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FLUORESCENCE SPECTRAL INDICES COMBINED WITH MACHINE LEARNING CLASSIFIERS FOR EARLY SCREENING OF COCOA SWOLLEN SHOOT DISEASE

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ABSTRACT

Cocoa swollen shoot disease (CSSD) is a major threat to cocoa production in West Africa, and its early detection remains difficult because current molecular methods are often destructive, costly, and time-consuming. This study investigated whether chlorophyll fluorescence spectral indices could discriminate healthy (SA, $n = 361$), asymptomatic infected (AS, $n = 284$), and symptomatic infected (SY, $n = 161$) cocoa leaves. Fluorescence spectra were acquired from 650 to 800 nm under 490 nm excitation, and 30 spectral indices were calculated from 806 preprocessed spectra. Their discriminatory ability was assessed using the Kruskal–Wallis test with Dunn–Bonferroni post-hoc comparisons, effect sizes, and false discovery rate correction. Nine supervised classifiers were evaluated using repeated stratified 10-fold cross-validation with fold-wise standardization. The mean spectra showed a progressive SA → AS → SY pattern, characterized mainly by increased red fluorescence around 688–691 nm and decreased far-red emission between 720 and 760 nm. All 30 indices significantly differed among classes ($p_{\text{FDR}} < 10^{-11}$), with the strongest effects observed for far-red descriptors, particularly F735_norm ($\epsilon^2 = 0.64$). For three-class classification, the multilayer perceptron achieved $92.5 \pm 0.7\%$ accuracy and $90.9 \pm 0.7\%$ balanced accuracy. In the healthy-versus-asymptomatic task, it achieved an AUC of 0.991 ± 0.002 , with 96.4% sensitivity and specificity. These findings indicate that chlorophyll fluorescence indices, particularly far-red descriptors, can detect pre-symptomatic spectral changes associated with CSSD and may support non-destructive field pre-screening.

Keywords: Cocoa swollen shoot disease; Chlorophyll fluorescence; Spectral indices; Asymptomatic infection; Machine learning; Early detection

1 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is one of the most economically important perennial crops in tropical regions. It provides livelihoods for millions of smallholder farmers and supplies the global chocolate industry [1],[2]. Côte d'Ivoire and Ghana alone account for nearly 60% of global cocoa production, making the sustainability of cocoa farming in West Africa an important issue for both economic development and food security [1-3]. Among the major biotic constraints affecting cocoa, Cocoa Swollen Shoot Disease (CSSD) is considered the most devastating viral disease in the region [4]. The disease is caused by a complex of cacao swollen shoot badnavirus species belonging to the family *Caulimoviridae*, which are transmitted in a semi-persistent manner by several mealybug species [4], [5].

The development of CSSD is associated with a range of symptoms, including vein clearing and banding, leaf chlorosis, swelling of stems and roots, reduced pod formation, and progressive canopy decline. Infected trees generally die within a few years [4], [6], [7]. Depending on the viral strain and the stage of infection, yield losses can exceed 50%. In areas experiencing severe outbreaks, disease management commonly involves the removal of infected trees and their neighbouring trees [6], [7]. CSSD has spread extensively across Ghana, Côte d'Ivoire, and Togo, where considerable molecular diversity among the associated viruses has been reported [8-11].

Early detection is particularly important for CSSD management because infected trees can remain symptomless for several months while continuing to act as virus reservoirs for mealybug-mediated transmission [4], [7], [11]. Diagnosis currently relies mainly on visual inspection, followed, when necessary, by molecular confirmation using polymerase chain reaction (PCR) [10]. Although PCR provides high sensitivity and specificity, its destructive nature, relatively high cost, and time requirements make it difficult to apply for large-scale field surveillance [12], [13]. There is therefore a need for rapid, non-destructive, and high-throughput methods capable of identifying potentially infected trees before visible symptoms develop [12-14].

Optical sensing has attracted increasing interest as a possible alternative because pathogen infection can induce biochemical, structural, and physiological changes that modify the optical properties of plant leaves [12], [13], [15]. Hyperspectral reflectance combined with machine learning has shown considerable promise for detecting plant diseases [14],[15]. However, reflectance measurements are strongly influenced by illumination geometry and primarily provide information related to leaf surface and internal structure [15]. Chlorophyll fluorescence offers a complementary approach because it originates from photosystem II and provides direct information on the functional state of the photosynthetic apparatus [16-18].

Under blue-light excitation, leaves typically exhibit two main chlorophyll fluorescence emission bands, centred at approximately 685–690 nm in the red region and 730–740 nm in the far-red region. Their relative intensities are mainly influenced by chlorophyll content and the resulting re-absorption of red fluorescence, while photochemical activity of photosystem II also contributes to the observed signal [17], [19-22]. Consequently, the red-to-far-red fluorescence ratio and related indices have been widely used as non-destructive indicators of chlorophyll concentration and plant stress [17], [19-23]. Since viral infections can modify chlorophyll content, pigment composition, photosystem II efficiency, and energy dissipation, changes in fluorescence emission may occur before the appearance of visible symptoms [24-27].

Despite this potential, chlorophyll fluorescence has received limited attention in the context of CSSD. Moreover, previous fluorescence-based studies on other plant–pathogen systems have often focused on a single classification algorithm, making it difficult to determine whether the reported performance reflects the information contained in the spectral data or the characteristics of a particular learning method [12], [26], [27]. Spectral indices provide a way to reduce data dimensionality while retaining biologically meaningful information, and they can also be incorporated into relatively low-cost and portable sensing systems [17], [21], [23].

Against this background, the present study aimed to: (i) calculate a comprehensive set of chlorophyll fluorescence spectral indices within the 650–800 nm range using a large dataset of cocoa leaves covering the CSSD infection gradient, from healthy to asymptomatic and symptomatic infection; (ii) assess and rank these indices using a non-parametric framework combining effect sizes, false-discovery-rate control [29], and Dunn's post-hoc pairwise comparisons [30]; (iii) systematically compare nine supervised classification algorithms using repeated stratified cross-validation to determine whether the discriminative information is primarily contained in the indices themselves rather than being dependent on a particular algorithm; and (iv) assess the practically important task of distinguishing healthy from asymptomatic leaves through ROC analysis [33], thereby estimating the potential of fluorescence indices for pre-symptomatic CSSD screening.

2 MATERIALS AND METHODS

2.1 Study sites, plant material and class assignment

The study was carried out at two sites in Côte d'Ivoire: the experimental greenhouse in Bouaflé and the northern campus of the Institut National Polytechnique Félix Houphouët-Boigny (INP-HB) in Yamoussoukro. Bouaflé, the administrative capital of the Marahoué Region, is located in central-western Côte d'Ivoire (6°57'–7°00' N, 5°45'–5°47' W), approximately 59 km west of Yamoussoukro and 306 km north-west of Abidjan. The area is an important cocoa-producing region crossed by the Marahoué River. Yamoussoukro, where the fluorescence measurements were carried out, is located in central Côte d'Ivoire (6°49'15" N, 5°16'34" W).

Young cocoa leaves were obtained from two main sources. Field samples were collected from smallholder cocoa plantations in Doyaokro (6°59'00" N, 5°45'00" W) and Garango (6°57'47" N, 5°48'31" W), both located in the Bouaflé sub-prefecture. Additional symptomatic and asymptomatic leaves were collected from CSSV-infected cocoa plants maintained under controlled conditions in the experimental greenhouse in Bouaflé.

The sanitary status of each source tree was determined by an experienced phytopathologist from the Centre National de Recherche Agronomique (CNRA, Divo). The assessment was based on a visual examination of the entire tree, considering the main features of the CSSD syndrome, including vein clearing and banding, chlorosis, stem and root swellings, and overall canopy condition. No molecular confirmation by PCR was performed. Consequently, the class labels used in this study correspond to the expert-assessed sanitary status of the source trees rather than to a molecularly confirmed infection status.

