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Detection of grapevine leaf stripe disease symptoms by hyperspectral sensor

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Summary. Hyperspectral sensors can measure reflectance in a wide range of the electromagnetic spectrum. These can be used as an indirect method for detecting plant disease, by comparing the specific spectral signatures between symptomatic and asymptomatic vegetation. Grapevine Leaf Stripe Disease (GLSD), including the Esca complex, is a very important Grapevine Trunk Disease (GTD) worldwide. With the objective of developing an innovative method for quantitative and qualitative analyses of symptomatic plants using remote sensing, this study measured and characterized the spectral behaviour of GLSD asymptomatic and symptomatic grapevine leaves using a hyperspectral sensor. Asymptomatic, initial and final GLSD symptomatic leaves were collected in two stages of the phenological cycle (before and after harvest) from a 'Merlot' vineyard in Veranópolis, Rio Grande do Sul, Brazil. Reflectance measurements (350 to 2,500 nm) were performed using a spectroradiometer. The spectral behaviour of vine leaves with GLSD symptoms changed especially in the visible light range; reflectance increased in the green edge (520-550 nm) and red edge (700 nm) associated with reduced photosynthetic pigments (especially chlorophyll *b*). At near-infrared, reflectance decreased, especially in leaves with advanced GLSD, due to cell structure loss and toxin accumulation induced by pathogens. Even at different intensities, leaf reflectance changed in initial and final GLSD symptoms and at different stages of the cycle. These results showed that proximal, non-destructive sensing techniques may be useful tools for detecting the changing spectral behaviour of grapevine leaves with GLSD, which could be used for disease identification and detection.

Key words: Esca, trunk diseases, spectroradiometer, 'Merlot'.

Introduction

The photosynthetic processes and cellular structures of tissues in plants attacked by pathogens undergo changes that modify the interaction between vegetation and electromagnetic radiation, and, consequently, the reflectance of leaves and canopies (Calcante *et al.*, 2012; Prabhakar *et al.*, 2012; Martinelli *et al.*, 2015; Mahlein, 2016). Plant pathogens trigger host protection mechanisms that lead to suboptimal growth, changing variables such leaf area index (LAI), chlorophyll content and surface temperature, and produce spectral signatures that are different from the healthy vegetation (Martinelli *et al.*, 2015).

Techniques using hyperspectral sensors are indirect methods for detecting plant diseases, since they measure vegetation reflectance in a wide range of the electromagnetic spectrum. Early detection of diseases in the field, before or at the beginning of symptom expression, is important for timely application of disease control measures. Zarco-Tejada *et al.* (2018) detected changes in regular functions of olive trees with *X. fastidiosa* infections using airborne imaging spec-

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troscopy and thermography. Their results revealed plant infection before appearance of visible symptoms, with accuracies of disease detection greater than 80%. In vineyards, Naidu *et al.* (2009) reported different reflectance measurements between leaves with or without *Grapevine leafroll-associated virus* infections. Spectral changes associated to downy mildew (*Plasmopara viticola*) in 'Cabernet Franc' leaves were also reported (Calcante *et al.*, 2012). Knauer *et al.* (2017) presented an advanced approach for classifying hyperspectral images based on combined spatial and spectral features to improve the detection of powdery mildew (*Erysiphe necator*) infection levels of 'Chardonnay' grape bunches. In addition, Di Gennaro *et al.* (2016) reported high correlation between the Normalized Difference Vegetation Index (NDVI) and symptoms of Grapevine Leaf Stripe Diseases (GLSD) in a 'Cabernet Sauvignon' vineyard.

