), while SVM-LIN reached 50% for diseased plants and 61% for healthy ones (overall accuracy of 56.6%). MAXLIKE correctly identified 45% of the diseased plants and 68% of the healthy ones

(accuracy of 56.8%). SVM-POLY showed lower performance, correctly classifying 41% of the diseased plants and 71% of the healthy ones, with an overall accuracy of 54.7%. In contrast, the MLP-SIG, MLP-LIN, and MLP-SMX models failed to differentiate between classes, classifying all samples as healthy plants.

In Castamay Section 2, six classifiers were able to distinguish between classes (Figure 4C1-C2). RF was again the most efficient, achieving 69% success for diseased plants and 63% for healthy plants, with an overall accuracy of 65%. SVM-RBF correctly classified 60% of diseased plants and 56% of healthy ones (overall accuracy of 57%). SVM-SIG achieved an accuracy of 53%, with success rates of 52 and 54% for diseased and healthy plants, respectively.

The SVM-LIN model obtained an accuracy of 54%, correctly classifying 50% of diseased plants and 57% of healthy ones. MAXLIKE reached an accuracy of 60%, correctly detecting 36% of diseased plants and 72% of healthy ones. SVM-POLY was the classifier with the lowest accuracy among the models considered useful, achieving 25% accuracy for diseased plants and 62% for healthy ones (overall accuracy of 50%). As in Section 1, the MLP-SIG, MLP-LIN, and MLP-SMX classifiers failed to distinguish between classes, classifying all canopies as healthy.