Based on this assessment, three leaf categories were considered:

- SA (healthy): leaves collected from trees assessed as healthy by the CNRA expert.
- AS (asymptomatic infected): leaves showing no visible symptoms but collected from trees assessed as infected with CSSV.
- SY (symptomatic infected): leaves displaying typical CSSD symptoms, including vein clearing, chlorosis, and leaf deformation, collected from infected trees.

Leaves showing damage caused by insects or environmental factors were not included in the present analysis. These samples had initially been considered as a fourth class in a preliminary four-class analysis. However, their fluorescence responses were highly heterogeneous and lacked sufficient specificity, as the measured signal appeared to depend largely on the proportion of damaged tissue rather than on a consistent systemic physiological response. Since the objective of the present study was to characterise the fluorescence changes associated specifically with the CSSD infection gradient, this class was excluded from the final analysis.

Immediately after collection, each leaf was placed in a separately labelled zip-lock bag and kept on ice to minimise physiological changes during transportation. The samples were subsequently transferred to the Laboratory of Instrumentation, Image and Spectroscopy (L2IS) at the northern campus of INP-HB, where the fluorescence spectra were recorded.

Following spectral acquisition, a quality-control procedure was applied to remove saturated and excessively noisy spectra. The resulting dataset contained 806 leaves, distributed among the three classes as reported in Table 1.

Table 1: Distribution of the 806 cocoa leaf samples among the three physiological classes.

Class code	Description	Number of leaves	Proportion (%)
SA	Healthy leaves	361	44.8
AS	Asymptomatic leaves from infected trees	284	35.2
SY	Symptomatic leaves from infected trees	161	20.0
Total		806	100

2.2 Fluorescence spectra acquisition and preprocessing

2.2.1 Spectra acquisition

Chlorophyll fluorescence spectra were recorded with a fibre-optic spectrometer (Ocean Optics usb 400) coupled to a blue light-emitting diode (LED) excitation source centred at 490 nm. A high-pass optical filter with a 650 nm cut-off wavelength was placed in front of the detection fibre to remove scattered excitation light, so that only the leaf fluorescence emission was recorded. The acquisition system was controlled by an Arduino Uno board and the SpectraSuite software (Figure 1).

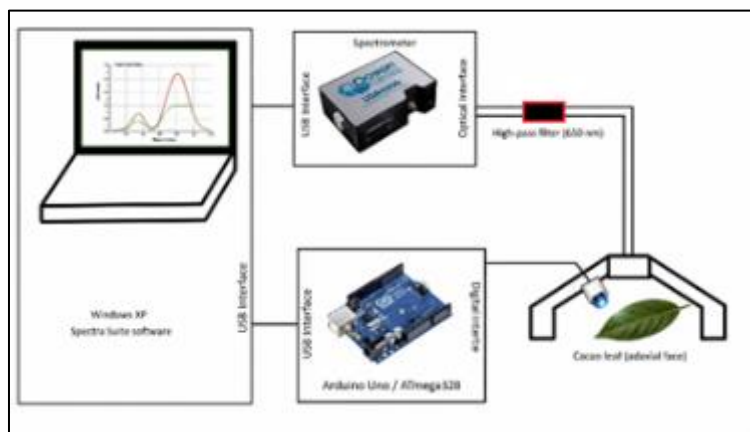


Figure 1: Fluorescence spectra acquisition system.

Measurements were performed on the adaxial surface of each leaf, at a fixed distance and angle, under identical illumination and integration-time settings. Each spectrum stored for a given leaf was the average of four consecutive acquisitions, which reduced fluctuations related to the excitation source and detector response and improved the signal-to-noise ratio. Spectra were exported as intensity–wavelength tables and processed in MATLAB

2.2.2 Spectral preprocessing

The same preprocessing procedure was applied to all spectra, following the sequence required for the subsequent analyses. First, the spectra were restricted to the 650–800 nm range. This spectral window covers the two main chlorophyll *a* fluorescence emission bands associated with photosystem II, namely the red band, with a maximum around 685–690 nm, and the far-red band, centred approximately at 730–740 nm [16], [17]. These regions contain the physiologically relevant fluorescence information, whereas the signal beyond 800 nm is considerably weaker and increasingly affected by detector noise. The wavelengths were arranged in ascending order, and the resulting spectral matrix (806 samples $\times$ *m* wavelengths) was checked to ensure dimensional consistency.

Missing or non-finite intensity values were then identified and replaced by linear interpolation along the wavelength axis. At the boundaries of the selected spectral range, nearest-neighbour extrapolation was used to avoid introducing artificial trends. The spectra were subsequently smoothed using a Savitzky–Golay filter [28] applied along the wavelength axis, with a seven-point window and a second-order polynomial. This approach was preferred to a conventional moving average because it reduces high-frequency instrumental noise while preserving the position, height, and width of the fluorescence emission bands. These characteristics are particularly important for the calculation of peak-related spectral indices. Any residual negative intensity values resulting from noise after smoothing were set to zero.

Since the acquisition geometry, excitation power, and integration time were kept strictly identical for all samples, no amplitude normalization was performed before calculating the spectral indices. The resulting index set therefore included both shape-based descriptors, such as ratios, normalized differences, and area fractions, which are relatively insensitive to overall signal intensity, and amplitude-based descriptors, such as band areas and peak intensities, which retain information related to the absolute fluorescence yield.

For the purpose of comparing the mean spectra of the different classes graphically, an additional normalization was applied only at this stage. Each smoothed spectrum was divided by its integrated intensity over the 650–800 nm range, calculated using the trapezoidal rule. This normalization allowed differences in spectral shape between classes to be visualized independently of variations in overall fluorescence amplitude.

Finally, fluorescence intensities at ten predefined wavelengths (680, 685, 690, 700, 710, 720, 730, 735, 740, and 750 nm) were extracted from each smoothed spectrum using linear interpolation. This ensured that all spectral indices were calculated at the same nominal wavelengths, irrespective of the exact pixel positions of the spectrometer.

2.3 Fluorescence spectral indices

Chlorophyll *a* fluorescence in the 650–800 nm range is characterized by two partially overlapping emission bands associated with photosystem II [16], [17]. Because red fluorescence is partly re-absorbed by chlorophyll within the leaf, whereas far-red fluorescence is much less affected by re-absorption, the relative contribution of these two bands is influenced by both chlorophyll content and the functional state of the photosynthetic apparatus. Accordingly, red-to-far-red ratios and related measures are widely used as indicators of chlorophyll concentration and plant stress [16]–[19], [21].

Thirty spectral indices, grouped into six families, were calculated from each smoothed spectrum (Table 2). These included seven simple band ratios, three normalized difference indices (NDIs), and eight integrated-area descriptors calculated using the trapezoidal rule over three spectral regions: the red region (650–700 nm), the transition region (700–720 nm), and the far-red region (720–800 nm). Six additional descriptors were derived from the fluorescence peaks, including peak intensities, peak wavelengths, and red-to-far-red peak ratios. The red peak was identified within the 670–700 nm range, while the far-red peak was searched within 720–760 nm. Two slope descriptors were also calculated for the 700–740 nm spectral flank, together with four band intensities normalized by the total spectral area.

To avoid numerical problems when a denominator approached zero, a small constant ($\varepsilon = 10^{-12}$) was added to all denominators. The resulting 30 indices were organized into a sample $\times$ index matrix, with each spectrum assigned to its corresponding leaf class. This matrix was then exported for the subsequent statistical analyses and supervised classification.

Table 2: Fluorescence spectral indices computed from the 650–800 nm region of each spectrum (n = 806), with their formulation, physiological significance and supporting references.

