



Detection of downy mildew infection in grapevine leaves using field spectroscopy and machine learning

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Abstract

Context Downy mildew, caused by *Plasmopara viticola*, remains one of the most damaging diseases affecting grapevines, especially in humid viticultural regions such as the “Vinhos Verdes” in northern Portugal. Traditional detection relies on visual inspection and laboratory techniques, which are subjective and reactive, often delaying effective intervention.

Aims This study aims to evaluate the potential of field spectroscopy combined with machine learning to detect downy mildew in *Vitis vinifera* cv. Loureiro field conditions. The focus is on providing an early, non-destructive detection method that can be used in precision viticulture, reducing the need for costly, widespread pesticide applications.

Methods and Key Results Leaf spectral reflectance data were collected along the 2023 and 2024 growing seasons using a portable spectroradiometer. Measurements were obtained from both untreated and fungicide-treated grapevines, covering different infection stages. Spectral signatures from 600 grapevine leaves were used to train and validate classification models using Partial Least Squares Linear Discriminant Analysis (PLS-LDA) and Random Forest (RF) models. Both RF and PLS-LDA models showed an overall accuracy of 95.1% when trained with all spectral features from the dataset. Red edge (700–750 nm) and visible (400–700 nm) wavelengths demonstrated the highest classification contribution. Moreover, the twenty most informative wavelengths for infection discrimination were identified for each model.

Conclusion The results confirm the effectiveness of field spectroscopy, making it possible to detect downy mildew symptoms in different stages of infection. This method offers a rapid, cost-effective, and sustainable tool for early disease detection, which can greatly benefit winegrowers by enabling more timely and specific interventions and reducing the reliance on chemical treatments.

Implications and Impacts This study demonstrates a novel approach to precision viticulture, offering winegrowers a rapid, cost-effective, and sustainable tool for early disease detection. The methodology not only promotes more disease management strategies but also aligns with environmental and regulatory goals.

Keywords *Plasmopara viticola* · Spectral signatures · Random forest · Partial least squares discriminant analysis · Disease detection · Precision viticulture

Introduction

Viticulture, in addition to its economic, social, and cultural significance, faces increasingly complex challenges from the intensification of fungal diseases whose dynamics are intensified by climate change and the growing demand for environmental sustainable practices imposed by consumers and regulators (Santos et al., 2020a). Consequently, the development of innovative strategies for vineyard monitoring and phytosanitary management of vineyards has become a central focus of research (Hasanaliyeva et al., 2022). This is particularly relevant for regions such as Northwest Portugal, where the pressure from downy mildew (*Plasmopara viticola*) is severe (Portela et al., 2025b). The region's Atlantic climate, marked by high precipitation and cool temperatures, favors the production of high-quality wines (Santos et al., 2020b). Among the most cultivated grape varieties, Alvarinho and Loureiro are of particular importance in the Lima sub-region. These varieties are distinguished by their high quality, aromatic intensity and freshness of its wines, qualities that define the identity of wines from this region (Oliveira et al., 2004; Vilela et al., 2018).

However, the humid climate and frequent rainfall also create favorable conditions for vineyard diseases, especially downy mildew (Portela et al., 2024). This disease is considered one of the most destructive threats for *Vitis vinifera* L., causing severe losses in yield and grape quality (Koledenkova et al., 2022). The pathogen depends on the presence of water and moderate temperatures for its development, which are common in humid viticultural areas (Portela et al., 2024, 2025b). Initial symptoms include yellowish or light-green oily spots on the upper leaf surface, while the underside displays a whitish layer caused by fungal sporulation (Clippinger et al., 2024). As the disease progresses, necrosis and premature leaf drop occur, reducing photosynthetic capacity and impairing plant growth. In severe infections, grape clusters are also affected, leading to total crop losses (Koledenkova et al., 2022).

Beyond the agronomic impact, controlling downy mildew imposes high costs on winegrowers, as management largely relies on systematic fungicide applications, often in a preventive manner (Hasanaliyeva et al., 2022). This approach requires continuous investment in plant protection products and labor, while also raising environmental and regulatory concerns regarding sustainability and chemical residues in the final product (Velasquez-Camacho et al., 2023). As a result, more efficient, selective, and environmentally responsible protection strategies are increasingly pursued (Viret & Gindro, 2025).

Traditional detection methods used for downy mildew diagnosis relied on visual observation of symptoms on leaves and grape clusters (Khakimov et al., 2022). Typical signs include the oily spots, discoloration, and sporulation on the leaf underside (Clippinger et al., 2024). Spot analyses of field samples have also been used as a diagnostic tool. However, these methods are inherently reactive and limited (Martinelli et al., 2015). On the one hand, there is a heavy dependence on the observer's experience, which can potentially introduce variability and subjectivity into the assessments and can delay interventions. In addition, conventional sampling only covers a limited vineyard area, making early detection of initial outbreaks difficult (Khakimov et al., 2022).

To address these limitations, scientific research has been exploring sensor-based technological approaches for vineyard monitoring (Fang & Ramasamy, 2015; Portela et al., 2024; Sankaran et al., 2010). Among these, remote and proximal sensing are particularly significant, including sensors such as multispectral (Portela et al., 2025b), hyperspectral (Nguyen et al., 2021; Yang et al., 2025), LiDAR (Ferreira et al., 2024; Yang et al., 2025) and thermal infrared data (Portela et al., 2025a). These sensors provide high-resolution data on plant physiological status, often before visible symptoms appear or spread (Gold et al., 2020). For instance, hyperspectral sensors (Oerke et al., 2023), mounted on unmanned aerial vehicles (UAVs) or mobile ground platforms (Cheshkova, 2022), capture reflectance changes in leaves caused by biochemical and structural alterations induced by biotic stress such as *Plasmopara viticola* infection.

Leaf reflectance spectroscopy, in particular, has shown promise for monitoring plant health (Khaled et al., 2018; Vélez et al., 2024). By identifying specific spectral regions that change during infection, it has potential to enable an early and non-destructive detection. This approach has also been applied to detect other plant pathosystems, such as *Flavescence dorée* in grapevines (Al-Saddik et al., 2019), yellow rust in wheat (Huang et al., 2007), *Cercospora* leaf spot, powdery mildew, and rust in sugar beet (Mahlein et al., 2010), and huanglongbing in citrus (Sankaran et al., 2011). These studies demonstrate its potential for improving diagnosis and disease management.

Spectral reflectance between 400 and 2500 nm contains information on both biochemical and structural properties of leaves (Carter & Knapp, 2001). This spectral range can be subdivided into regions with different optical properties (Vélez et al., 2024). The visible spectrum (400–700 nm) is dominated by a pigment absorption, such as chlorophyll (Renzullo et al., 2006). The red edge (700–750 nm) corresponds to the abrupt transition zone between strong red absorption and high near infrared (NIR) reflectance (Cotrozzi, 2022), and is sensitive to chlorophyll concentration, canopy density, and plant stress (Marin-Ortiz et al., 2024). The NIR region (750 to 1100 nm) mainly reflects leaf structural characteristics (Pithan et al., 2021), while the short-wave infrared (SWIR) region (1100 and 2500 nm) provides information on water content (Braga et al., 2021).

In viticulture, multivariate analysis techniques are often used to interpret spectral data. Partial Least Squares Discriminant Analysis (PLS-DA) and Random Forest (RF) have both proven to be effective in grapevine disease detection, as demonstrated in recent studies using hyperspectral imaging and machine learning (Hernández et al., 2021; Lacotte et al., 2022). PLS-DA reduces the dimensionality of spectral data and identifies latent variables that distinguish healthy from infected leaves, making it suitable for datasets with high collinearity and continuous spectral regions (Couture et al., 2018; Zhang et al., 2020). RF, based on ensembles of decision trees, provides a high predictive performance and robustness under varying environmental conditions, making it widely applied in vineyard monitoring and remote sensing (Kanaley et al., 2024; Wei et al., 2021b). Using both approaches allows not only accurate classification but also the identification of the most informative wavelengths, contributing to a more efficient vineyard management (García-Vera et al., 2024).

Portable spectroradiometers are well adapted for vineyard fieldwork, as they provide rapid, non-destructive, and repeated measurements (Vélez et al., 2024). This equipment has been applied to evaluate plant water status (Diago et al., 2018; Tardaguila et al., 2017), nutrient availability (Rodríguez-Pérez et al., 2021), fertilization (Horf et al., 2022; Najdenko et al., 2024; Sinfield et al., 2010), and disease severity such as powdery mildew in vine-

yards (Vélez et al., 2024). Their application to downy mildew detection represents a logical extension, with the benefit of real-time monitoring, avoiding the need laboratory sample processing (Khaled et al., 2018), and throughout the growing season (Imran et al., 2023), a prerequisite for effective monitoring and to implement precise interventions.

Despite recent progresses in sensor technologies and disease-detection algorithms, their validation under real field conditions remains limited, particularly for grape varieties of regional importance such as Loureiro. This study aims to address this gap by evaluating the performance of leaf reflectance spectroscopy combined with multivariate models for the detection of *Plasmopara viticola* in a real field context, contributing to the digital and sustainable transition of regional viticulture. The specific objectives of this study are: the assessment of the feasibility of field spectroscopy for detecting downy mildew symptoms in Loureiro grapevine leaves; to develop and validate partial least squares discriminant analysis (PLS-DA) and random forest (RF) models for the identification of spectral patterns associated with infection; and to identify the informative wavelengths that contribute for the grapevine downy mildew discrimination. By addressing these objectives, this article aims to support early, non-destructive detection of downy mildew and to provide recommendations for more efficient and sustainable vineyard management.