Worldwide, grapevine trunk diseases (GTD's) have been described as one of the main problems in vineyards, due to production losses, decline and death of plants (Bertsch *et al.*, 2013; Kaplan *et al.*, 2016; Gramaje *et al.*, 2018). Among GTD's, Petri disease (*Phaemoniella chlamydospora* and *Phaeoacremonium* spp.) and Black-foot (*Cylindrocarpon* spp., *Ilyonectria* spp., *Campylocarpon* spp. and others) are frequently associated with decline and death of young grapevine plants (less than 5 years old) (Úrbez-Torres *et al.*, 2014), while fungi of the Esca complex are the major disease agents in established vineyards. This complex includes the GLSD associated mainly with wood vascular infections by *Phaeomoniella chlamydospora* and *Phaeoacremonium* spp., and white rot associated especially with *Fomitiporia mediterranea, Phelinus* spp. and other basidiomycetes. The term 'Esca' commonly refers to white rot occurring in the trunks and branches of mature standing vines. 'Esca proper', usually encountered in mature vineyards, indicates the co-occurrence of GLSD and Esca on individual vines (Mugnai *et al.*, 1999; Bertsch *et al.*, 2013; Cloete *et al.*, 2016; Fontaine *et al.*, 2016).

In Brazil, occurrence of Esca associated pathogens was reported by Garrido *et al.* (2004) in vineyards of the Serra Gaúcha, an important grape and wine producing region in that country. In the same region, Almança *et al.* (2013) detected occurrence of *Phaeomoniella chlamydospora* for the first time. Esca diseases caused by *Phaeoacremonium aleophilum, P. parasiticum*, and *Phaeomoniella chlamydospora* have also been reported in Vale do São Francisco, a viticultural region

in northeastern Brazil (Correia *et al.*, 2013). Because viticulture is an important agricultural activity in Brazil, and it is possible to develop an innovative method for quantitative and qualitative analyses of symptomatic plants using sensors, the study described in this paper aimed to measure and characterize the spectral behaviour of GLSD asymptomatic and symptomatic grapevines leaves using a hyperspectral sensor.

Materials and methods

The experimental area used for this study was a commercial 'Merlot' vineyard in Veranópolis, in the Serra Gaúcha viticultural region, Rio Grande do Sul, Brazil. The regional climate is classified as Cfb (Köppen, 1948), i.e., humid subtropical, with average temperature less than 22ºC in the warmest month of each year. In the study region, the vegetative grapevine cycle occurs from September (budding) to June (leaf fall), with the manual grape harvest carried out in February. The vineyard was 20 years old, the vine rows were north-south oriented and the canopy was conducted using the vertical training system. Vines with and without GLSD symptoms were monitored for two consecutive years, ensuring from which plants the leaf samples could be taken for measurements.

Fully expanded leaves (each with central vein between 7 and 12 cm length) were collected from monitored plants in January and May 2016, during, respectively, the pre- and post-harvest stages of the vine cycle. The collected leaves were grouped into three categories, asymptomatic (Figure 1a), or with initial (Figure 1b) or final (Figure 1c) GLSD symptoms. Leaves with initial GLSD symptoms had mildly yellow spots and onset of leaf discolouration (Figure 1b), while those with final GLSD had chlorosis and/or intervening necrosis, with reddish-purple discoloration surrounded by yellowish discoloration, characterizing the standard "tiger stripes" symptom (Figure 1c). For each group, five to ten leaves were collected, labeled, stored in paper bags, and kept at room temperature until laboratory analysis.