Family	Index	Formula	Physiological significance	Reference
Ratios	F685/F735	F_{685}/F_{735}	Chlorophyll content indicator	[18], [19]
	F690/F740	F_{690}/F_{740}	Plant stress indicator	[16], [17]
	F680/F740	F_{680}/F_{740}	Red/far-red balance (short-wavelength flank)	[16]
	F700/F735	F_{700}/F_{735}	Transition/far-red balance	[18]
	F730/F685	F_{730}/F_{685}	Inverse chlorophyll indicator	[19]
	F735/F685	F_{735}/F_{685}	Inverse chlorophyll indicator	[18], [19]
	F740/F690	F_{740}/F_{690}	Photosynthetic efficiency	[16]
NDI	NDI _{685/735}	$(F_{735} - F_{685}) / (F_{735} + F_{685})$	Normalized red/far-red contrast	[18]
	NDI _{690/740}	$(F_{740} - F_{690}) / (F_{740} + F_{690})$	Normalized red/far-red contrast	[18]
	NDI _{700/750}	$(F_{750} - F_{700}) / (F_{750} + F_{700})$	Normalized far-red contrast	[18]
Areas	A_Red	$\int_{650}^{700} F \, d\lambda$	Integrated red emission	[17], [21]
	A_Trans	$\int_{700}^{720} F \, d\lambda$	Integrated transition emission	[17], [21]
	A_FarRed	$\int_{720}^{800} F \, d\lambda$	Integrated far-red emission	[17], [21]
	A_Total	$\int_{650}^{800} F \, d\lambda$	Total emitted fluorescence	[17], [21]
	A_Red/A_FarRed	$A_{\text{Red}}/A_{\text{FarRed}}$	Band energy partition	[16]
	A_FarRed/A_Red	$A_{\text{FarRed}}/A_{\text{Red}}$	Band energy partition	[16]
	Fraction_Red	$A_{\text{Red}}/A_{\text{Total}}$	Red share of total emission	—
	Fraction_FarRed	$A_{\text{FarRed}}/A_{\text{Total}}$	Far-red share of total emission	—
Peaks	Peak_Red_Intensity	$\max F(\lambda), 670\text{--}700 \text{ nm}$	Red emission maximum	[17]

	Peak_FarRed_Intensity	$\max F(\lambda), 720\text{--}760\text{ nm}$	Far-red emission maximum	[17]
	$\lambda_{\max, \text{Red}}$	$\operatorname{argmax} F(\lambda), 670\text{--}700\text{ nm}$	Red peak position (spectral shift)	[17]
	$\lambda_{\max, \text{FarRed}}$	$\operatorname{argmax} F(\lambda), 720\text{--}760\text{ nm}$	Far-red peak position	[17]
	PeakRatio_Red/FarRed	Peak_Red/Peak_FarRed	Peak-based red/far-red ratio	[16], [19]
	PeakRatio_FarRed/Red	Peak_FarRed/Peak_Red	Peak-based far-red/red ratio	[16], [19]
Slope	Slope _{700–740}	$(F_{740} - F_{700})/40$	Fluorescence gradient	[16]
	RelativeSlope _{700–740}	$\text{Slope}_{700\text{--}740}/F_{700}$	Relative gradient	—
Area-normalized	F685_norm	F_{685}/A_{Total}	Shape-only red intensity	[17]
	F690_norm	F_{690}/A_{Total}	Shape-only red intensity	[16]
	F735_norm	F_{735}/A_{Total}	Shape-only far-red intensity	[16]
	F740_norm	F_{740}/A_{Total}	Shape-only far-red intensity	[16]

2.4 Statistical analysis

Because the distributions of the spectral indices were not assumed to follow a normal distribution and the three classes were not equally represented, differences among classes were evaluated using the non-parametric Kruskal–Wallis test for each index. For each test, the H statistic and corresponding p-value were calculated. The epsilon-squared effect size was then estimated as $\epsilon^2 = (H - k + 1)/(n - k)$, where $k = 3$ represents the number of classes and $n = 806$ the total number of samples. This measure was used to estimate the proportion of rank variance associated with class membership while accounting for sample size. As 30 indices were tested, the resulting p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false-discovery-rate procedure [29]. The indices were subsequently ordered according to decreasing ϵ^2 values.

Some indices are mathematically equivalent because they are exact monotonic transformations of one another. For example, F_{685}/F_{735} , F_{735}/F_{685} , and $NDI_{685/735}$ are directly related and therefore produce identical rank-based statistics. These indices were consequently considered as equivalence groups when interpreting the results, rather than as independent sources of statistical evidence.

For each index, the overall Kruskal–Wallis test was followed by pairwise comparisons between the three classes (SA–AS, SA–SY, and AS–SY) using Dunn’s rank-based procedure with Bonferroni adjustment [30]. Particular emphasis was placed on the SA–AS comparison because the ability to distinguish these two groups is directly relevant to the assessment of pre-symptomatic detection.

In addition, mean spectra were calculated for each class after normalizing individual spectra by their total integrated area. This additional normalization was used only to facilitate comparison of spectral shapes between classes while minimizing the influence of differences in overall fluorescence amplitude.

2.5 Multiclass supervised classification:

Rather than assessing a single learning algorithm, we compared nine supervised classifiers representing the main families of classification methods using the same predictor matrix. The models included linear discriminant analysis (LDA), multinomial logistic regression based on one-versus-all linear models, and support vector machines (SVM) with either a linear or radial-basis-function (RBF) kernel [31]. Both SVM approaches were implemented using an error-correcting output code framework with one-versus-one coding. We also evaluated k-nearest neighbours (k-NN; $k = 7$, Euclidean distance), a single decision tree with a minimum leaf size of 5, and a Random Forest consisting of 200 bagged trees with a minimum leaf size of 3 [32]. The remaining classifiers were naïve Bayes with kernel density estimation and a multilayer perceptron with two hidden layers containing 20 and 10 neurons, respectively. The latter used L2 regularization ($\lambda = 10^{-3}$) and was trained for a maximum of 500 iterations.

Model performance was assessed using stratified 10-fold cross-validation repeated five times with different random partitions. The same partitions were used for all classifiers so that their performance could be compared under identical data splits. Within each fold, the mean and standard deviation used for standardization were calculated exclusively from

the training subset and then applied to the corresponding test subset. This procedure prevented information from the test data from being incorporated into the training process.

Several complementary performance measures were obtained from the cross-validated confusion matrices, including overall accuracy, balanced accuracy (defined as the mean recall across classes), macro-averaged F1-score, and recall and precision for each class. For each classifier, the results are reported as mean $\pm$ standard deviation across the five repetitions.

The relative contribution of individual spectral indices to the classification was further investigated using permutation-based predictor importance from a Random Forest model. This analysis was performed with an ensemble of 300 trees using curvature-based predictor selection and was trained on the complete standardized dataset. The resulting out-of-bag importance measures were used to assess the contribution of each index to the observed class discrimination.

2.6 Early-detection task: healthy versus asymptomatic leaves

Because the main operational objective of CSSD surveillance is to detect infected trees before visible symptoms develop, we performed a dedicated binary classification using only the SA ($n = 361$) and AS ($n = 284$) classes, giving a total of 645 leaves. The same nine classifier families were evaluated using the protocol described above, namely five repetitions of stratified 10-fold cross-validation with standardization performed independently within each fold. For each classifier, overall accuracy, sensitivity (the proportion of AS leaves correctly identified), specificity (the proportion of SA leaves correctly classified), and the area under the receiver-operating-characteristic curve (AUC) were calculated. The AUC was derived from the cross-validated class scores, with AS defined as the positive class [33]. All performance measures are reported as mean $\pm$ standard deviation across the five repetitions.