Materials and methods

Study area and experimental design

The study was conducted during the 2023 and 2024 growing seasons in a 2.2-hectare vineyard of *Vitis vinifera* cv. Loureiro, located in the Lima sub-region of the demarcated Vinhos Verdes region, in Ponte de Lima, Viana do Castelo, northwestern Portugal (41°47'34.2"N, 8°32'20.6"W), at an altitude of 50 m (Fig. 1a). This vineyard plot was selected due to its recurrent history of high downy mildew incidence, representing conditions of high disease pressure for the study. Within the vineyard, a specific area was selected for experimental

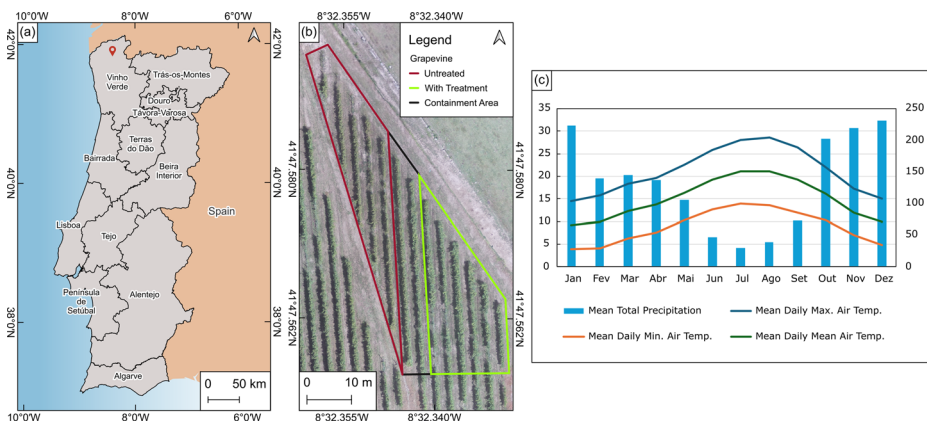


Fig. 1 Location of the study area in mainland Portugal (a); experimental design showing untreated (red) and treated (green) vineyard rows (b); and climate normals from Ponte de Lima for the period 1991–2020 (c), obtained from the Portuguese Institute for Sea and Atmosphere (IPMA, Lisbon, Portugal) (colour figure online)

purposes and divided into two blocks of 0.14 ha each (Fig. 1b). One block received phytosanitary treatments (green polygon in Fig. 1b), while the other remained untreated (red polygon in Fig. 1b). Each block consists of six rows, with the two central rows serving as a buffer zone to minimize interference between treatments.

The Vinhos Verdes region is characterized by a humid climate, classified as warm-summer Mediterranean climate (Csb) according to the Köppen climate classification, with frequent precipitation and high relative humidity (Fig. 1c), conditions that favors the development of *Plasmopara viticola*.

Infection of grapevines and phytosanitary treatments

In untreated rows (Fig. 1b), downy mildew developed naturally throughout the growing seasons. From the first sampling dates (20 July 2023 and 28 June 2024), symptoms intensified, with widespread of oily lesions, necrotic spots, leaf necrosis, canopy reduction, and impaired bunch development at later dates. In the treated rows, fungicides were applied approximately every 15 days, with adjustments based on weather forecasts, such as rainfall, which influences pathogen dissemination (Portela et al., 2025b). To reduce the risk of cross-resistance, active substances were rotated according to regulatory guidelines. Table 1 summarizes the application schedule and active substances used. Both treated and untreated rows were subjected to the same canopy management operations.

Field spectroscopy data acquisition

Spectral data were collected using an ASD FieldSpec® 4 spectroradiometer (Analytical Spectral Devices, Boulder, Colorado, USA), operating from 350 to 2500 nm range (Fig. 2b). Although reflectance values are reported at 1 nm wavelength intervals, this spacing results from an interpolation performed by instrument software. According to the manufacturer specifications, the true spectral resolution is approximately 3 nm in the visible and near-infrared region and 6–10 nm in the shortwave infrared region (SWIR), depending on the wavelength. Measurements were achieved with an internally illuminated contact probe equipped with a built-in tungsten–halogen light source, which provide stable illumination

Table 1 Application dates and active substances used in the phytosanitary treatment block

Application Date	Difference (Days)	Active Substance
6 June 2023	–	Cymoxanil + folpet
12 June 2023	6	Metalaxyl-M + folpet
19 June 2023	7	Cymoxanil + folpet
3 July 2023	14	Copper oxychloride + iprovalicarb
22 July 2023	19	Cymoxanil + folpet
2 August 2023	11	Copper sulfate + calcium
14 August 2023	12	Copper sulfate + calcium
20 June 2024	–	Mandipropamid + zoxamide
27 June 2024	7	Fosetyl + folpet + cymoxanil
05 July 2024	7	Cymoxanil + folpet
12 July 2024	7	Cymoxanil + folpet
26 July 2024	14	Copper hydroxide
16 August 2024	21	Copper hydroxide



Fig. 2 Field spectral data acquisition performed directly on grapevine leaves (a) and spectroradiometer setup (b)

geometry and reduces the influence of ambient light. The spectroradiometer was warmed up for at least 30 min before data acquisition to ensure signal stability.

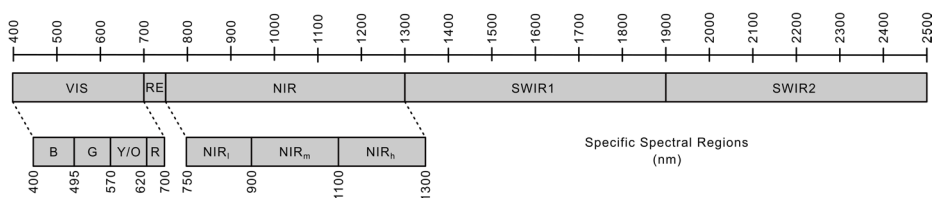
Leaf reflectance was measured in situ by positioning the contact probe perpendicular to the leaf surface and applying consistent pressure to ensure an optical contact without deforming or excessively compress the leaf tissue. An opaque black background was placed beneath the leaf to suppress transmitted light and maintain uniform background conditions. Measurements were taken on flat areas of the leaf blade, avoiding major veins to reduce structural artefacts. Reflectance calibration was performed using a certified white reference panel. A white reference and dark current measurement were recorded at the beginning of each sampling session and repeated when needed to correct for potential instrumental drift. For each leaf, three consecutive spectra were collected and averaged to reduce random noise and improve measurement stability.

Measurements were conducted directly on attached leaves, preserving their natural physiological state (Fig. 2a). Data were collected during over nine field campaigns across two growing seasons (Table 2). In 2023, sampling occurred on 20 and 27 July, and on 3, 8, and 21 August. In 2024, measurements occurred on 28 June, 12 and 29 July, and 12 and 26 August. During most campaigns, 30 symptomatic and 30 asymptomatic leaves were sampled per block, resulting in a total of 600 leaves, evenly distributed between growing seasons and infection classes. The set of averaged spectra for each leaf was used to represent the spectral variability associated with different infection stages, from early oily spots to necrosis.

Leaf phytosanitary status was determined directly in the field through visual inspection performed by experienced researchers trained in grapevine disease identification. Leaves were classified as symptomatic when typical downy mildew symptoms were observed, including oil spots, chlorosis, necrotic lesions, or sporulation on the abaxial leaf surface. Leaves showing no visible signs of infection at the time of assessment were classified as asymptomatic. Sampling was conducted independently at each field campaign, and leaves were not permanently marked. As a result, different leaves were measured at each sampling date, while measurements were consistently performed within the same experimental rows and under comparable phenological and environmental conditions. This approach ensured a representative characterization of disease presence and symptom variability throughout

Table 2 Number of leaves analyzed, spectral readings per leaf, and total readings for each sampling date and treatment, with corresponding BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) phenological stage (Lorenz et al., 1995)

Grow- ing season	Date	Untreated (no. leaves/readings)	Treated (no. leaves/readings)	Total (no. leaves/readings)	BBCH Code
2023	20 July	30 (90)	30 (90)	60 (180)	79
	27 July	30 (90)	30 (90)	60 (180)	80
	3 August	30 (90)	30 (90)	60 (180)	81
	8 August	30 (90)	30 (90)	60 (180)	81
	21 August	30 (90)	30 (90)	60 (180)	83
2024	28 June	30 (90)	30 (90)	60 (180)	79
	12 July	29 (87)	30 (90)	59 (177)	80
	29 July	30 (90)	29 (87)	59 (177)	81
	12 August	30 (90)	31 (93)	61 (183)	81
	26 August	31 (93)	30 (90)	61 (183)	85
	August				

**Fig. 3** Specific spectral regions analyzed, including the visible (VIS), red edge (RE), near infrared (NIR), and short-wave infrared (SWIR1, and SWIR2) domains, as well as the Blue (B), Green (G), Yellow-Orange (Y/O), Red (R), near Infrared low (NIR_l), near infrared medium (NIR_m) and near infrared high (NIR_h) sub-regions (colour figure online)

the growing seasons, while avoiding potential physiological alterations associated with repeated handling or marking.

Data analysis and processing

The spectral signatures (400–2500 nm) acquired in the field were processed using ViewSpectPro software (ASD Inc., Boulder, Colorado, USA) and exported to Excel format for analysis in R (version 4.5.2; R Foundation for Statistical Computing, Vienna, Austria). The response variable was binary (infected vs. non-infected), defined according to the phyto-sanitary status of each leaf. Data import and initial processing were performed using the *readxl*, *dplyr*, *tidyr*, *ggplot2*, and *writextl* R packages.