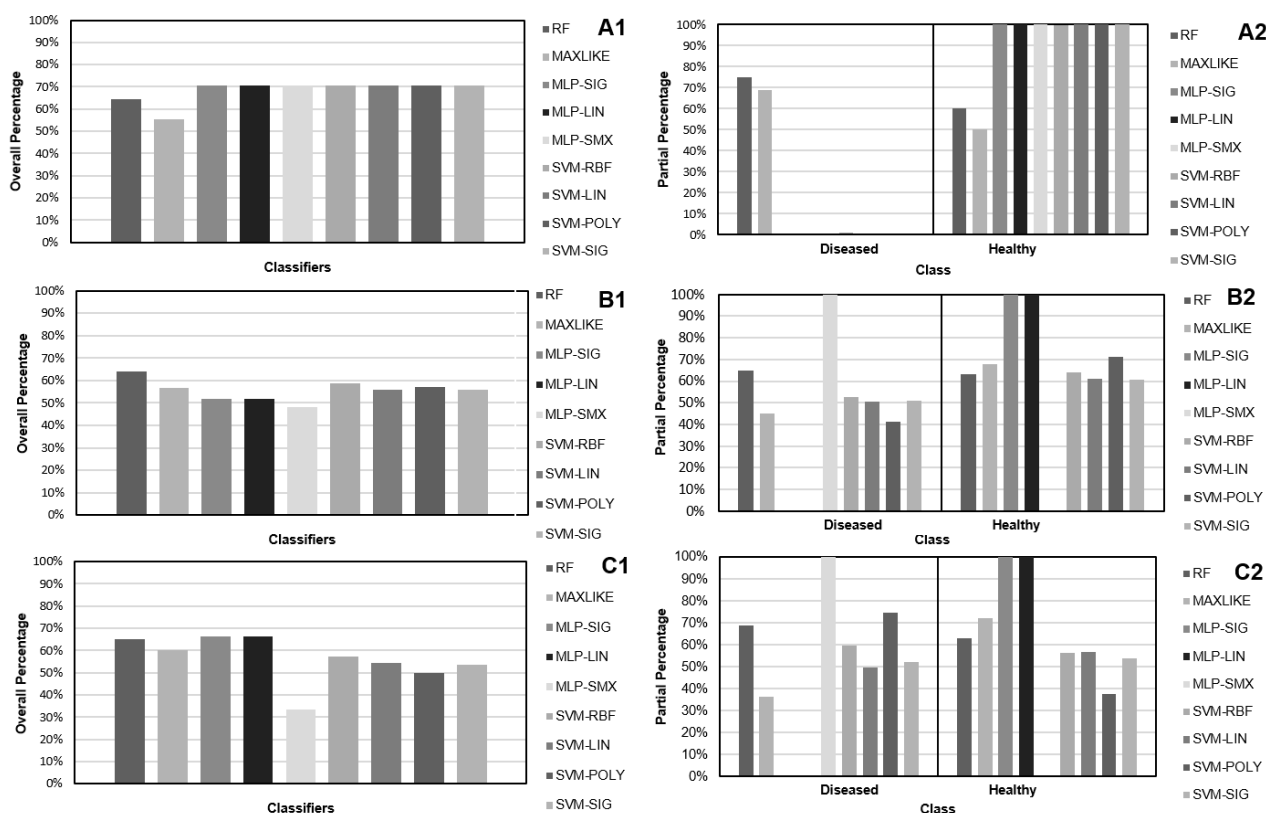


Figure 4. Hard classification for the three crop fields. Champotón field: **A1)** Overall accuracy considering all correctly classified pixels; **A2)** Percentage distribution of pixels by class (healthy and diseased plants). Castamay Section 1: **B1)** Overall accuracy considering all correctly classified pixels; **B2)** Percentage distribution of pixels by class (healthy and diseased plants). Castamay Section 2: **C1)** Overall accuracy considering all correctly classified pixels; **C2)** Percentage distribution of pixels by class (healthy and diseased plants).

Hard classification enables the assignment of a definitive class to each cell, facilitating the generation of thematic maps for precision agriculture and the detection of crop diseases or stress (Guerri *et al.*, 2023). In this study, the performance of hard classifiers varied across fields and algorithms, with Random Forest (RF) proving the most robust.

In Champotón, RF and MAXLIKE were the only methods that adequately distinguished between the classes. The strong performance of RF can be attributed to its ability to handle high dimensionality and noise (Belgiu and Drăguț, 2016; Zhou, 2022). In contrast, although SVM-RBF achieved a high overall accuracy (71%), it correctly classified only 1% of diseased plants, indicating a bias toward the dominant class. This phenomenon is common in imbalanced datasets (Mountrakis *et al.*, 2011). MLP models and other SVM variants classified 100% of the canopies as healthy, indicating limited sensitivity to the subtle spectral differences associated with asymptomatic infections (He and Chen, 2021).

In the Castamay sections, six classifiers distinguished between healthy and diseased plants. RF achieved the highest overall accuracy. These results are consistent with previous agricultural applications (Zhou, 2022; Belgiu and Drăguț, 2016). SVM-RBF, SVM-SIG, SVM-LIN, SVM-POLY, and MAXLIKE showed overall accuracies between 50% and 70%. Although lower than that of RF, these models proved useful when there was greater spectral differentiation between classes (Mountrakis *et al.*, 2011; Guerri *et al.*, 2023). In contrast, the MLP-SIG, MLP-LIN, and MLP-SMX models failed to separate the classes, classifying all crowns as healthy; this aligns with findings by He and Chen (2021) regarding the need for specific adjustments to neural network architecture and training to improve performance in hyperspectral classification.

Soft classification. Based on the results obtained from the hard classification at the Champotón site, the RF, MAXLIKE, and SVM-RBF models were selected for evaluation in the soft classification analysis. These models were the only ones capable of consistently detecting diseased trees, although SVM-RBF's performance was limited. This analysis evaluated the effect of the membership threshold on detection percentages by class (healthy and diseased) and on total error. RF was the best-performing classifier. At a membership threshold of 56%, it correctly identified 50 of the 63 diseased plants with only 16 classification errors, achieving an overall accuracy of 94% (Figure 5C). This result reflects a good balance between sensitivity and accuracy, detecting a high proportion of diseased plants while maintaining a low false-positive rate. MAXLIKE performed best at a membership threshold of 50%, correctly classifying 46 diseased plants while making 83 errors, resulting in an overall accuracy of 69% (Figure 5D).