Reflectance measurements for each leaf were performed using an ASD Fieldspec 3 Pro spectroradiometer (leaf clip sensor), to obtain three spectra from 350 to 2,500 nm in each of two parts of the adaxial leaf surface. For the symptomatic leaves, the data were acquired by positioning the leaf clip on the lesions that characterized the initial and final GLSD
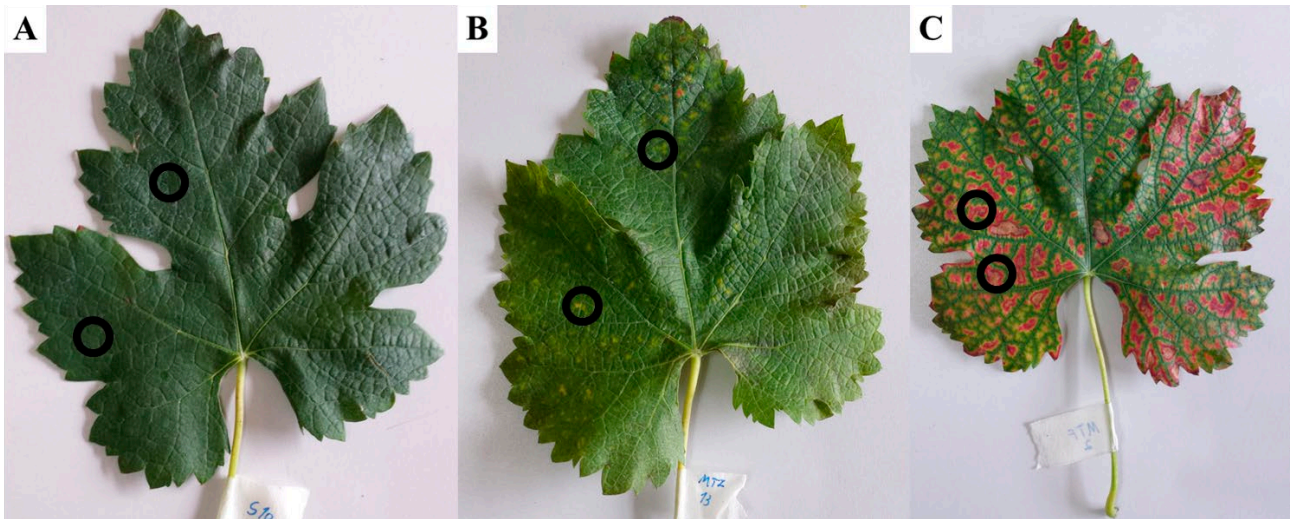
Figure 1. Grapevine leaves from a 'Merlot' vineyard in Serra Gaúcha region, Brazil. (A) asymptomatic; (B) with initial symptoms and (C) with final symptoms of Grapevine Leaf Stripe Disease (GLSD. Open black circles indicate leaf clip sensor positions during spectroradiometric measurements.

symptoms. The average spectral profile of each leaf category was derived from six measurements to determine the average normalized spectrum for each category and month. For normalizing the spectrum, each wavelength reflectance was divided by the spectrum area, which is the sum of the reflectance over the entire wavelength range. The differences between symptom categories were detected by subtracting the asymptomatic leaves from the leaves with initial or final GLSD symptoms. Thus, at wavelengths in which the reflectance of the symptomatic leaves was greater than asymptomatic, the difference-spectrum indicated negative reflectance differences.

The chlorophyll index of the same leaves used in the spectroradiometer analyses were also measured using an optical portable chlorophyll meter (Falker Clorofilog). The chlorophyll index is proportional to chlorophyll absorbance at wavelengths 635 and 660 nm and can be expressed as total chlorophyll and chlorophylls *a* and *b*. The chlorophyll data were submitted to variance analysis (one way ANOVA), and when mean differences were statistically significant ($P<0.05$) they were compared by Tukey test ($P=0.05$).

Results

The different spectral behaviours of the treatments are shown in Figure 2. Figures 2a and 2b show the average spectra for the three leaf symptom categories before and after harvest, while Figures 2c and 2d show the comparative spectral behaviour of symptomatic leaves, using the asymptomatic leaf spectrum as reference. The spectral behaviour of leaves with GLSD symptoms was distinct from that for the asymptomatic leaves in January (Figure 2a and 2c) and March (Figure 2b and 2d). This was especially in the visible part of the spectra (400 to 700 nm), where an increase of reflectance in symptomatic leaves was detected, and also in the near infrared part (700 to 1,200 nm), where a decrease in the reflectance characterized the leaves with advanced disease symptoms.

In the optical (visible) spectral region, the leaf reflectance changed little in the blue wavelengths for the initial and final GLSD symptomatic leaves, but changes were more noticeable in the green and red wavelengths (Figures 2c and 2d). Reflectance increased in GLSD symptomatic leaves in the 520–550 nm range, being, respectively, 41 and 25% greater in leaves with initial or final GLSD symptoms, compared to the average reflectance in asymptomatic leaves (Figure 2e). At the green edge (520 nm) specifically, reflectance increased between 42% (initial symptom leaves) and 33% (final), in leaves with GLSD symptoms.