Spectral data were organized by growing season and sampling date (Table 2) and segmented into predefined spectral regions (Fig. 3): VIS (400–700 nm), RE (700–750 nm), NIR (750–1300 nm), SWIR1 (1300–1900 nm), SWIR2 (1900–2500 nm), VIS_RE (400–750 nm), VIS_RE_NIR (400–1300 nm), RE_NIR (700–1300 nm), R_RE (620–750 nm), Blue (400–495 nm), Green (495–570 nm), Red (620–700 nm), Yellow_Orange (570–620 nm), NIR_l (750–900 nm), NIR_m (900–1100 nm) and NIR_h (1100–1300 nm).

To obtain an independent assessment of model generalization, 30% of all samples were allocated as an isolated external test set using stratified sampling based on growing season (2023 and 2024), sampling date, and infection status. This ensured that both classes and all acquisition periods were represented. The remaining 70% of the data were used as training set for model development.

Three modelling scenarios were considered: a scenario using all data, the training set included samples from the two growing seasons; and scenarios using only samples from 2023 or 2024, where the training set was restricted to samples collected in 2023 or 2024, respectively. For the year-specific scenarios, a leave-one-year-out (LOYO) evaluation was also performed by training models on one growing season and applying them to all samples from the other season.

Both RF and PLS-DA were implemented using dedicated modelling pipelines. RF models were fitted using the *ranger* algorithm through the *caret* package. For each scenario and spectral feature set, RF training was performed repeated cross-validation with ten folds and three repeats, applied only to the training set. The hyperparameters tuned were the number of variables randomly selected at each split (*mtry*), the minimum node size and the number of trees (*ntree*), evaluated over a grid of values of 100, 300, and 500. Model training used class probabilities and the “Accuracy” metric to compare configurations. Cross-validated accuracy was used to select the best hyperparameter combination. A final RF model was then refitted on the complete training set with the selected hyperparameters and applied to the independent external test set. For the LOYO evaluation, the same repeated cross-validation procedure was applied using training samples from one year and all samples from the other season as test set.

PLS-DA models were implemented in two steps using the *pls* and *MASS* R packages, for PLS and linear discriminant analysis (LDA), respectively. PLS regression was fitted to the spectral matrix and a numeric response (1 = infected, 0 = non-infected). For each scenario (all data or data from a single growing season) and feature set (spectral regions), repeated cross-validation (ten folds, three repeats) was performed on the training set. For each resample, a PLS model with a maximum number of components was trained, and the optimal number of latent variables was selected based on the minimum root mean square error of prediction (RMSEP). The cross-validation also evaluated two additional hyperparameters: predictor scaling (enabled or disabled) and the LDA tolerance parameter (1×10^{-4} or 1×10^{-5}). Latent scores from the selected PLS model were computed, and components with insufficient variance in either class were removed. LDA was then fitted to the retained scores and evaluated on the validation subset. The configuration with the highest cross-validated accuracy was selected as the global best parameters. A final PLS-LDA model was then fitted on the full training set using these parameters and applied to the external test set and, for year-specific training, to the LOYO test samples.

Model performance was evaluated using metrics derived from the confusion matrix (Table 3), including overall accuracy, Cohen’s Kappa, and class-specific metrics Precision, Recall, F1-scores for infected and non-infected leaves. These metrics were computed from the true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) obtained from the external or LOYO test sets. Feature importance was also quantified for each model. For RF, variable importance was obtained with the *varImp* function from *caret*, based on impurity reduction. The resulting importance values were normalized so that their sum equaled 1 and were used to rank wavelengths by relative importance. For PLS-LDA,

Table 3 Performance metrics used for evaluating the classification models. TP: true positives; TN: true negatives; FP: false positives; FN: false negatives; p_o = observed agreement; p_e = expected agreement by chance

Metric	Definition	Formula
Accuracy	Proportion of correctly classified samples	$\frac{TP+TN}{TP+TN+FP+FN}$
Precision (infected)	Correctly predicted infected leaves among all predicted infected leaves	$\frac{TP}{TP+FP}$
Recall (infected)	Correctly identified infected leaves among all truly infected leaves	$\frac{TP}{TP+FN}$
F1-score (infected)	Harmonic mean of Precision and Recall for infected leaves	$2 \left(\frac{Precision_{inf} \times Recall_{inf}}{Precision_{inf} + Recall_{inf}} \right)$
Precision (not infected)	Correctly predicted non-infected leaves among all predicted non-infected leaves	$\frac{TN}{TN+FP}$
Recall (not infected)	Correctly identified non-infected leaves among all truly non-infected leaves	$\frac{TN}{TN+FN}$
F1-score (not infected)	Harmonic mean of Precision and Recall for non-infected leaves	$2 \left(\frac{Precision_{noninf} \times Recall_{noninf}}{Precision_{noninf} + Recall_{noninf}} \right)$
Cohen's Kappa	Agreement between predictions and reference, corrected for chance	$\frac{p_o - p_e}{1 - p_e}$

variable importance was assessed using variable importance in projection (VIP) scores computed from the PLS loading weights and loadings. For both RF and PLS-LDA, the 20 most relevant wavelengths were identified. All trained models, performance tables and wavelength importance rankings were exported in Excel format using *writextl*.

Results

Data characterization

This subsection presents the obtained mean spectral signatures and their corresponding variability for untreated and treated leaves across the analyzed part of electromagnetic spectrum (400–2500 nm). The data are shown separately for each growing season (2023 and 2024) and by sampling date. The results are structured according to main spectral regions analyzed in this study: VIS (400–700 nm), RE (700–750 nm), NIR (750–1300 nm), SWIR1 (1300–1800 nm), and SWIR2 (1800–2500 nm).

Spectral reflectance analysis

When the complete dataset is considered (Fig. 4), both groups followed similar reflectance in the VIS region, with reflectance increasing from the blue to red and peaking near 700 nm. The variation ranges of untreated and treated leaves overlap in the blue and green regions. In the RE region, reflectance increases and untreated leaves present slightly higher mean values and reduced overlap between groups. In the NIR region, reflectance reaches its highest values. Both groups show parallel and stable values, with a clear separation between mean reflectance signatures. The overlap between variation ranges was limited at shorter wavelengths but increased at longer ones. In SWIR1, reflectance gradually decreased for both groups, with separated mean spectral signatures, but some overlap remained, especially from 1400 to 1600 nm. In SWIR2, reflectance continues to decrease and the mean

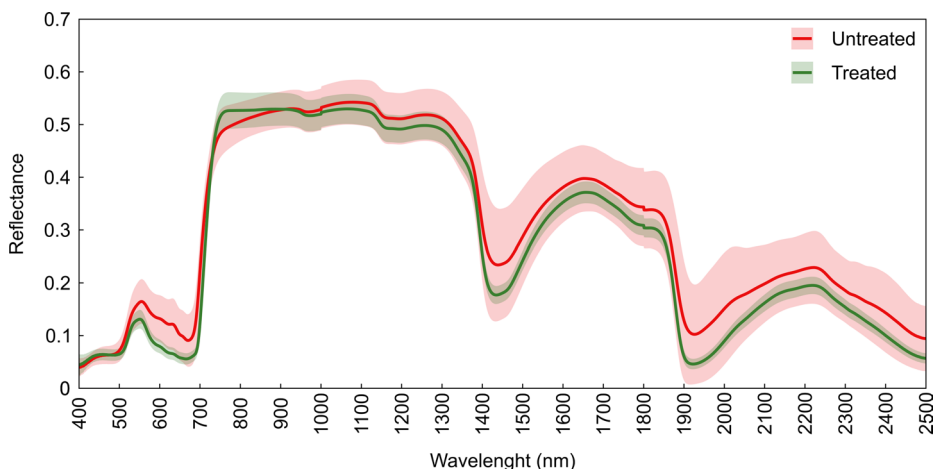


Fig. 4 Mean spectral reflectance of untreated (red) and treated (green) leaves across the 400–2500 nm wavelength range. Shaded areas represent standard deviation (colour figure online)

spectral signatures diverge slightly, although the untreated group displayed broader variation ranges that overlapped with the treated group.

When the spectral are examined by growing season (Fig. 5), different patterns are observed. In 2023 (Fig. 5a), the mean reflectance in the VIS region is similar between groups, with small differences in the blue, green, and red bands. A higher variability was observed in spectral signatures from untreated leaves, particularly around 650 nm. In the RE region, reflectance increases compared to VIS, with a clear separation between groups, more evident in the variation of untreated leaf spectra from 710 to 740 nm. In the NIR region, both groups show high reflectance values and parallel trajectories, with untreated leaves presenting higher mean values (from 850 nm onwards) and greater variation. In SWIR1, mean reflectance values were separated but followed a similar trend. In the SWIR2 interval, reflectance continues to decline and shows a large variability, especially in the untreated group. In 2024, the VIS reflectance of treated and untreated leaves is more similar,