3 RESULTS AND DISCUSSION

3.1 A continuous spectral gradient from healthy to symptomatic leaves

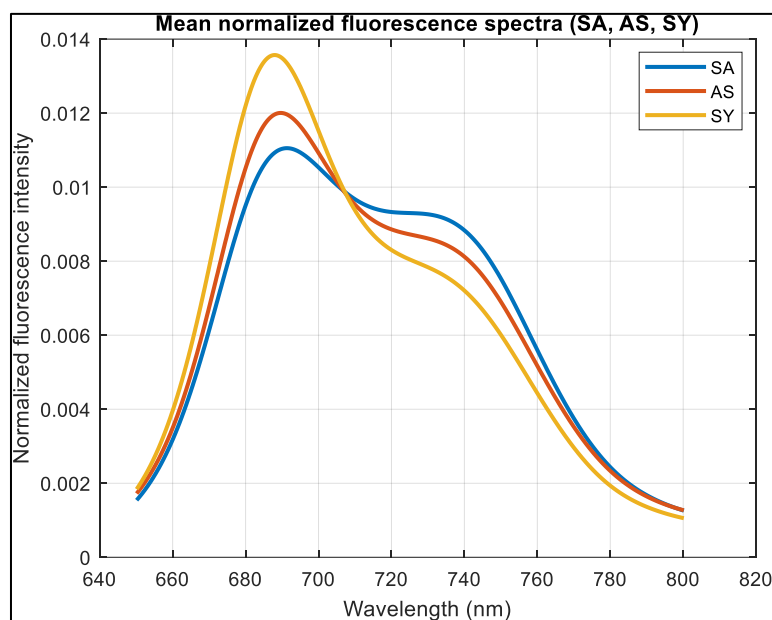


Figure 2: Mean area-normalized fluorescence spectra (650–800 nm) of the three leaf classes (SA, healthy; AS, asymptomatic infected; SY, symptomatic infected).

The area-normalized mean spectra (Figure 2) showed a clear red maximum between 688 and 691 nm, followed by a broad far-red shoulder extending from approximately 720 to 745 nm in all three classes. Rather than forming distinct groups, the spectra followed a continuous progression from SA to AS and then SY. Symptomatic leaves (SY) exhibited the highest red peak (≈ 0.0136 , with a maximum at ≈ 688 nm) together with the lowest far-red emission (≈ 0.0075 at 740 nm). Healthy leaves (SA) showed the opposite pattern, with the lowest red peak (≈ 0.0111 , maximum at ≈ 691 nm) and the highest far-red plateau (≈ 0.0093 between 720 and 735 nm). Asymptomatic leaves (AS) consistently fell between these two classes, with a red peak of approximately 0.0120 at ≈ 689 nm and a far-red intensity of ≈ 0.0085 at 740 nm. The three spectra intersected at approximately 707 nm, indicating opposite changes in the red region (< 707 nm) and the far-red region (> 707 nm). A small blue shift of the red maximum, of about 3 nm, was also observed along the SA-to-SY transition.

This spectral progression is consistent with the well-established effect of decreasing chlorophyll content on leaf fluorescence. Because chlorophyll strongly re-absorbs red fluorescence within the leaf but has a much weaker effect on far-red emission, chlorophyll loss generally results in an increased red-to-far-red ratio and a shift of the red fluorescence maximum towards shorter wavelengths [16-19]. Chlorosis is one of the characteristic symptoms of CSSD [4], making the position of SY at the end of this spectral gradient consistent with the expected progression of the disease. More importantly, the intermediate position of AS suggests that changes in chlorophyll content may already be occurring before visible symptoms become apparent. This interpretation agrees with previous reports showing that viral infections can modify pigment composition and photosystem II function at an early stage of infection, before the onset of visible symptoms [24], [26], [27].

3.2 Statistical ranking of the indices and pairwise separation of the classes

All 30 fluorescence indices showed highly significant differences among the three classes after false-discovery-rate correction ($p_{\text{FDR}} \leq 9.8 \times 10^{-12}$; Table 3). This result confirms that the SA $\rightarrow$ AS $\rightarrow$ SY progression observed in the mean spectra (Figure 2) is also reflected across the full index space. However, the strength of this discrimination varied considerably, with epsilon-squared effect sizes spanning roughly one order of magnitude, from 0.640 to 0.061.

The largest effect sizes were mainly associated with far-red emission and with the balance between red and far-red fluorescence. The area-normalized far-red intensity F735_norm showed the strongest effect ($H = 515.7$; $\epsilon^2 = 0.640$), followed by the transition-band area A_Trans ($\epsilon^2 = 0.632$), the red peak intensity ($\epsilon^2 = 0.631$), and the far-red peak descriptors, including Peak_FarRed_Intensity and the two peak-ratio indices ($\epsilon^2 = 0.629$). The conventional band ratios commonly used in plant stress studies also ranked among the most discriminant descriptors: F730/F685 ($\epsilon^2 = 0.620$), F685/F735 and its monotonic equivalents ($\epsilon^2 = 0.613$), and F690/F740 and its equivalents ($\epsilon^2 = 0.602$) [16-19]. Thus, the eight highest-ranked descriptors all describe, either directly or indirectly, how fluorescence is distributed between the red and far-red regions, a relationship strongly influenced by chlorophyll re-absorption [16], [19]. In contrast, the far-red peak position $\lambda_{\text{max,FarRed}}$ showed a much smaller effect ($\epsilon^2 = 0.196$), while the integrated red-band area A_Red was the least discriminant descriptor ($\epsilon^2 = 0.061$). The class-related variation therefore appears to be concentrated primarily in the far-red region and in the red/far-red balance, whereas the integrated red emission alone changes relatively little between classes.

Two further points are worth noting from Table 3. First, because the acquisition geometry, excitation power, and integration time were kept strictly identical across all samples, the absolute-amplitude descriptors Peak_Red_Intensity ($\epsilon^2 = 0.631$) and A_Total ($\epsilon^2 = 0.575$) were almost as discriminant as the shape-based ratios. This indicates that the overall fluorescence yield contains information related to the infection status, a component that would have been lost if amplitude normalization had been applied before calculating the indices. Second, the red peak position $\lambda_{\text{max,Red}}$ also showed a substantial effect size ($\epsilon^2 = 0.585$). This provides statistical support for the blue shift of the red maximum observed in the mean spectra and is consistent with the reduction in chlorophyll re-absorption associated with declining chlorophyll content [16-19].

Finally, several indices were found to be exact monotonic transformations of one another. For example, F685/F735, F735/F685, and $\text{NDI}_{685/735}$ produced strictly identical rank-based statistics. These equivalence groups, indicated by superscript letters in Table 3, highlight a substantial structural redundancy within the index space. In practical terms, this suggests that most of the discriminant information could be retained using a relatively small set of two to three non-redundant descriptors.

Table 3: Kruskal-Wallis statistics, epsilon-squared effect sizes and FDR-corrected p-values for the 30 fluorescence indices, ranked by decreasing effect size.

Rank	Index	H	ϵ^2	p_{FDR}
1	F735_norm	515.7	0.640	3.1×10^{-111}
2	A_Trans	509.3	0.632	3.7×10^{-110}
3	Peak_Red_Intensity	508.6	0.631	3.7×10^{-110}
4	Peak_FarRed_Intensity ^a	506.8	0.629	4.4×10^{-110}
4	PeakRatio_FarRed/Red ^a	506.8	0.629	4.4×10^{-110}
4	PeakRatio_Red/FarRed ^a	506.8	0.629	4.4×10^{-110}
7	F730/F685	499.9	0.620	1.2×10^{-108}
8	F740_norm	497.0	0.616	4.6×10^{-108}