While overall accuracy increased as the threshold was raised, the ability to detect diseased plants declined sharply, limiting practical application when early detection of infection is required. For SVM-RBF, the best result was obtained with a membership threshold of 29%, correctly classifying 79% of diseased plants and 60% of healthy plants, with an overall accuracy of 65% (Figure 5E). However, when the threshold was raised to 31%, overall accuracy increased to 77%, but the detection of diseased plants fell to 10%, while the correct classification of healthy plants reached 99%.

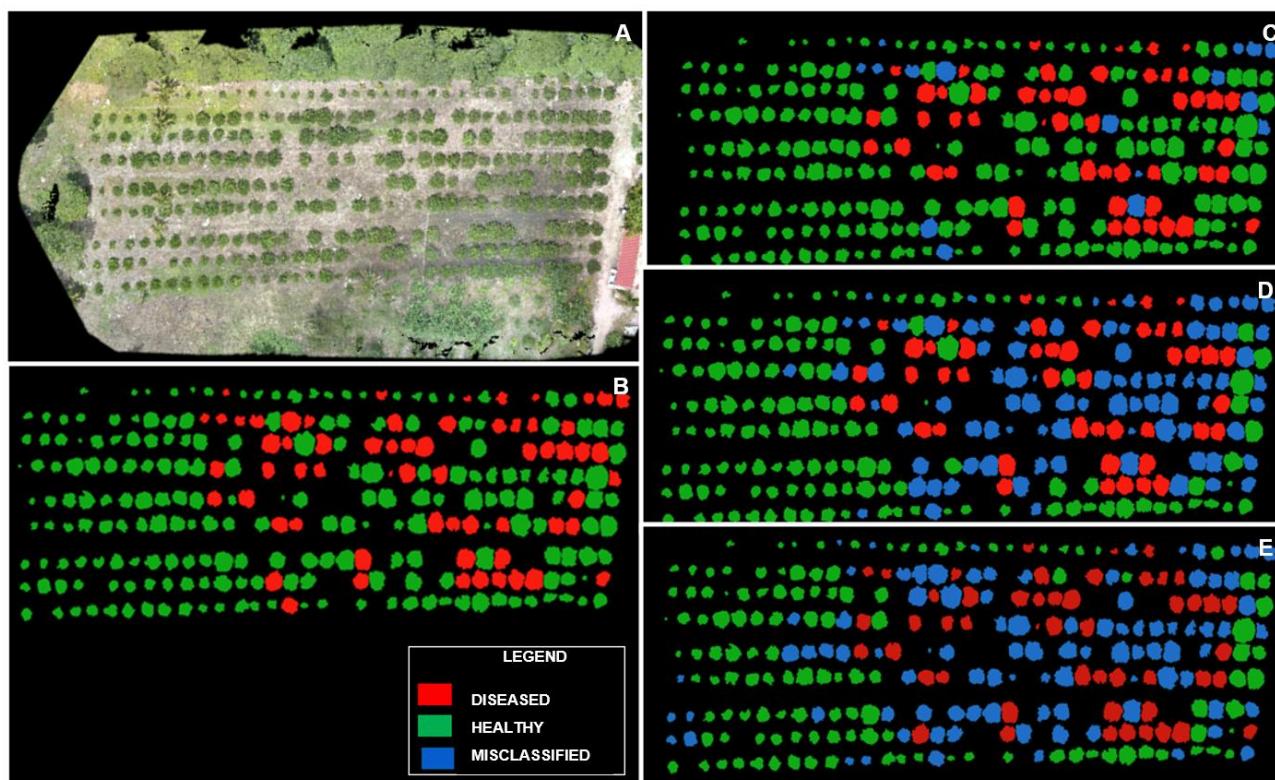


Figure 5. Champotón field in Campeche, Mexico, with a Persian lime crop. Soft classification of the best results on probability scales by algorithm. **A)** RGB drone image of the Persian lime field; **B)** Training site, data obtained from field analyses using immunostrips; **C)** RF with a 56% threshold; **D)** MAXLIKE with a 50% threshold; **E)** SVM-RBF with a 29% threshold.