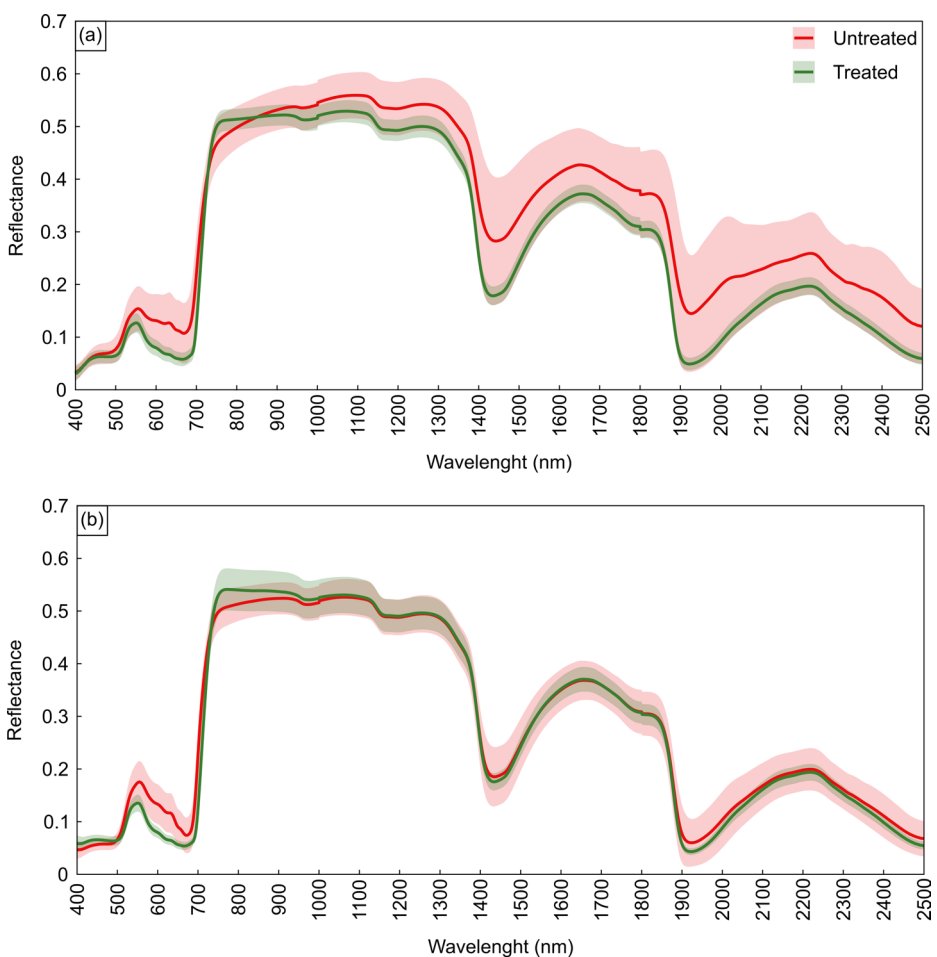


Fig. 5 Mean spectral reflectance of untreated (red) and treated (green) leaves across the 400–2500 nm wavelength range for the 2023 (a) and 2024 (b) growing seasons. Shaded areas represent the standard deviation (colour figure online)

with lower intra-group variability than in 2023 (Fig. 5b). The RE region shows separation between groups but with a reduced overlap. NIR reflectance remains high, with treated leaves showing lower variability. In SWIR1 and SWIR2, the groups diverge, but variability intervals are generally smaller than in 2023.

When analyzed by sampling date, additional temporal patterns emerge. During the 2023 season, on 20 July 2023, VIS reflectance is similar, with greater dispersion in the untreated group. In RE, untreated leaves show higher reflectance. In NIR, both groups follow parallel trajectories, with higher mean reflectance in untreated leaves. In SWIR1 and SWIR2, the variability range of untreated leaves overlaps the treated leaves. On 27 July 2023, VIS reflectance remains similar. In RE, a separation in the mean values is observable, with higher reflectance in untreated leaves. In the NIR region the values present a moderate variability. In SWIR1 and SWIR2, reflectance decreases in both groups, but a separation between the mean values is observed. On 3, 8, and 21 August 2023, VIS reflectance is similar, diverging mainly in the red wavelengths, while RE reflectance shows a clear separation, with untreated leaves generally presenting a higher mean reflectance. In NIR, untreated leaves show an upward shift towards longer wavelengths. In SWIR1, mean reflectance values diverge, whereas in SWIR2, differences are smaller but variability is higher in untreated leaves.

For the 2024 season, on 28 June, VIS reflectance is higher in untreated leaves in the green (495–570 nm) and red (620–750 nm) regions, with a broader amplitude. The RE region shows a lower reflectance for treated leaves. In NIR, both groups follow parallel trajectories, with lower dispersion for treated leaves. In SWIR1, mean reflectance values show a moderate separation, and in SWIR2, separation is smaller, although untreated leaves show more variability. On 12 July, the VIS pattern is similar, with increasing variability in the blue part (400–495 nm). The RE region maintains higher mean reflectance for untreated leaves. In NIR, reflectance are separated, with treated leaves showing lower values and less variability. In SWIR1, separation is also observed and treated leaves remain more stable, while in SWIR2, differences between group means are reduced. On 29 July, VIS reflectance largely overlaps. In RE, untreated leaves show higher mean reflectance. In NIR, both groups follow parallel patterns, with greater variability in untreated leaves. In SWIR1 and SWIR2, separation is noticeable, with slightly higher variation in untreated leaves. On 12 August, VIS reflectance follows the same pattern, with lower variability for untreated leaves. In RE, treated leaves show lower mean reflectance values and reduced variability. In NIR, separation between groups is consistent, with treated leaves showing an overall lower reflectance. In SWIR1, mean reflectance separation continues, while in SWIR2 treated leaves show less variability. On 23 August, VIS and RE reflectance patterns are similar to previous dates. In NIR, treated leaves show a higher mean reflectance and variation. In SWIR1 and SWIR2, variability increases for treated leaves compared with earlier campaigns, while untreated leaves maintain higher variability.

Spectral signature at different downy mildew infection stages

Downy mildew infection in grapevine leaves progresses through distinct stages, beginning with the appearance of oil spots, followed by the development of initial necrotic spots, advanced necrosis, and finally, complete necrosis of the affected tissue. Figure 6 presents representative images of leaves at these stages together with their corresponding spec-

tral signatures extracted from symptomatic areas. The spectral response of a healthy leaf (Fig. 6a) shows the characteristic features of functional plant tissue. In the VIS region, reflectance is low in the blue and red regions with a peak in the green part. The RE region displays the typical sharp increase in reflectance associated with intact photosynthetic structures. Reflectance in the NIR region is high and stable, followed by a gradual decline in the SWIR region.

As the downy mildew infection progresses, progressive modifications are observed in the VIS, RE, and NIR regions. In the early infection stages, such as the oil spot stage (Fig. 6b), reflectance increases in the green and red wavelengths. At the initial necrotic oil spots stage (Fig. 6c), the transition between green and red bands begins to flatten and changes appear in the NIR region. Changes are most pronounced in the RE region, where the typical steep increase in reflectance becomes attenuated. In the advanced necrotic stage (Fig. 6d), changes become more evident. Reflectance in the green part decreases while the red part increases. The RE transition flattens further and the NIR region shows a reduction in reflectance. In fully necrotic tissue (Fig. 6e), the spectral signature differs from the healthy leaf. VIS reflectance is significantly altered, with higher values in the red band and loss of the typical green peak. The RE transition is nearly absent, and the NIR region shows a higher reflectance.

Classification results

Model performance

The overall performance of the PLS-LDA and RF models was evaluated using global classification metrics (Table 4 and Tables 1 and 2 in the supplementary material). The PLS-LDA model achieved an accuracy of 95.1%. For the infected class, the model showed a precision of 0.977 and a recall of 0.923, resulting in an F1-score of 0.949. For the not infected class, a precision of 0.927 and recall was 0.978, with an F1-score of 0.952. These results indicate a balanced performance between classes. The RF model showed a similar behavior, also reaching 95.1% accuracy. For infected leaves, precision was 0.988 and recall was 0.912, resulting in an F1-score of 0.949. For the not infected class, precision was 0.918 and recall was 0.989, producing an F1-score of 0.952. Both models demonstrated similar and stable performance, maintaining high accuracy and balanced predictions for infected and not infected samples.

When models were trained using individual spectral regions and their combinations, the PLS-LDA models showed good performance across several parts. The highest accuracies were obtained for VIS_RE_NIR and RE_NIR (both 96.7%), followed by the general spectrum (95.1%) and NIR (94.0%). High values were also observed for Green (91.2%) and R_RE (91.2%) and in VIS and RE (both 90.7%). Moderate performance was observed for VIS_RE (89.0%) and SWIR1 (89.6%), while lower accuracies were obtained for Red (88.5%), SWIR2 (85.2%), Yellow-Orange (84.6%), and Blue (82.4%). The lowest values were found in the NIR subregions NIR_m (72.5%) and NIR_h (73.6%).