9	F685/F735 ^b	494.0	0.613	1.5×10^{-107}
9	F735/F685 ^b	494.0	0.613	1.5×10^{-107}
9	NDI _{685/735} ^b	494.0	0.613	1.5×10^{-107}
12	F690/F740 ^c	485.8	0.602	7.1×10^{-106}
12	F740/F690 ^c	485.8	0.602	7.1×10^{-106}
12	NDI _{690/740} ^c	485.8	0.602	7.1×10^{-106}
15	F680/F740	481.5	0.597	5.5×10^{-105}
16	F700/F735	473.8	0.587	2.5×10^{-103}
17	$\lambda_{\text{max,Red}}$	471.5	0.585	7.1×10^{-103}
18	F685_norm	471.4	0.585	7.1×10^{-103}
19	F690_norm	466.0	0.578	1.0×10^{-101}
20	A_Total	463.7	0.575	3.1×10^{-101}
21	Fraction_Red	462.0	0.573	6.8×10^{-101}
22	RelativeSlope ₇₀₀₋₇₄₀	459.8	0.570	2.0×10^{-100}
23	A_FarRed	451.1	0.559	1.4×10^{-98}
24	A_Red/A_FarRed ^d	449.9	0.558	2.4×10^{-98}
24	A_FarRed/A_Red ^d	449.9	0.558	2.4×10^{-98}
26	Slope ₇₀₀₋₇₄₀	444.7	0.551	3.2×10^{-97}
27	Fraction_FarRed	431.2	0.535	2.6×10^{-94}
28	NDI _{700/750}	421.6	0.523	3.0×10^{-92}
29	$\lambda_{\text{max,FarRed}}$	159.1	0.196	2.9×10^{-35}
30	A_Red	50.7	0.061	9.8×10^{-12}

Dunn-Bonferroni post-hoc comparisons (Table 4) established that, for the top-ranked indices, all three pairwise contrasts were highly significant. For the six best descriptors, the operationally critical SA-AS contrast reached $p \leq 5.3 \times 10^{-56}$, the SA-SY contrast $p \leq 2.9 \times 10^{-94}$ and the AS-SY contrast $p \leq 1.6 \times 10^{-11}$. The SA-AS separation therefore does not merely reflect the extreme position of the symptomatic class in the omnibus test, but a genuine spectral difference between healthy and pre-symptomatic leaves.

Table 4: Dunn-Bonferroni post-hoc pairwise p-values for the six top-ranked fluorescence indices.

Index	SA vs AS	SA vs SY	AS vs SY
F735_norm	1.1×10^{-57}	6.5×10^{-96}	4.1×10^{-12}
A_Trans	2.0×10^{-57}	2.0×10^{-94}	1.0×10^{-11}
Peak_Red_Intensity	5.3×10^{-56}	2.9×10^{-95}	1.6×10^{-12}
Peak_FarRed_Intensity ^a	7.2×10^{-57}	3.6×10^{-94}	7.9×10^{-12}
F730/F685	2.8×10^{-56}	8.6×10^{-93}	1.4×10^{-11}
F740_norm	1.0×10^{-54}	3.8×10^{-93}	2.9×10^{-12}

Three aspects of the post-hoc results are particularly noteworthy. First, and most importantly from the perspective of early detection, the SA-AS comparison was significant for all 30 indices, without exception. This remained true even for the least discriminant descriptor, A_Red ($p = 7.8 \times 10^{-11}$). Thus, the pre-symptomatic spectral change is not restricted to a small number of selected indices but is reflected throughout the fluorescence emission. This broad response

supports the interpretation that the observed differences are related to underlying physiological changes rather than to isolated or incidental spectral effects [24], [26], [27].

Second, the three pairwise comparisons showed a remarkably consistent pattern across the index set, with the strongest significance generally observed for SA–SY, followed by SA–AS and then AS–SY (SA–SY $\ll$ SA–AS $\ll$ AS–SY in terms of p-value magnitude). The very small p-values for the SA–SY comparison, reaching approximately 10^{-96} , are expected given the clear separation between the two ends of the infection gradient. More informative is the comparison between the two adjacent stages. For the most discriminant indices, the SA–AS contrast reached $p \approx 10^{-57}$, whereas the corresponding AS–SY contrast was around 10^{-12} . Although asymptomatic leaves occupy an intermediate position in the mean spectra (Figure 2), they are already clearly separated from the healthy baseline and appear spectrally closer to the symptomatic class. This asymmetry is partly influenced by the smaller size of the SY group ($n = 161$), but it nevertheless indicates that a substantial part of the spectral alteration occurs early during infection, before visible symptoms develop. This early shift is particularly relevant for pre-symptomatic screening [4], [7].

Third, only two comparisons among the 30×3 pairwise tests were non-significant, and both involved the AS–SY contrast for the two lowest-ranked indices: A_Red ($p = 1$) and $\lambda_{\max, \text{FarRed}}$ ($p = 1$). These two descriptors can therefore distinguish healthy from infected leaves, but they do not provide sufficient resolution to separate the asymptomatic and symptomatic stages. Their relatively weak omnibus effect sizes in Table 3 ($\epsilon^2 = 0.061$ for A_Red and 0.196 for $\lambda_{\max, \text{FarRed}}$) are consistent with this limitation. Taken together, these results provide independent support for the conclusion that progression from AS to SY is primarily reflected in changes in far-red fluorescence intensity and in the red/far-red balance, rather than in the integrated red emission alone or in the position of the fluorescence peaks.

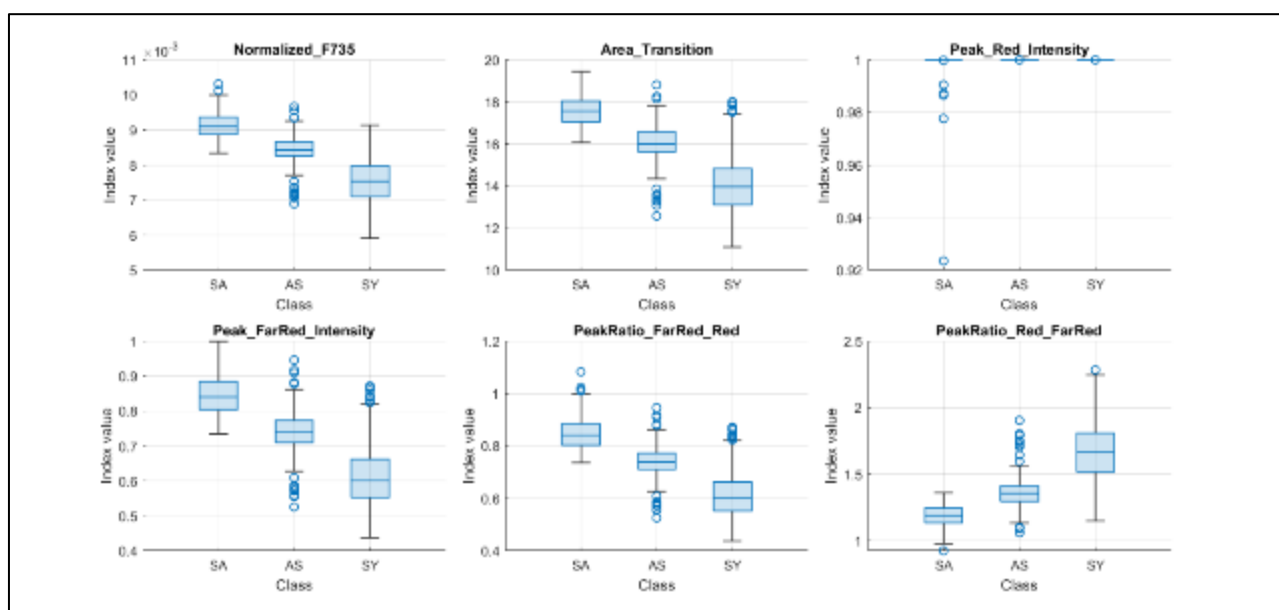


Figure 3: Box plots of the six top-ranked fluorescence indices (F735_norm, A_Trans, Peak_Red_Intensity, Peak_FarRed_Intensity, PeakRatio_FarRed/Red, PeakRatio_Red/FarRed) for the three classes.

The box plots of the six best indices (Figure 3) illustrate the monotonic class gradient, with AS distributions systematically intermediate between SA and SY, in agreement with the post-hoc structure. The AS class exhibited outliers extending towards both the SA and the SY ranges, suggesting a continuum of infection stages within this class, some leaves being still close to the healthy state and others already approaching the symptomatic state a pattern consistent with progressive viral colonisation of the canopy prior to symptom expression [24], [26], [27].