In Castamay Section 1, the RF model showed a progressive decrease in overall accuracy as the membership threshold increased. At a 50% threshold, it achieved 91% accuracy, with 92% accuracy in identifying diseased plants and 89% in identifying healthy ones (Figure 6C). At a 55% threshold, detection of diseased plants fell to 54%, while detection of healthy plants rose to 100%, and overall accuracy dropped to 77%. Finally, at 60%, only 10% of diseased plants were identified, and overall accuracy fell to 55%, with an overall error rate of 45%. SVM-RBF showed more unstable behavior. At a 50% threshold, it achieved an overall accuracy of 66%, with 63% accuracy in identifying diseased plants and 69% in identifying healthy ones (Figure 6D). Increasing the threshold to 53% lowered accuracy to 61%, with only 33% of diseased plants correctly classified. At a 55% threshold, detection of diseased plants dropped to 8%, although healthy plants were classified correctly in their entirety. The SVM-LIN model achieved an overall accuracy of 58% at a 50% threshold, with 59% correct identification of diseased plants and 57% for healthy ones (Figure 6E). Raising the threshold to 52% improved accuracy to 63%, although the detection rate for diseased plants decreased to 37%.

At a 53% threshold, accuracy dropped to 59%, with only 24% of diseased plants correctly classified. SVM-SIG showed 56% accuracy at the 50% probability level, with 57% of diseased and 55% of healthy plants correctly classified (Figure 6F). Increasing the threshold to 52% improved accuracy to 62%, but the detection rate for diseased plants fell to 37%. At a 53% threshold, accuracy dropped to 53%, with only 13% of diseased plants correctly detected. MAXLIKE, at a 50% threshold, correctly classified 57% of diseased and 63% of healthy plants (overall accuracy of 60%) (Figure 6G). At 51%, it achieved 62% accuracy, with 51% of diseased and 72% of healthy plants correctly classified. Raising the

threshold to 52% reduced the accuracy to 59%, with a 35% detection rate for diseased plants. SVM-POLY achieved 64% accuracy at the 50% level, with 51% diseased and 77% of healthy plants correctly classified (Figure 6H). At a 51% threshold, accuracy dropped to 59%, and at 52%, the detection rate for diseased plants fell to 22%.