When analyzing models trained by growing season and evaluated in the external dataset, PLS-LDA achieved its best 2023 performance in VIS_RE_NIR (94.5%), followed by the whole spectrum (92.3%) and RE_NIR (91.2%). High results were also observed for Green (91.8%), NIR (90.7%), and Red (89.0%). VIS_RE and R_RE reached 88.5%, VIS reached 86.8%, using Blue lead to 85.2%, and NIR_l 88.5%. Lower accuracies occurred when using

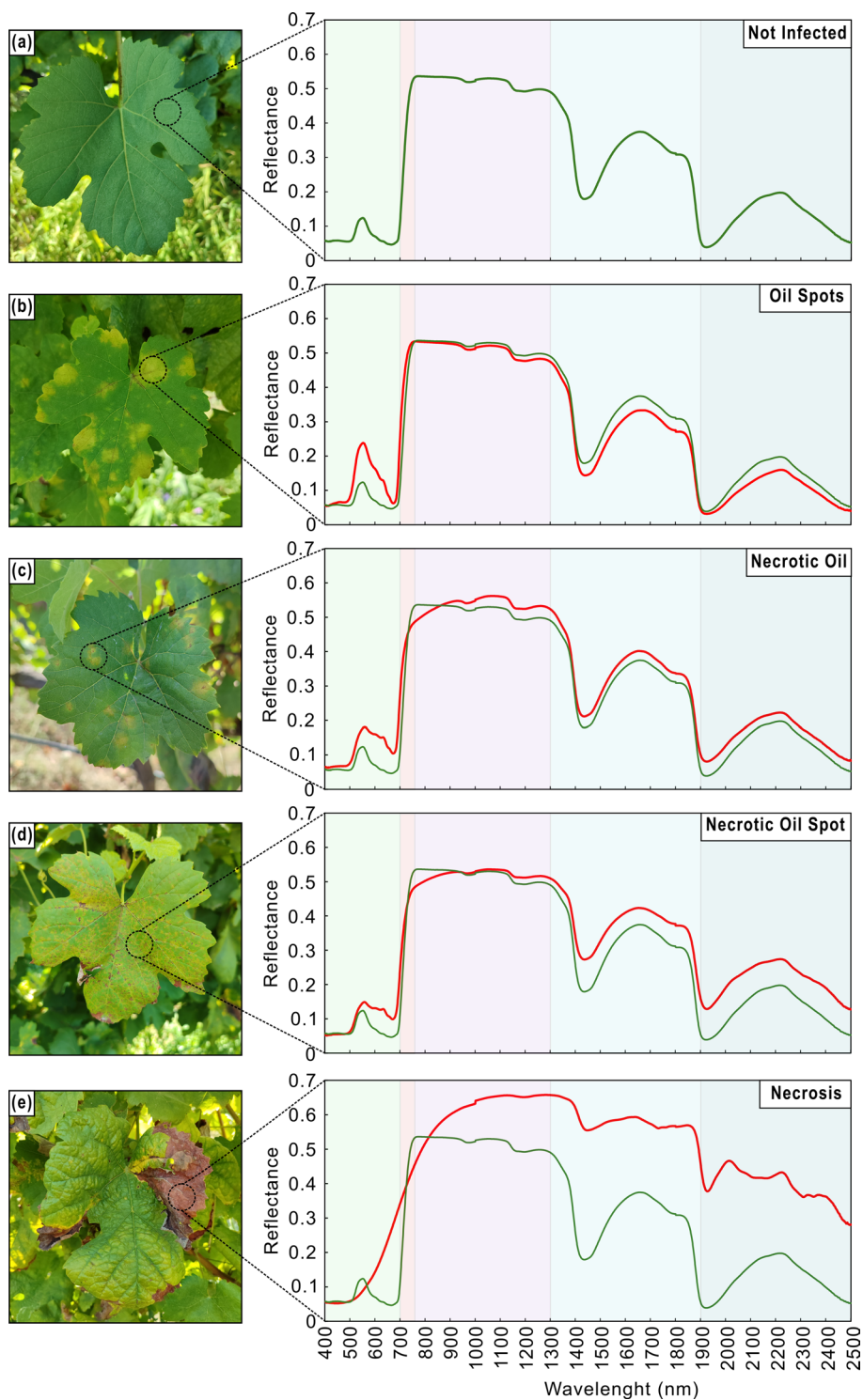


Fig. 6 Photographs and representative spectral signatures of grapevine leaves (cv. Loureiro) at different downy mildew infection stages: healthy (a), oil spots (b), initial necrotic oil spots (c), advanced necrotic oil spots (d), and fully necrotic tissue (e). The green line represents the typical spectral signature of a healthy leaf and the red line the signatures at each infection stage. Background colors indicate the analyzed spectral intervals: visible (400–700 nm), red edge (700–750 nm), near infrared (750–1300 nm), and short-wave infra-red (1300–1800 nm, and 1800–2500 nm)

reflectance data in SWIR1 (75.3%), SWIR2 (78.0%), NIR_m (70.3%), and NIR_h (67.6%). In 2024, the highest accuracies were obtained for NIR_l and RE_NIR (both 92.9%), followed by NIR (91.8%) and VIS, RE, and R_RE (all 91.2%). VIS_RE achieved 89.0% and Green 88.5%, while using the whole spectrum achieved 86.8%. Lower values were obtained for SWIR1 (86.8%), SWIR2 (81.3%), Blue (83.0%), NIR_m (69.8%), and NIR_h (60.4%). These patterns show that PLS-LDA performs more consistently in VIS, RE, and NIR_l related bands, with reduced accuracy in higher NIR sub-regions.

For RF models trained using data from spectral regions and their combinations, strong performance was observed mainly in VIS-related ranges and combined spectral regions. Apart from the overall accuracy obtained using the whole spectrum (95.1%), it was by VIS_RE_NIR and RE (both with 94.0%), and VIS and VIS_RE (both 93.4%). High performance was also observed for R_RE and RE_NIR (both 92.9%) and Red (91.2%). Moderate results were obtained for Blue (87.9%), SWIR2 (82.4%), and SWIR1 (81.9%), whereas lower accuracies were observed for NIR (79.7%) and in the NIR sub-regions NIR_l (79.1%), NIR_m (72.0%), and NIR_h (71.4%).

Table 4 Overall accuracy of partial least squares discriminant linear analysis (PLS-LDA) and random forest (RF) models in detecting downy mildew infection in spectral signatures from grapevine leaves, evaluated for total dataset and separately for the 2023 and 2024 growing seasons. Results presented for typical spectral ranges, spectral combinations, and individual wavelength regions

Spectral range / region (nm)	PLS-LDA (Total)	RF (Total)	PLS-LDA (2023)	RF (2023)	PLS-LDA (2024)	RF (2024)
Typical spectra						
General (400–2500)	0.951	0.951	0.923	0.929	0.868	0.923
VIS (400–700)	0.907	0.934	0.868	0.918	0.912	0.940
RE (700–750)	0.907	0.940	0.907	0.885	0.912	0.896
NIR (750–1300)	0.940	0.797	0.907	0.731	0.918	0.720
SWIR1 (1300–1900)	0.896	0.819	0.753	0.725	0.868	0.791
SWIR2 (1900–2500)	0.852	0.824	0.780	0.742	0.813	0.780
Spectral combinations						
VIS_RE (400–750)	0.890	0.934	0.885	0.923	0.890	0.934
VIS_RE_NIR (400–1300)	0.967	0.940	0.945	0.923	0.885	0.929
RE_NIR (700–1300)	0.967	0.929	0.912	0.907	0.929	0.918
R_RE (620–750)	0.912	0.929	0.885	0.896	0.912	0.923
Spectral sub-regions						
Blue (400–495)	0.824	0.879	0.852	0.769	0.830	0.714
Green (495–570)	0.912	0.907	0.918	0.868	0.885	0.874
Red (620–700)	0.885	0.912	0.890	0.857	0.857	0.901
Yellow-Orange (570–620)	0.846	0.846	0.786	0.835	0.863	0.846
NIR _l (750–900)	0.923	0.791	0.885	0.670	0.929	0.736
NIR _m (900–1100)	0.725	0.720	0.703	0.665	0.698	0.676
NIR _h (1100–1300)	0.736	0.714	0.676	0.643	0.604	0.604

RF models trained with data from 2023, it achieved the highest accuracies when using the whole data (92.9%), VIS_RE and VIS_RE_NIR (both 92.3%) and VIS (91.8%). RE_NIR reached 90.7%, R_RE 89.6%, RE 88.5%, and Red reached 85.7%. Lower performance was observed in Blue (76.9%), SWIR1 (72.5%), SWIR2 (74.2%), and NIR (73.1%). The lowest values were in the NIR sub-regions, particularly NIR_l (67.0%), NIR_m (66.5%), and NIR_h (64.3%). In 2024, RF models maintained high accuracy in VIS (94.0%) and VIS_RE (93.4%), followed by VIS_RE_NIR (92.9%), the whole spectrum and R_RE, both with 92.3%. RE_NIR reached 91.8%, Red (90.1%) and RE 89.6%. Lower accuracies are observed in SWIR1 (79.1%), SWIR2 (78.0%), Blue (71.4%), and NIR (72.0%). As in 2023, the lowest performance was in NIR sub-regions, particularly NIR_l (73.6%), NIR_m (67.6%), and NIR_h (60.4%). The RF models performed well in VIS and RE combinations but showed a reduced effectiveness in the NIR and SWIR regions, especially in higher NIR sub-regions.