3.3 Comparison of nine classifiers on the three-class problem

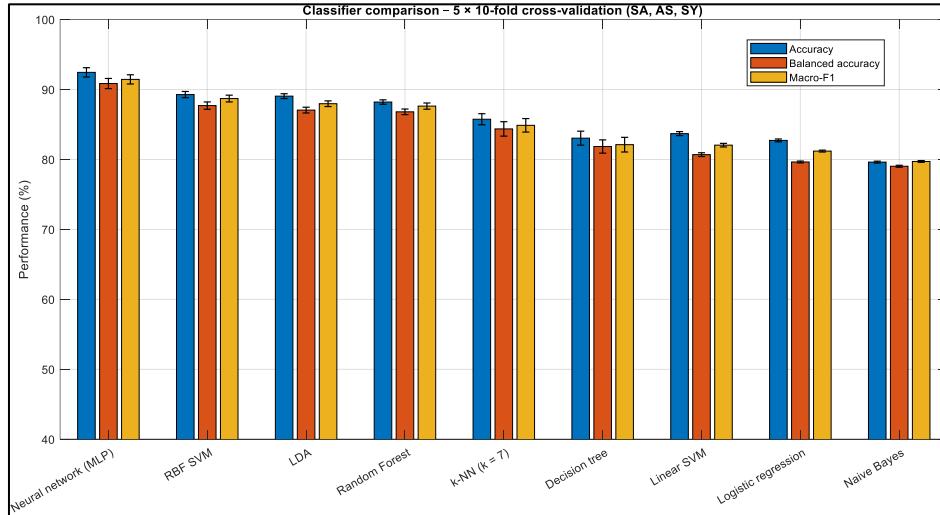


Figure 4: Accuracy, balanced accuracy and macro-F1 (mean $\pm$ SD over 5×10 -fold cross-validation) of the nine classifiers on the three-class problem.

Table 5: Performance of the nine classifiers on the three-class problem (mean $\pm$ SD over five repetitions of stratified 10-fold cross-validation).

Classifier	Accuracy (%)	Balanced accuracy (%)	Macro-F1 (%)	Recall SA (%)	Recall AS (%)	Recall SY (%)
Neural network (MLP)	92.5 $\pm$ 0.7	90.9 $\pm$ 0.7	91.5 $\pm$ 0.7	96.4	92.3	83.9
RBF SVM	89.3 $\pm$ 0.4	87.7 $\pm$ 0.5	88.7 $\pm$ 0.5	95.1	86.1	82.0
LDA	89.1 $\pm$ 0.3	87.1 $\pm$ 0.4	88.0 $\pm$ 0.4	94.7	87.7	78.8
Random Forest	88.2 $\pm$ 0.3	86.8 $\pm$ 0.4	87.6 $\pm$ 0.4	94.7	83.1	82.6
k-NN (k = 7)	85.8 $\pm$ 0.8	84.4 $\pm$ 1.0	84.9 $\pm$ 1.0	93.1	79.3	80.7
Linear SVM	83.7 $\pm$ 0.3	80.7 $\pm$ 0.3	82.1 $\pm$ 0.3	90.6	84.3	67.2
Decision tree	83.1 $\pm$ 1.0	81.9 $\pm$ 0.9	82.1 $\pm$ 1.0	89.0	78.0	78.5
Logistic regression	82.7 $\pm$ 0.2	79.6 $\pm$ 0.1	81.2 $\pm$ 0.1	90.1	82.9	66.0
Naive Bayes	79.6 $\pm$ 0.1	79.0 $\pm$ 0.1	79.7 $\pm$ 0.1			

Across the nine classifiers, overall accuracy ranged from 79.6 % to 92.5 % (Table 5, Figure 4). The MLP achieved the highest accuracy (92.5 $\pm$ 0.7 %), together with a balanced accuracy of 90.9 $\pm$ 0.7 % and a macro-F1 score of 91.5 $\pm$ 0.7 %. It was followed by the RBF SVM (89.3 $\pm$ 0.4 % accuracy) and LDA (89.1 $\pm$ 0.3 %), with a difference of approximately 3 percentage points between the MLP and these two classifiers. The results can be broadly grouped into three performance ranges: MLP, RBF SVM, LDA, and Random Forest yielded accuracies between 88 % and 93 %, k-NN and the decision tree between 83 % and 86 %, while linear SVM, logistic regression, and Naive Bayes remained between 80 % and 84 %.

The small standard deviations across the five repetitions ($\leq 1.05\%$) indicate that the results were relatively stable despite changes in the random partitioning of the data. The performance of the non-linear models, particularly the RBF SVM, Random Forest, and MLP, was generally higher than that of the linear classifiers, suggesting that the class structure is not fully captured by linear decision boundaries in the index space. LDA was the strongest of the linear methods, with an accuracy of 89.1 %, although it remained more than 3 percentage points below the MLP.

The class-specific recalls provide further insight into these differences. The MLP correctly identified 96.4 % of SA leaves, 92.3 % of AS leaves, and 83.9 % of SY leaves. By comparison, logistic regression and linear SVM showed a much larger drop in recall for the SY class, reaching only 66.0 % and 67.2 %, respectively. This pattern suggests that the distinction between asymptomatic and symptomatic leaves is more difficult to represent with a linear decision boundary than the separation of the other classes. Naive Bayes produced the lowest overall accuracy (79.6 %), which may be related to the dependence among the spectral indices and the resulting violation of its conditional-independence assumption.

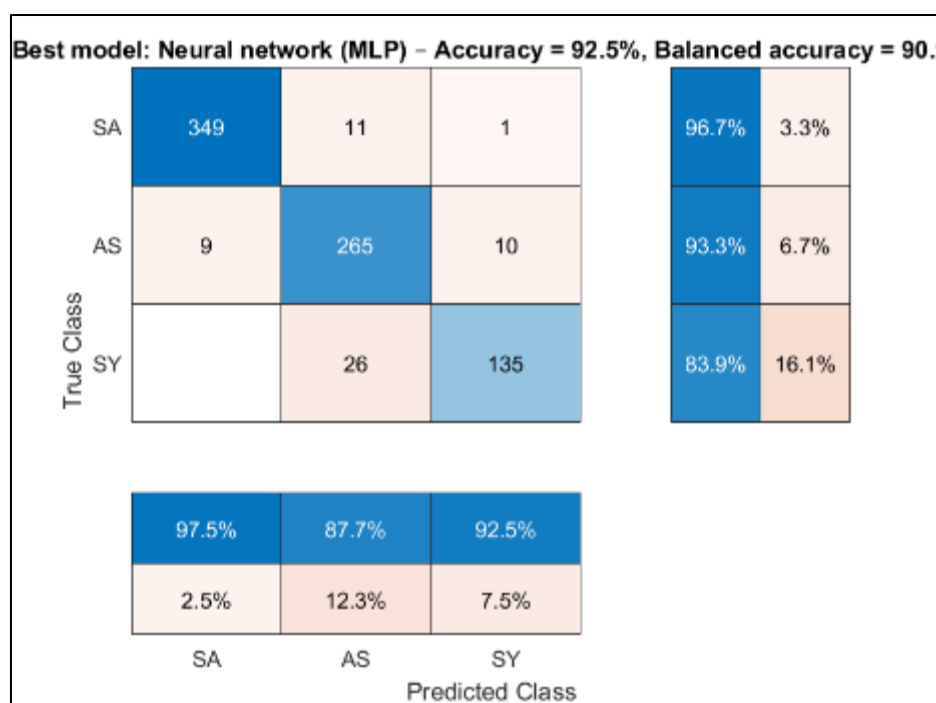


Figure 5: Cross-validated confusion matrix of the best classifier (neural network, MLP), with row-normalized recall and column-normalized precision.