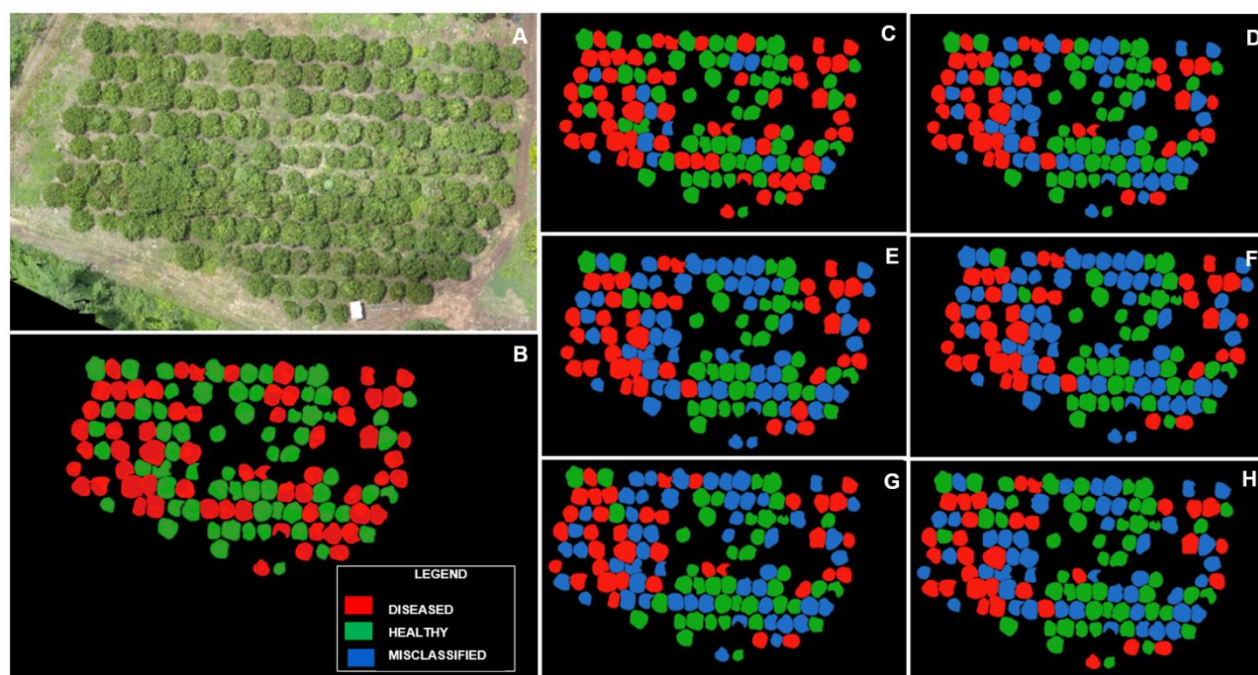


Figure 6. Castamay Section 1 in Campeche, Mexico, with a Persian lime crop. Soft classification of the best results based on the algorithm probability scales. **A)** RGB drone image of the Persian lime crop; **B)** Training site, data obtained from field analyses using immunostrips; **C)** RF with 50% threshold; **D)** SVM-RBF with 50% threshold; **E)** SVM-LIN with 50% threshold; **F)** SVM-SIG with 50% threshold; **G)** MAXLIKE with 50% threshold; **H)** SVM-POLY with 50% threshold.

In Castamay Section 2, the RF classifier demonstrated outstanding performance. At a 50% probability threshold, it achieved 99% accuracy, with 100% of diseased plants and 98% of healthy plants correctly classified (Figure 7C). This performance was maintained at the 55% threshold, with only a slight decrease in the detection of diseased plants (97%). From the 58% threshold onwards, detection of diseased plants dropped to 50%, and at 60%, it fell to 28%, although the classification of healthy plants remained at 100%. SVM-RBF showed less stability. At a 50% threshold, overall accuracy was 74%, with correct classification rates of 84% for diseased plants and 69% for healthy ones (Figure 7D). Raising the threshold to 51% increased accuracy to 76%, although the detection rate for diseased plants dropped to 59%. At a 55% threshold, detection of diseased plants fell to 19%, while healthy plants were classified correctly in all cases. SVM-SIG correctly classified 97% of diseased plants and 15% of healthy ones at a 49% threshold, yielding an overall accuracy of 45%. At 50%, accuracy improved to 51% (56% for diseased and 48% for healthy plants) (Figure 7E), and at 51%, it reached 63%, although detection of diseased plants dropped to 25%. The SVM-LIN classifier achieved 91% and 19% accuracy for diseased and healthy subjects, respectively, at a 49% threshold, resulting in an overall accuracy of 45%. Upon increasing the threshold to 50%, overall accuracy rose to 59% (63% for diseased and 57% for healthy subjects) (Figure 7F). At 51%, overall accuracy was 60%, though detection of diseased subjects dropped to 28%. MAXLIKE achieved 62% overall accuracy (compared to 49%), with 44% of diseased and 72% of healthy subjects correctly classified. At 50%, accuracy increased to 67% (34% for diseased and 87% for healthy

subjects) (Figure 7G). At 51%, accuracy was 66%, although detection of diseased subjects fell to 22%. SVM-POLY showed strong initial performance: at a 50% threshold, it detected 91% of diseased and 56% of healthy subjects (69% overall accuracy). With a 51% threshold, accuracy rose to 74%, with 78% of diseased and 72% of healthy subjects correctly classified (Figure 7H). Increasing the threshold to 52% caused detection of diseased subjects to drop to 53% and detection of healthy subjects to rise to 81%, resulting in an overall accuracy of 71%.