To evaluate temporal transferability, the applied LOYO validation (Table 5 and Table 3 in the supplementary material). In this procedure, models were trained using data from one growing season (2023 or 2024) and tested on the independent dataset from the other season. For the PLS-LDA models trained with 2023 data, the highest accuracies were observed for Green (97.7%) and R_RE (97.3%), followed by Red (95.0%) and RE (94.3%). High values were also observed for models trained with all spectral features (92.3%) and VIS_RE_NIR (90.7%). Moderate performance was achieved in VIS_RE (89.7%), RE_NIR (89.0%), VIS (87.7%), and NIR_l (87.7%). Accuracy decreased in NIR (85.7%) and Blue (88.0%), with the lowest values observed in SWIR2 (71.7%), SWIR1 (57.0%), NIR_m (57.3%) and

Table 5 Classification accuracy obtained using the Leave-One-Year-Out (LOYO) validation approach for PLS-LDA and Random Forest (RF) models. Models were trained using data from one year (2023 or 2024) and tested on the independent dataset from the other year. Results are presented for different spectral ranges, spectral combinations, and sub-regions

Spectral range / region (nm)	Training (2023) / Testing (2024)		Training (2024) / Testing (2023)	
	PLS-LDA	RF	PLS-LDA	RF
Typical spectra				
General (400–2500)	0.923	0.903	0.760	0.847
VIS (400–700)	0.877	0.937	0.827	0.880
RE (700–750)	0.943	0.843	0.847	0.820
NIR (750–1300)	0.857	0.550	0.840	0.613
SWIR1 (1300–1900)	0.570	0.637	0.787	0.740
SWIR2 (1900–2500)	0.717	0.690	0.700	0.763
Spectral combinations				
VIS_RE (400–750)	0.897	0.957	0.867	0.873
VIS_RE_NIR (400–1300)	0.907	0.950	0.853	0.853
RE_NIR (700–1300)	0.890	0.860	0.823	0.837
R_RE (620–750)	0.973	0.907	0.850	0.837
Spectral sub-regions				
Blue (400–495)	0.880	0.693	0.843	0.580
Green (495–570)	0.977	0.887	0.797	0.800
Red (620–700)	0.950	0.877	0.783	0.820
Yellow-Orange (570–620)	0.800	0.840	0.763	0.813
NIR _l (750–900)	0.877	0.580	0.867	0.647
NIR _m (900–1100)	0.573	0.467	0.523	0.513
NIR _h (1100–1300)	0.547	0.503	0.517	0.550

NIR_h (54.7%). For models trained with 2024 data and tested on 2023 samples, the overall performance decreased. The highest accuracies were obtained for VIS_RE (86.7%), NIR_l (86.7%), and VIS_RE_NIR (85.3%), followed by R_RE (85.0%) and RE (84.7%). VIS achieved 82.7%, RE_NIR reached 82.3% and Green 79.7%, while using all wavelengths resulted in 76.0%. Lower performance was again in SWIR2 (70.0%) and in the NIR sub-regions, particularly NIR_m (52.3%) and NIR_h (51.7%). These results show a reduction in temporal robustness when models are trained on 2024 and were applied to 2023 data.

For RF models, training with data from 2023 and testing on 2024 samples obtained a high accuracy in VIS_RE (95.7%), VIS_RE_NIR (95.0%), and VIS (93.7%). Good performance was also obtained for Green (88.7%), Red (87.7%), and R_RE (90.7%), while using all spectral features resulted in 90.3%. Model performance decreased in the NIR region (55.0%), SWIR1 (63.7%), and the NIR sub-regions, particularly NIR_l (58.0%), NIR_m (46.7%), and NIR_h (50.3%). When trained on 2024 and tested on 2023, the RF models showed moderate performance. The highest accuracies were observed for VIS (88.0%), VIS_RE (87.3%), and VIS_RE_NIR (85.3%), followed by the dataset using all features (84.7%) and Red and RE (both with 82.0%). Lower performance is registered in SWIR1 (74.0%), SWIR2 (76.3%), and NIR (61.3%), with lower accuracies in the remainder NIR sub-regions.

Both models showed better temporal generalization when trained with 2023 data and tested on samples from 2024. VIS-based spectral regions, and combinations (VIS_RE and VIS_RE_NIR) were among the most stable, whereas the NIR and SWIR regions, showed a more limited temporal transferability.

Main wavelengths contributing for classification

The twenty most informative wavelengths identified in models trained with all wavelengths showed different selection patterns (Table 6). PLS-LDA (Fig. 7a) selected two compact wavelength groups, consisting of bands in the blue region (400–405 nm) and a contiguous set of wavelengths in the RE region from 701 and 714 nm. This pattern shows that the model relied on a combination of short-wavelength VIS signals, which are sensitive to pigment-related changes variations, and RE wavelengths associated with structural and chlorophyll-related changes. In contrast, the RF model (Fig. 7b) selected a broader and more heterogeneous set of wavelengths. Important features appeared in the red region (690–699 nm) and in the RE (701–712 nm), and the model also incorporated wavelengths from the visible spectrum, particularly within the green–red range, including wavelengths around

Table 6 Top 20 most relevant wavelengths are order by importance score for partial least squares discriminant linear analysis (PLS-LDA) and random forest (RF) models in discriminating downy mildew infection in grapevine leaves. Results are presented for the combined dataset, as well as separately for the 2023 and 2024 seasons

Dataset	PLS-LDA (nm)	RF (nm)
Total	400, 402, 401, 403, 404, 708, 707, 709, 706, 710, 705, 711, 704, 405, 712, 703, 713, 702, 714, 701	703, 711, 697, 695, 595, 706, 616, 701, 699, 708, 710, 709, 694, 712, 702, 625, 690, 637, 698, 691
2023	705, 704, 706, 707, 703, 708, 702, 709, 701, 710, 700, 711, 699, 400, 712, 698, 401, 713, 697, 402	702, 705, 703, 696, 701, 704, 695, 697, 699, 706, 693, 694, 698, 700, 707, 690, 692, 708, 709, 691
2024	400, 401, 402, 403, 404, 405, 406, 409, 408, 407, 411, 410, 412, 413, 414, 415, 416, 417, 418, 682	709, 701, 697, 704, 586, 638, 700, 602, 616, 607, 633, 695, 713, 706, 590, 702, 634, 703, 582, 707

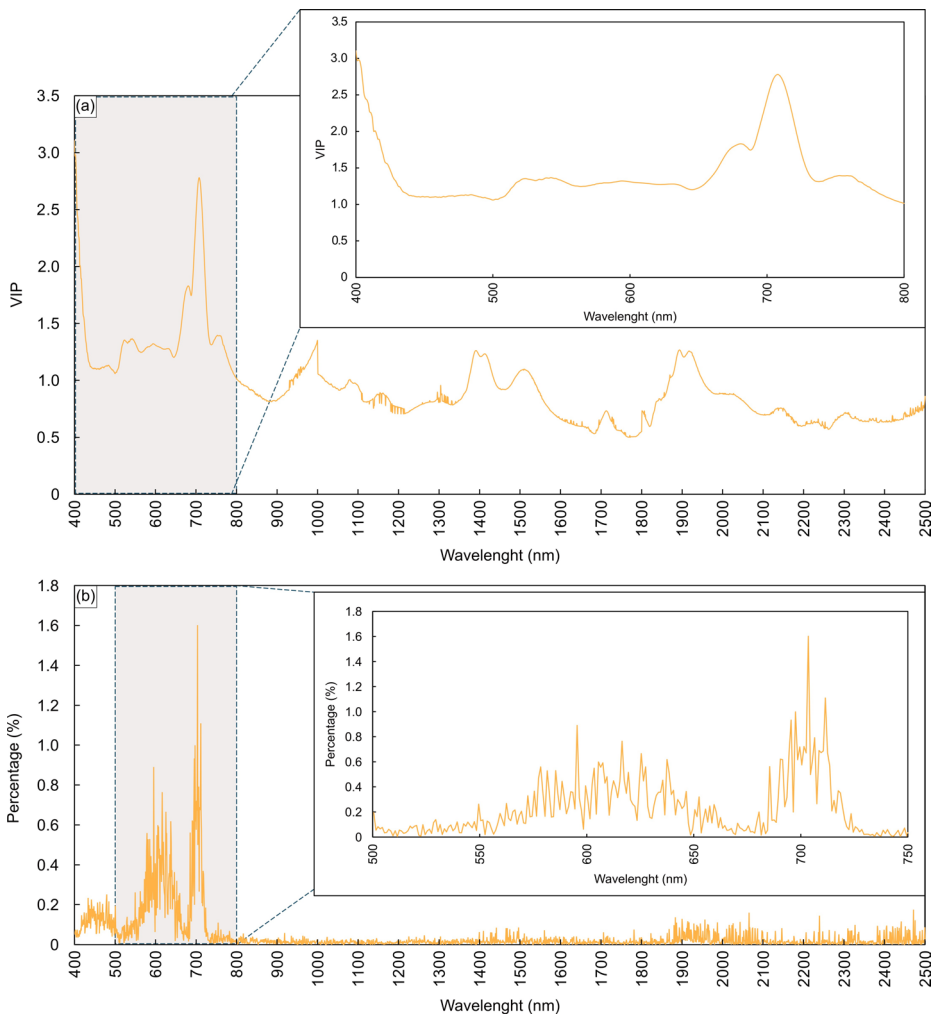


Fig. 7 Top 20 most relevant wavelengths, across the entire data set, for classification of downy mildew infection in grapevine leaves using the variable importance in projection (VIP) for partial least squares discriminant linear analysis **(a)** and random forest feature importance **(b)** (colour figure online)

595, 616, 625, and 637 nm. This distribution indicates that RF model used both RE-related structural information and additional pigment-related information expressed in the visible domain.

When using only data from the 2023 season (Table 6), PLS-LDA again selected wavelengths in the visible blue region (400–402 nm), red region (697–699 nm) and a compact RE sequence from 700 to 713 nm, matching the pattern observed in the full dataset. The RF importance values in 2023 were concentrated almost entirely in the red (690–699 nm) and RE (700–709 nm) regions, with minimal contribution from other spectral intervals. For the 2024 season, the behavior of PLS-LDA changed, selecting a continuous series of visible wavelengths from 400 to 418 nm and only one wavelength in the red region (682 nm). RF,

in contrast, maintained a wider selection pattern, assigning importance to red wavelengths around 695–699 nm and RE wavelengths between 700 and 713 nm, while also incorporating several visible wavelengths within the green-red range (582–638 nm). The comparison between the models shows that PLS-LDA relied more on visible information in 2024, whereas RF continued to select a broad combination of visible, red and RE wavelengths.

Discussion

Spectral patterns associated with downy mildew infection

The spectral analysis showed differences between healthy grapevine leaves and those infected with *Plasmopara viticola*, with characteristic patterns emerging in the VIS, RE, NIR, SWIR1 and SWIR2 regions (Figs. 4 and 5). These differences demonstrate the progressive biochemical and structural changes associated with symptom development.