The confusion matrix of the best-performing model (Figure 5) provides further insight into the classification errors. Only one SA sample out of 361 was misclassified as SY, while no SY sample was assigned to the SA class. Most errors occurred between adjacent classes, namely SA and AS (11 + 9 = 20 misclassifications) and AS and SY (10 + 26 = 36 misclassifications). This pattern is also reflected in the class-specific recalls: SA reached 96.7 %, AS 93.3 %, and SY 83.9 %. The lower recall for SY was mainly due to the 26 SY samples (16.1 %) that were classified as AS.

The distribution of errors is consistent with the continuous nature of the spectral changes observed across the SA → AS → SY gradient. Misclassifications were concentrated between neighbouring classes rather than between the two extremes, suggesting that the spectral differences evolve progressively with infection status. In this context, the remaining errors appear to reflect the gradual transition between physiological states rather than a limitation specific to the spectral-index representation. A similar qualitative pattern was observed across the nine classifiers, although with higher error rates (see supplementary confusion matrices). The persistence of this confusion structure across different models indicates that the observed pattern is largely determined by the underlying data rather than by the choice of classifier.

3.4 Identification of the Most Discriminant Indices

The Random Forest permutation analysis (Figure 6; complete ranking available in the file importance_RF_indices_SA_AS_SY.csv) identified Peak_Red_Intensity as the most influential predictor, with an OOB importance of 1.24. It was followed by Normalized_F735 (0.77), PeakRatio_Red_FarRed (0.73), NDI_700_750 (0.69), and Ratio_F690_F740 (0.67). Normalized_F740 (0.67) and Fraction_FarRed (0.67) formed the next group of highly ranked predictors. Despite differences in their formulation, these indices capture a common spectral feature: the distribution of fluorescence between the red and far-red regions. This information is represented either by the absolute fluorescence

intensity around 735 nm (Normalized_F735), by ratios between the red peak near 685 nm and the far-red region around 735–740 nm (PeakRatio_Red_FarRed and Ratio_F690_F740), or by differential measures contrasting the two spectral regions (NDI_700_750 and Fraction_FarRed).

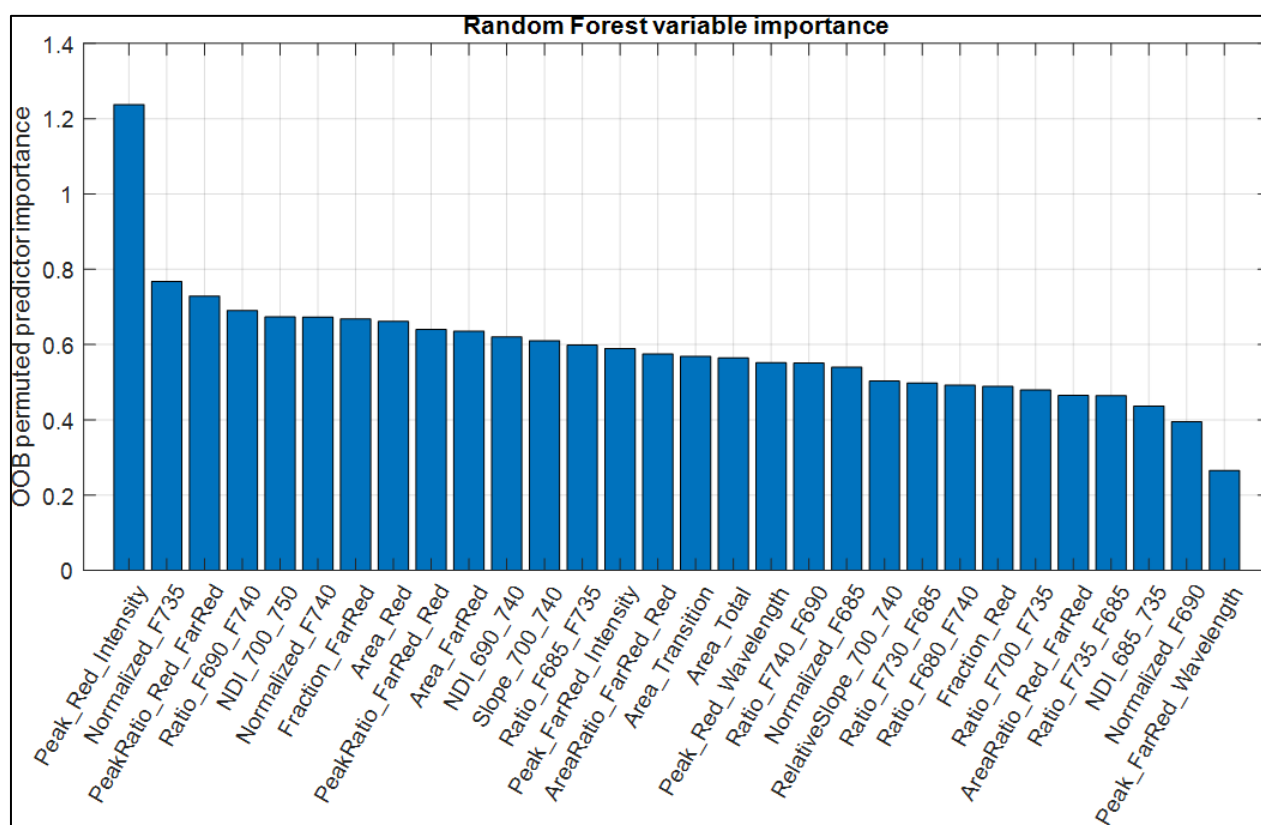


Figure 6: Out-of-bag permuted predictor importance of the Random Forest for the 30 fluorescence indices.

This multivariate ranking is consistent with the univariate ϵ^2 results presented in Table 3, in which F735_norm and the red/far-red ratios also showed the strongest discrimination. The agreement between these two approaches is noteworthy because they capture different aspects of the data: ϵ^2 measures the strength of class differences for each index individually, whereas Random Forest permutation importance evaluates the contribution of each predictor within a multivariate classification model. Their convergence therefore provides additional support for the central role of the far-red/red fluorescence balance in discriminating the SA → AS → SY gradient.

At the lower end of the ranking, Normalized_F690 (0.39) and Peak_FarRed_Wavelength (0.27) contributed relatively little additional information once the more discriminant red/far-red descriptors were included. The broad range of intermediate importance values, with most indices between approximately 0.4 and 0.7, also points to substantial redundancy within the 30-index set. Rather than relying on a single additional predictor, these indices appear to provide overlapping or complementary information that contributes to refining the decision boundaries.

Of particular interest, Normalized_F735 and PeakRatio_Red_FarRed were both among the three highest-ranked descriptors in the univariate ϵ^2 analysis (Table 3) and in the multivariate Random Forest analysis (Figure 6). Their consistent ranking across the two approaches makes them strong candidates for a reduced-band sensing configuration. In principle, a field device measuring fluorescence around 685 and 735 nm could directly derive these indices and retain a substantial part of the discriminatory information captured by the complete 30-index dataset. However, Peak_Red_Intensity was the single most important Random Forest predictor and requires an additional measurement of absolute fluorescence intensity, together with appropriate calibration. This should therefore be considered when designing a simplified field-sensing system.

3.5 Early detection: healthy versus asymptomatic leaves

Table 6: Performance of the nine classifiers on the SA-versus-AS screening task (mean $\pm$ SD over five repetitions of stratified 10-fold cross-validation), ranked by decreasing AUC (positive class: AS).