Soft classification allows the assignment of a class membership probability rather than a categorical decision, which is particularly useful in agricultural contexts where symptoms may be subtle or invisible (Foody, 2002). This analysis evaluated the impact of the membership threshold on overall accuracy, total error, and per-class detection rates.

In Champotón, RF was the most efficient classifier, achieving a 79% success rate for diseased plants and a 6% error rate. This behavior has been documented in agricultural applications due to the algorithm's ability to handle non-linear relationships (Belgiu and Drăguț, 2016; Rodríguez-Galiano *et al.*, 2012). MAXLIKE performed best at a 50% membership threshold, detecting 46 diseased plants and achieving an overall accuracy of 69%, albeit with higher error rates. SVM-RBF showed intermediate performance, demonstrating sensitivity to both threshold selection and class imbalance (Mountrakis *et al.*, 2011).

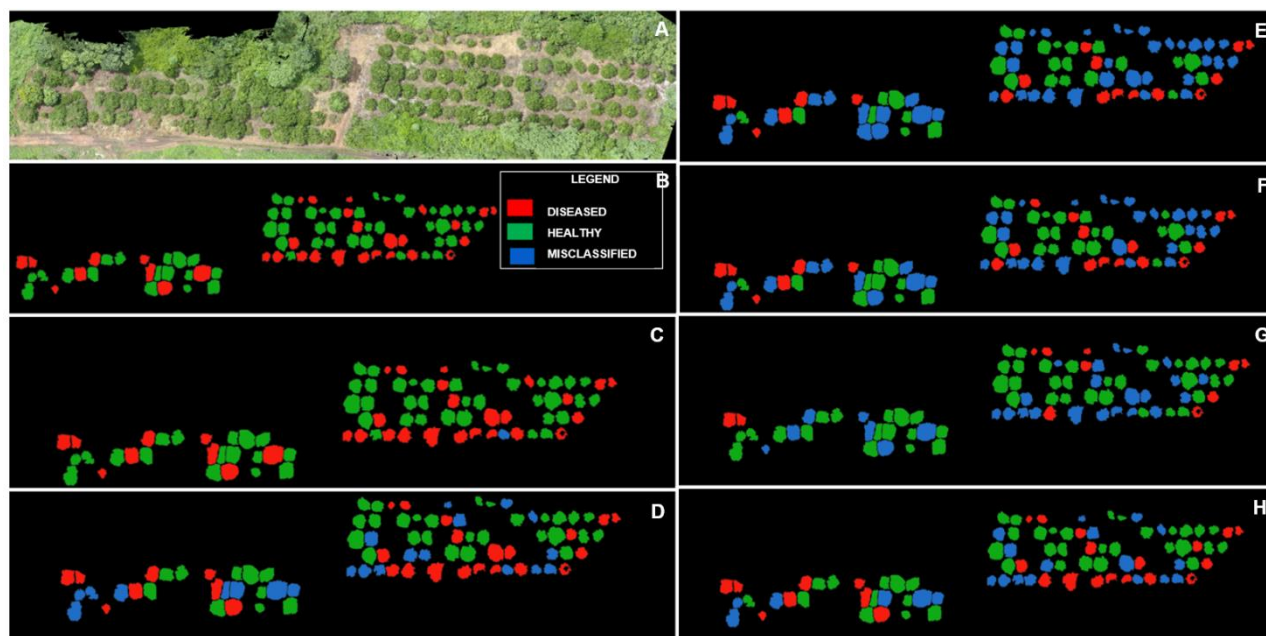


Figure 7. Castamay Section 2, Campeche, Mexico, with a Persian lime crop. Soft classification of the best results based on algorithmic probability scales. **A)** Drone-captured RGB image of the Persian lime field; **B)** Training site, with data obtained from field analyses using immunostrips; **C)** Random Forest results at a 50% membership threshold (MT); **D)** SVM-RBF with 50% MT; **E)** SVM-SIG with 50% MT; **F)** SVM-LIN with 50% MT; **G)** MAXLIKE with 50% MT; **H)** SVM-POLY with 51% MT.