In the VIS region, differences may be associated with the presence and integrity of photosynthetic pigments such as chlorophyll and carotenoids (Falcioni et al., 2023). Healthy leaves showed the typical reflectance profile of strong absorption in the blue and red bands and high reflectance in the green, due to selective light absorption for photosynthesis (Sims & Gamon, 2002). Infected leaves gradually lost this pattern, showing higher blue and red reflectance, consistent with pigment degradation and photosystem dysfunction (Lacotte et al., 2022), confirming that VIS wavelengths provide early indicators of compromised photosynthetic activity (Al-Saddik et al., 2019), which pigment breakdown leads to reduced absorption and, consequently, higher reflectance (Nguyen & Sung, 2025). The RE region showed a greater variability between infected and non-infected leaves. The classic steep slope between reflectance absorption in red and the increase in NIR verified in healthy leaves is a suitable indicator of intact mesophyll structure (Oerke et al., 2016) and chloroplast functional status. The slope decrease with the presence of infection is documented as an early and reliable indicator of stress (Galvan et al., 2023).

In the NIR region, leaf reflectance is less dependent on chemical composition and is linked to the physical structure of the leaf, such as the density and organization of internal tissues (Maimaitiyiming et al., 2017). In healthy leaves, high reflectance is due to strong internal light scattering (Neuwirthová et al., 2017), while infected leaves presented reductions associated to cell collapse, loss of turgor, and tissue disorganization, events that occur in intermediate to advanced stages of infection (Calamita et al., 2021; Oerke et al., 2023). The spectral signature becomes less pronounced, with a clear decrease in the typical signature of functional leaves (Stamford et al., 2024). While the separation between groups in this region is not as pronounced as in the RE region, NIR provides information on disease progression at intermediate to advanced stages (Pithan et al., 2021). In the SWIR region, reflectance patterns were associated with water content and biochemical composition (Rapaport et al., 2015). An increase in reflectance was observed as the infection progressed, indicating water loss, necrosis, and cell wall disintegration (Oerke & Steiner, 2025).

The progression of downy mildew symptoms (Fig. 6) was reflected in systematic changes in leaf reflectance due to physiological damage to leaf tissue (Clippinger et al., 2024; Simkin et al., 2022). Healthy leaves displayed the typical vegetation signature (Fig. 6a), with strong VIS absorption, a sharp RE slope, high NIR reflectance (Wei et al., 2021a), and low SWIR

(Oerke & Steiner, 2025). As infection advanced from oil spots (Fig. 6b) to necrotic lesions (Fig. 6c and d) and full necrosis (Fig. 6e), pigment loss (Castro & Sanchez-Azofeifa, 2008; Clippinger et al., 2024), structural degradation (Li et al., 2023), and dehydration became more notorious, producing flattened VIS (Zhao et al., 2016) and RE wavelengths, declining NIR reflectance (Mahlein et al., 2012b), and increased reflectance in SWIR (Li et al., 2023). These stage-specific profiles demonstrate the ability of reflectance spectroscopy to monitor disease progression. In early necrotic lesions, VIS reflectance increases due to pigment loss and cellular degradation (Junges et al., 2020), RE transition attenuates indicating structural dysfunction (Oerke et al., 2016), NIR reflectance declines with mesophyll collapse (Mahlein et al., 2012b), and SWIR reflectance rises due to cell wall disorganization and water loss (Ge et al., 2019). As necrosis advances, VIS reflectance rises from pigment degradation (Jiang et al., 2018), RE nearly disappears, marking photosynthetic failure (Flexas et al., 2009; Terentev et al., 2022). NIR reflectance decreases, and SWIR reflectance remains high with reduced variability, indicating cellular disorganization and dehydration (Oerke & Steiner, 2025). In fully necrotic tissue, VIS reflectance diverges from typical red absorption (Falcioni et al., 2024). The RE transition is practically non-existent, revealing complete functional collapse (Terentev et al., 2022; Xu et al., 2019), and NIR reflectance flattens (750–800 nm), confirming internal structural collapse (Yu et al., 2025). SWIR reflectance remains elevated and erratic, which is indicative of severe degradation and irreversible cell death (Oerke & Steiner, 2025).

Comparison between PLS-LDA and RF models

The employment of machine learning approaches have shown to be essential for interpreting the spectral information of dataset, enabling a successful classification (Talaat et al., 2025). Both PLS-LDA and RF provided accurate classifications (Table 4), but their behavior differed depending on the spectral region and growing season data used for training. When models were trained using data from individual seasons, PLS-LDA showed greater variation between years, with accuracies in 2023 ranging from 0.67 to 0.95 and in 2024 from 0.60 and 0.93, depending on the spectral region. Performance was highest for broader spectral combinations that included VIS, RE and NIR, which capture pigment-related and structural information. These seasonal differences may be related to variations in symptom expression and spectral separability between growing seasons, especially in the RE–NIR transition region (Fig. 5). RF showed greater variability across spectral regions but comparable maximum performance between seasons, with values ranging from 0.64 to 0.93 in 2023 and 0.60 to 0.94 in 2024. This behavior aligns RF's capacity to integrate non-linear patterns from dispersed wavelengths, even within more heterogeneous contexts (Sivaganesh et al., 2025).

The analysis by typical spectral regions shows that PLS-LDA was effective out especially in the RE (0.91) and NIR (0.94) regions, comparable values in 2024 for RE (0.91) and NIR (0.92), consistent with studies demonstrating the effectiveness of these models in distinguishing spectral signatures of plant diseases in the NIR region (Couture et al., 2018). RF performed well when using VIS (0.93) and RE (0.94), but showed a reduced performance in NIR and SWIR, suggesting sensitivity to structural variability and noise in these regions (Oerke & Steiner, 2025; Upadhyay et al., 2025). For spectral combinations, the highest accuracies were obtained by PLS-LDA in VIS_RE_NIR, RE_NIR and by RF in VIS_RE and R_RE. However, combinations including NIR also demonstrated high

performance, reinforcing the observations from Zhang et al. (2020) regarding the robustness of NIR-based ratios in disease detection. As for the specific regions of the spectrum, PLS-LDA demonstrated robustness in NIR_l (0.923 for the whole dataset; 0.929 in 2024) and Green (0.912 for the whole dataset; 0.885 in 2024), while RF, although having a good performance in Red (0.912) and Green (0.907) regions, the models showed performance declines in the NIR_m (0.720 total; 0.676 in 2024) and NIR_h (0.714 total; 0.604 in 2024) subregions. These losses suggest that RF, despite its greater versatility in heterogeneous contexts, has a reduced efficiency in NIR sub-regions characterized by more subtle patterns or those that are strongly dependent on spectral continuity. These differences suggest that the linear, latent-variable structure of PLS-LDA is advantageous when relevant information is concentrated within continuous spectral intervals (Shi et al., 2018), while RF is better in dispersed wavelength contributions.

Temporal generalization assessed through LOYO validation (Table 5) showed that models trained on 2023 generalized better to 2024 than the reverse. For PLS-LDA, the highest cross-year accuracies were obtained in Green and R_RE, followed by Red and RE, highlighting the stability of VIS and RE information between seasons. RF showed a similar trend, with high performance in VIS_RE, VIS_RE_NIR, and VIS. In contrast, both models showed reduced performance in the NIR and higher NIR sub-regions. When models were trained on 2024 and tested on 2023, performance decrease for both methods. For PLS-LDA, only moderate accuracies were maintained in VIS, VIS_RE, and RE, while decreases occurred in NIR. RF showed a similar pattern, preserving relatively good performance only in VIS, whereas most spectral combinations and NIR-based regions decreased to moderate or low accuracies. The asymmetric behavior between transfer directions suggests that the 2024 season was spectrally more homogeneous, whereas 2023 encompassed a wider range of conditions, which is corroborated with the results presented in Fig. 5. This explains the better generalization of models trained on 2023 and highlights the importance of interannual variability for model robustness. The consistent superiority of VIS and VIS-RE information across both scenarios further indicates that pigment-related features provide more stable temporal signals, while NIR and longer wavelengths are more affected by structural and environmental changes, limiting their cross-year transferability.

The analysis of the most relevant wavelengths demonstrates the existence of different approaches between PLS-LDA and RF in classifying spectral signatures associated with downy mildew (Fig. 7 and Table 6). For models trained in both classifiers, no single band exceeded a high relative importance, but regions like RE and VIS were the most influential, aligning with previous studies that highlight the relevance of these regions for plant disease detection (Terentev et al., 2022; Thomas et al., 2018). The highest concentration of selected wavelengths was observed around the RE region (approximately 700–714 nm), which was repeatedly identified across datasets and models. However, a greater contribution of visible wavelengths was observed in the 2024 dataset, indicating seasonal differences in spectral separability. Although SWIR exhibited relatively lower discriminatory capacity in the trained models, likely due to the high variability observed in untreated leaves, this spectral region is well known to encode information related to biochemical and structural leaf properties and has been shown to support classification tasks in multivariate approaches, as highlighted by studies on plant disease detection and vegetation spectroscopy (Mahlein et al., 2012a).

PLS-LDA concentrated its selection on the RE (701–714 nm), favoring adjacent wavelengths sensitive to chlorophyll and structural changes (Wan et al., 2022). However, in 2023, it integrated RE (700–713 nm) and VIS (400–402 nm and 697–699 nm) wavelengths, reflecting early infection stages where VIS changes precede visible symptoms (Lowe et al., 2017; Terentev et al., 2022). RE bands retained their importance, indicating balanced use of VIS and RE, which align with recommendations for combining both regions to improve detection across stages (Dave et al., 2022; Thomas et al., 2018). In 2024, the model shifted toward a stronger reliance on VIS wavelengths (400–418 nm), with no bands selected within the RE region, aligning with seasonal differences in spectral separability and symptom expression (Wan et al., 2022).