Model	Accuracy (%)	Sensitivity AS (%)	Specificity SA (%)	AUC
Neural network (MLP)	96.4 $\pm$ 0.6	96.4 $\pm$ 0.8	96.4 $\pm$ 0.7	0.991 $\pm$ 0.002
LDA	93.5 $\pm$ 0.2	91.3 $\pm$ 0.5	95.2 $\pm$ 0.2	0.981 $\pm$ 0.001
Random Forest	91.8 $\pm$ 0.2	88.1 $\pm$ 0.3	94.8 $\pm$ 0.5	0.966 $\pm$ 0.001
RBF SVM	91.8 $\pm$ 0.4	86.6 $\pm$ 0.9	96.0 $\pm$ 0.2	0.954 $\pm$ 0.003
k-NN (k = 7)	89.3 $\pm$ 0.3	84.6 $\pm$ 0.5	93.0 $\pm$ 0.7	0.950 $\pm$ 0.002
Logistic regression	88.4 $\pm$ 0.3	86.4 $\pm$ 0.3	90.0 $\pm$ 0.3	0.948 $\pm$ 0.001
Linear SVM	89.6 $\pm$ 0.4	87.8 $\pm$ 0.7	91.0 $\pm$ 0.4	0.946 $\pm$ 0.001
Decision tree	88.6 $\pm$ 0.5	86.0 $\pm$ 1.4	90.7 $\pm$ 1.1	0.919 $\pm$ 0.012
Naive Bayes	83.2 $\pm$ 0.1	81.5 $\pm$ 0.2	84.4 $\pm$ 0.1	0.909 $\pm$ 0.001

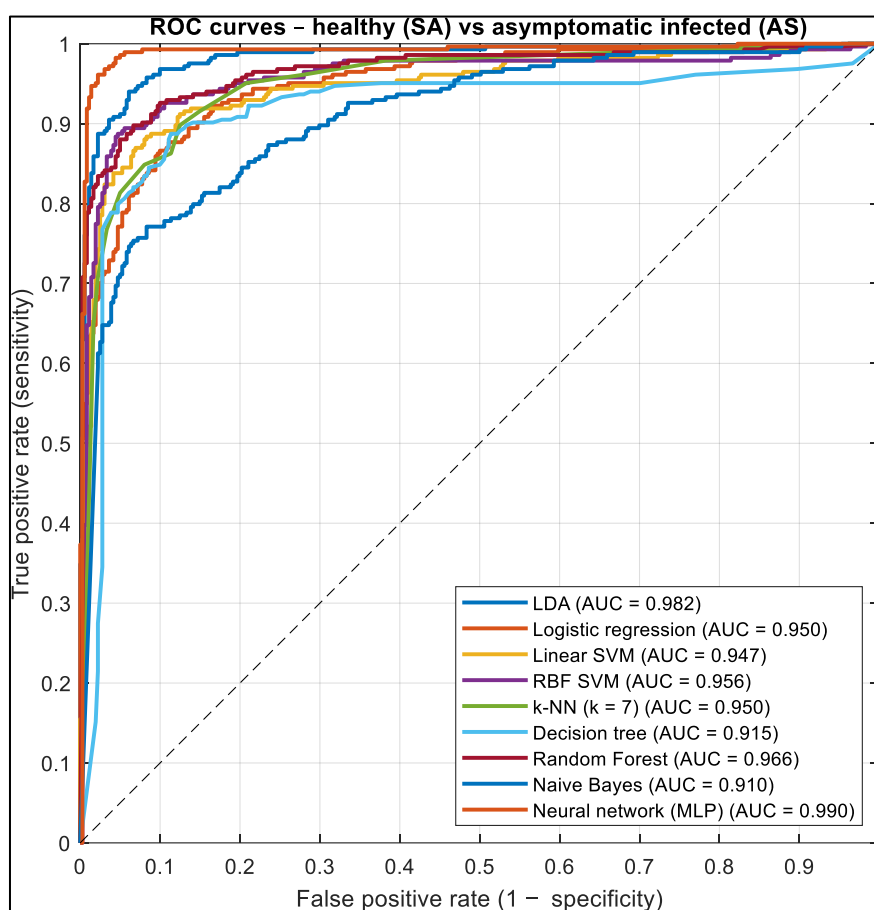


Figure 7: ROC curves of the nine classifiers for the discrimination of healthy (SA) versus asymptomatic infected (AS) leaves (positive class: AS; first cross-validation repetition).

The binary screening task represents the most demanding and operationally relevant classification setting because the SA and AS classes cannot be reliably distinguished by visual inspection alone. Despite this difficulty, all nine classifiers achieved an AUC above 0.90. The multilayer perceptron gave the highest performance, with an AUC of 0.991 ± 0.002 , together with a sensitivity of 96.4 ± 0.8 % for asymptomatic leaves and a specificity of 96.4 ± 0.7 % (Table 6, Figure 7). LDA also performed strongly, with an AUC of 0.981, a sensitivity of 91.3 %, and a specificity of 95.2 %. These results indicate that the fluorescence indices contain substantial information for separating healthy from asymptomatic leaves

and support their potential use as a field pre-screening approach, provided that the classification performance is further validated under operational field conditions.

From an epidemiological perspective, sensitivity to AS leaves is particularly important because asymptomatic infected trees may remain sources of virus for mealybug-mediated transmission [4], [7]. With the MLP, fewer than 4 % of asymptomatic leaves were missed, while fewer than 4 % of healthy leaves were incorrectly flagged. The ROC analysis (Figure 7) also provides flexibility in selecting an operating threshold according to the intended surveillance strategy. For example, in a campaign where minimizing missed infections is prioritized, a higher false-positive rate could be accepted because flagged trees could subsequently undergo confirmatory molecular testing [10]. The appropriate threshold would therefore depend on the relative costs of false negatives, false positives, and confirmatory testing.

The performance observed here falls within, and in some cases exceeds, the range reported for optical detection of viral and other biotic stresses in other pathosystems. Previous studies commonly report accuracies of approximately 70–90 % when asymptomatic stages are included [14], [15], [26], [27]. The present results are particularly relevant because the classification relies on a relatively compact set of interpretable fluorescence indices rather than on a high-dimensional hyperspectral representation. Nevertheless, comparisons across studies should be made cautiously because datasets, disease stages, acquisition conditions, validation strategies, and performance metrics differ substantially between studies.

4 CONCLUSION

This study evaluated 30 chlorophyll fluorescence spectral indices derived from the 650–800 nm region for discriminating healthy, asymptomatic infected, and symptomatic infected cocoa leaves. The analysis was based on 806 spectra and combined a non-parametric statistical framework incorporating effect sizes, false-discovery-rate correction, and Dunn–Bonferroni post-hoc comparisons with a systematic evaluation of nine supervised classifiers. Model performance was assessed using five repetitions of stratified 10-fold cross-validation, with standardization performed within each fold to avoid data leakage.

The three leaf conditions followed a continuous spectral gradient that was mainly expressed through the balance between red and far-red fluorescence. This pattern is consistent with progressive changes in chlorophyll content from healthy to symptomatic leaves. Far-red-related descriptors provided the strongest discrimination, with the area-normalized far-red intensity F735_norm showing the largest effect size ($\epsilon^2 = 0.640$), followed by the far-red/red peak descriptors ($\epsilon^2 \approx 0.63$). The post-hoc analysis further showed that asymptomatic infected leaves were significantly different from healthy leaves across all 30 indices, providing evidence that measurable fluorescence changes occur before the appearance of visible symptoms.

Among the nine classifiers, the multilayer perceptron achieved the highest performance in the three-class problem, with 92.5 % accuracy and 90.9 % balanced accuracy. Seven of the nine classifiers exceeded 82 % accuracy, indicating that the discriminatory information was not restricted to a particular learning algorithm but was consistently present in the fluorescence-index space. This consistency is relevant for the development of compact sensing systems, where a limited number of interpretable spectral features may be preferable to a full-spectrum representation.

In the dedicated healthy-versus-asymptomatic screening task, the multilayer perceptron reached an AUC of 0.991, with 96.4 % sensitivity at 96.4 % specificity. LDA also performed strongly, achieving an AUC of 0.981. These results indicate that chlorophyll fluorescence indices have substantial potential for detecting infection before visible symptom development. In a practical surveillance workflow, such a system could be used for preliminary screening, with flagged trees subsequently subjected to confirmatory molecular diagnosis.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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