In Castamay Section 1, RF once again achieved the best performance; however, raising the threshold reduced sensitivity to the diseased class, consistent with findings by Kavzoglu *et al.* (2024), who note that RF tends to favor the majority class. This behavior suggests that in plots where infection may be in early stages or exhibit faint spectral signatures, even slight increases in the threshold can hinder the detection of infected trees. In this regard, SVM-RBF and other models showed sharp drops in sensitivity with slight

increases in threshold; for instance, the detection rate for diseased trees using SVM-RBF fell from 63% to 8% when the threshold was raised from 50% to 55%. SVM-POLY achieved 64% overall accuracy at the 50% threshold, while MAXLIKE reached 62% at its optimal threshold.

In Castamay Section 2, RF correctly classified 100% of the diseased trees and 98% of the healthy ones, achieving an overall accuracy of 99% and maintaining stable performance even when the threshold was raised to 55% (97% for diseased trees). This stability aligns with studies highlighting RF's ability to balance sensitivity and specificity in agricultural contexts (Narmilan *et al.*, 2022). From a biological perspective, this behavior may be linked to greater spectral differentiation between healthy and infected trees in this section—possibly due to a more advanced stage of infection or more pronounced physiological stress induced by the pathogen. In contrast, SVM-RBF, SVM-SIG, and SVM-LIN demonstrated low stability; for instance, at a 49% threshold, SVM-SIG achieved a 97% success rate for diseased trees but only 15% for healthy ones, resulting in an overall accuracy of 45%. MAXLIKE and SVM-POLY showed intermediate performance, although SVM-POLY stood out for its greater consistency, maintaining an overall accuracy ranging from 74 to 51%. Overall, the results confirm that RF was the most robust classifier for soft classification, whereas SVM models require careful calibration of the membership threshold to avoid a loss of sensitivity toward the minority class (Belgiu and Drăguț, 2016; Mountrakis *et al.*, 2011).

CONCLUSIONS

The integration of RGB and multispectral imagery acquired via UAV-borne remote sensors, combined with supervised machine learning algorithms, proved a viable strategy for detecting Closterovirus tristeza in Persian lime trees, even under latent infection and in the absence of visible symptoms. The results demonstrate that spectral information, combined with classification methods, enables discrimination between healthy and diseased plants at the individual-tree scale.

Among the evaluated algorithms, Random Forest demonstrated the most robust and stable performance across the various scenarios and fields analyzed—in both hard classification (65%) and soft classification (94%)—confirming its ability to handle non-linear relationships and spectral variability in real agricultural environments. In contrast, other classifiers, particularly those based on SVM, showed greater sensitivity to the choice of membership threshold and to class imbalance, limiting their operational consistency under field conditions.

Soft classification proved to be a particularly useful tool by allowing the sensitivity-specificity trade-off to be adjusted via the membership threshold—a feature relevant to phytosanitary surveillance applications, where the early detection of infected trees is a priority. Overall, these findings support the use of remote sensing and machine learning-based approaches as a practical, scalable alternative for monitoring CTV in commercial citrus orchards, with the potential to assist in field-level pathogen management and control strategies.

Limitations. Sample size, the number of replicates, and environmental variability represent limitations that could be addressed in future studies. Furthermore, this study did not include verification of pathogenicity using Koch's postulates; therefore, the findings should be interpreted as associations.

Conflict of interest. The authors declare that they have no conflicts of interest regarding the research, authorship, and/or publication of this Article.

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