In turn, RF showed a more dispersed selection, combining RE (701–712 nm) and VIS (595–699 nm), consistent with its multiregional strategy (Fernandes et al., 2025; Wei et al., 2021b). In 2023, it selected a continuous spectral range spanning the red region (690–699 nm) and the RE domain (700–709 nm), supporting the detection through spectral complementarity (Li et al., 2024; Zhang et al., 2020). The RF model trained with data from 2024 continued integrating RE and VIS, with a broader distribution of visible wavelengths (582–697 nm) together with several RE (700–713 nm), reflecting the value of combining chlorophyll-sensitive RE with VIS for detecting pigment and structural changes (Mohd Shafr et al., 2006).

This methodological difference suggests that PLS-LDA is effective when relevant signals are concentrated in a continuous region of the spectrum, while RF benefits from integrating dispersed information across different regions (Zhang et al., 2020). PLS-LDA captures structured, collinear patterns, whereas RF improves flexibility but may be more sensitive to noise and variability (Zhang et al., 2024). The patterns observed in this study, with PLS-LDA showing a stronger concentration of relevant wavelengths in the RE when using data from both growing seasons and only from 2023, and a shift toward VIS in 2024 and RF selecting features across VIS and RE regions, support this complementary behavior. A combined strategy, exploiting the robustness of PLS-LDA in the RE and the spectral diversity of RF, could improve the detection of downy mildew across different disease stages, as supported by recent studies advocating hybrid or ensemble approaches for hyperspectral disease detection (García-Vera et al., 2024; Portela et al., 2024; Wang et al., 2025).

The wavelength ranges identified as most relevant for downy mildew detection in this study, particularly within the VIS and RE regions, are widely recognized as being sensitive to general physiological and structural changes in plant tissues. Similar spectral domains have been reported in the detection of other plant diseases and stress conditions across different crops. Consequently, while the results obtained in this study demonstrate the capacity of these spectral regions to highlight the presence of *Plasmopara viticola* infection under the experimental conditions evaluated, they do not allow, at this stage, allow the attribution of spectral specificity to downy mildew. Distinguishing disease-specific spectral responses from general stress signals remains a central challenge in optical plant disease detection. Further studies combining multiple biotic and abiotic stress factors, as well as different pathosystems and cultivars, will be required to identify and validate spectral features that are associated with grapevine downy mildew.

Sensor selection, monitoring considerations and limitations

The spectral patterns observed in this study align with previous work showing that VIS, RE and NIR wavelengths contain information for detecting foliar diseases in grapevines and other crops. For example, Vélez et al. (2024), identified differences at 450 nm, 700 nm and several NIR/SWIR (1050, 1425, 1650, 2250 nm) bands when detecting powdery mildew (*Erysiphe necator*). PLS regression showed a strong predictive power ($R^2=0.74/0.71$; $MSE<13\%$), and the PMVI index, based on the normalized difference of reflectance values at 755 and 675 nm, correlated well with infection severity. Pithan et al. (2021) reported that both downy and powdery mildew cause reflectance changes, with up to 20% reflectance variation in ratios such as 443/695 and 1900/2435 nm, due to tissue degradation and water loss in Cabernet Sauvignon leaves.

Similar trends were observed in other crops. In tomato and kiwi, Pereira et al. (2024) identified RE (700–740 nm) and NIR (750–900 nm) as key bands for disease detection, with optimized indices and Gaussian process classification (GPC) improving early diagnosis. In wheat, Sanaeifar et al. (2025) used UAV-based hyperspectral imagery and robust PLS regression (rPLS) to detect bacterial leaf streak, reducing bands from 244 to 29 while maintaining accuracy. Yuan et al. (2022) also demonstrated effectiveness in discriminating diseases such as powdery mildew, yellow rust, and aphids in wheat using only three bands (570, 680, 750 nm) and RTVI (Ratio Triangular Vegetation Index) with a support vector machine, achieving 83% accuracy. In peanuts, Guan et al. (2022) demonstrated that hyperspectral reflectance in leaves, plants, and fields, identified 540, 660, and 770 nm, as most relevant for detecting leaf spot caused by *Cercosporidium personatu*, with KNN and logistic regression showing strong class separability. For cassava, Owomugisha et al. (2020) used VIS–NIR (400–1000 nm) spectroscopy to detect viral infections within greenhouse environments, with RE, VIS (620–680 nm), and NIR (750–900 nm) most effective in advanced stages. These results confirm the robustness of VIS–NIR regions for non-destructive disease diagnosis across diverse crops.

Reflectance spectroscopy, combined with machine learning provides an approach for detecting downy mildew in field conditions because of its sensitivity to biochemical and structural changes before visual symptoms appear (Singh et al., 2025). Its non-destructive nature and capacity for rapid data acquisition and high reproducibility (Thomas et al., 2018) make it sustainable alternative to traditional visual or lab-based methods (Martinelli et al., 2015). Given the distinct spectral responses of grapevine leaves to *Plasmopara viticola* infection spectrum (Mishra et al., 2020), the results presented in this study demonstrate suitability for the implementation of monitoring strategies that are based on specialized optical sensors. These include proximity sensors and portable spectroradiometers (Portela et al., 2024), tractor-mounted or ground-based platforms (Molnár & Tamás, 2025), and UAV-based remote sensing (Portela et al., 2025b), manned aircrafts (Strati et al., 2025), or satellites (Kanaley et al., 2024).

The choice of sensor and spectral configuration should depend on the monitoring objectives and on the infection stage to be detected or characterized (Pereira et al., 2025; Portela et al., 2024). Hyperspectral sensors, with high spectral resolution (Junges et al., 2020), are well-suited for exploratory studies and for detecting early spectral changes VIS and RE regions (Thomas et al., 2018). Multispectral sensors, despite a typical lower spectral resolution, can also be effective when bands are selected to target VIS, RE and NIR wavelengths

(DadrasJavan et al., 2019), to improve efficiency, reduce processing demands, and augment adaptability to mobile (Mishra et al., 2020) or remote platforms (Portela et al., 2025b). In this context, advances in sensor miniaturization, and autonomous platforms, make it technically feasible to make near real-time phytosanitary monitoring systems possible. The proposed approach, which combines reflectance spectroscopy with machine learning, has the potential to support agronomic decision-making, support precise interventions and, ultimately, promotes sustainable, resilient precision viticulture resilient to fungal diseases.

Although the results indicate that spectroscopy can support operational downy mildew detection, several considerations need to be highlighted. The models in this study were not fully optimized because the primary objective was to compare spectral regions and modelling strategies under controlled conditions, rather than to maximize predictive performance. Further improvements could be achieved using more extensive hyperparameter tuning or alternative modelling architectures, such as using vegetation indices. Moreover, all data were collected in a single vineyard, on one cultivar and under consistent high disease pressure. While these conditions enable a reasonable symptom development and a simplified interpretation, they limit the general applicability of the results. Spectral responses may vary among cultivars, training systems, soil conditions and climates, other biotic and abiotic factors, and the transferability of the models to other conditions should be evaluated. Despite these limitations, the patterns identified in this study indicate that VIS, RE and low-NIR wavelengths provide important information for detecting downy mildew infection, while NIR and SWIR regions may show greater sensitivity to environmental and structural variability. These observations are relevant for guiding sensor selection and for designing monitoring strategies that rely on the most stable and informative parts of the spectrum.

Conclusions

This study demonstrated that field reflectance spectroscopy combined with machine learning provides an effective and non-destructive approach for detecting of *Plasmopara viticola* infections in *Vitis vinifera* cv. Loureiro. Spectral changes observed in VIS, RE, NIR and SWIR regions reflect the biochemical and structural changes induced by infection. The VIS region demonstrated relevance for early stages of infection, with changes in blue, green and red wavelength associated with initial pigment loss. The RE region was among the most discriminative capacity across nearly evaluated all stages, providing sensitive indicators for parameters such as chlorophyll content and mesophyll structure. The NIR region was more important at advanced stages, capturing reductions in internal scattering caused by cell collapse and loss of turgidity. This spectral complementarity highlights the value of combining multiple spectral domains for detection throughout the disease cycle.

Both classification models achieved high levels of overall accuracy. PLS-LDA performed well when spectral information was concentrated in contiguous regions, whereas RF showed greater stability across growing seasons and variable spectral conditions. The identification of the most discriminative wavelengths can provide basis for the design of multispectral sensors that integrate multiple imaging sensors in specific VIS, RE, and NIR wavelengths. For research or early detection, hyperspectral sensors are preferable. For operational large-scale vineyard monitoring, multispectral sensors calibrated to the specific wavelengths highlighted in this study can provide cost-effective solutions for mobile ground-based plat-

forms and UAV-based monitoring. Such systems may could reduce the need for systematic fungicide applications and support more sustainable viticulture practices.

Future work should address automatic classification of infection stages to support multi-temporal and spatial monitoring of disease progression. Extending the approach to develop models for sensors onboard aerial platforms (UAVs or manned aircrafts) and ground-based platforms would facilitate operational use in precision viticulture. Combining optical data with complementary sensing technologies, such as thermal infrared, RGB, multi-spectral and hyperspectral measurements, may improve monitoring efficiency at different spatial scales. With the advancing of these strategies, reflectance spectroscopy can support phytosanitary decision-making and contribute to reducing production costs.

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Declarations

Conflict of interest The authors declare no competing interests.

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