Figure 2 shows that leaf reflectance was different in the red spectrum range. Leaf reflectance increased in the presence of GLSD symptoms in the 650 nm
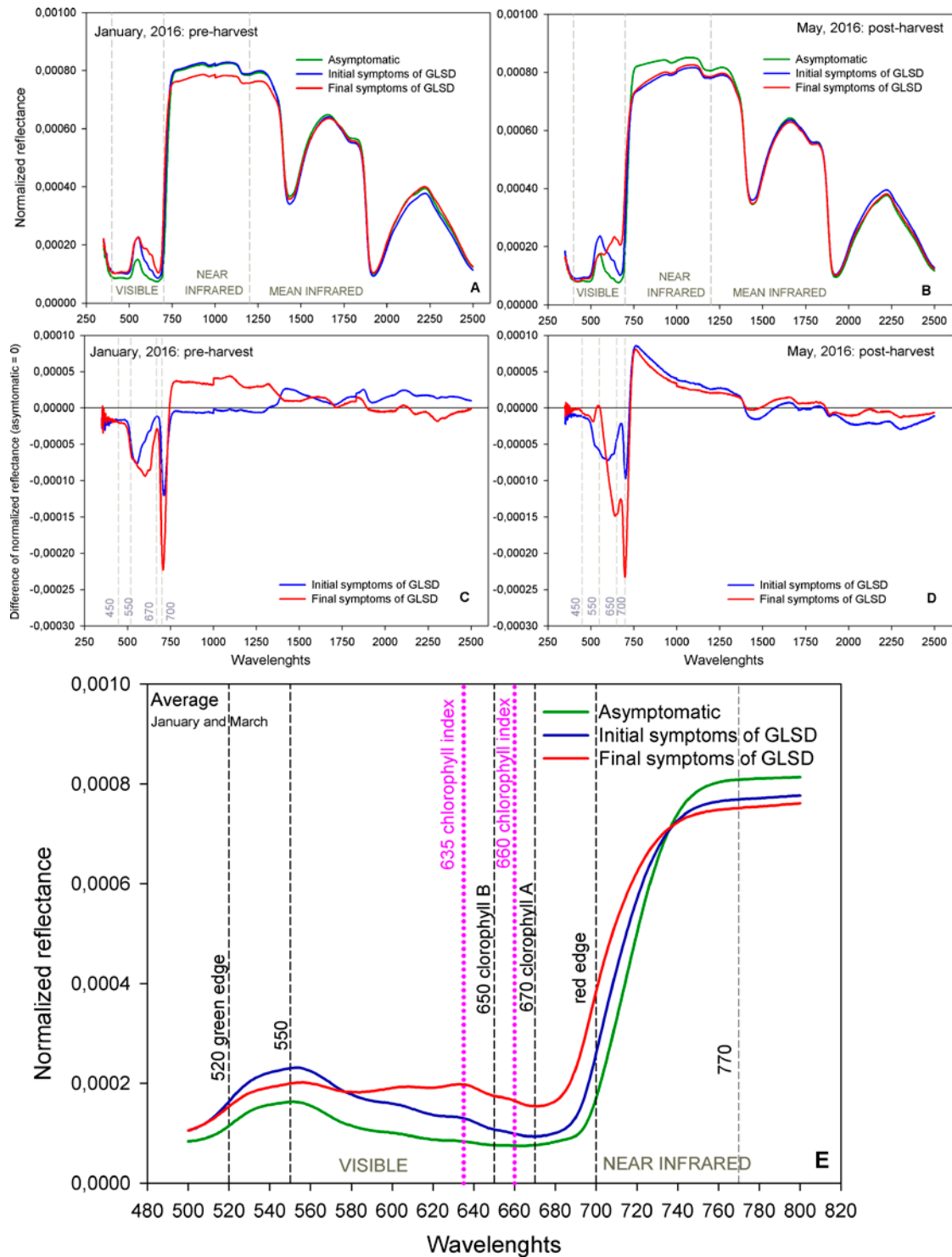
Figure 2. Spectral behaviour of asymptomatic grapevine leaves and with initial and final symptoms of grapevine leaf stripe disease (GLSD): normalized reflectance and difference of reflectance (250 to 3,500 nm) in January (A and C) and March (B and D); and normalized reflectance (500 to 800 nm) from asymptomatic and symptomatic GLSD grapevine leaves in average of these two months.
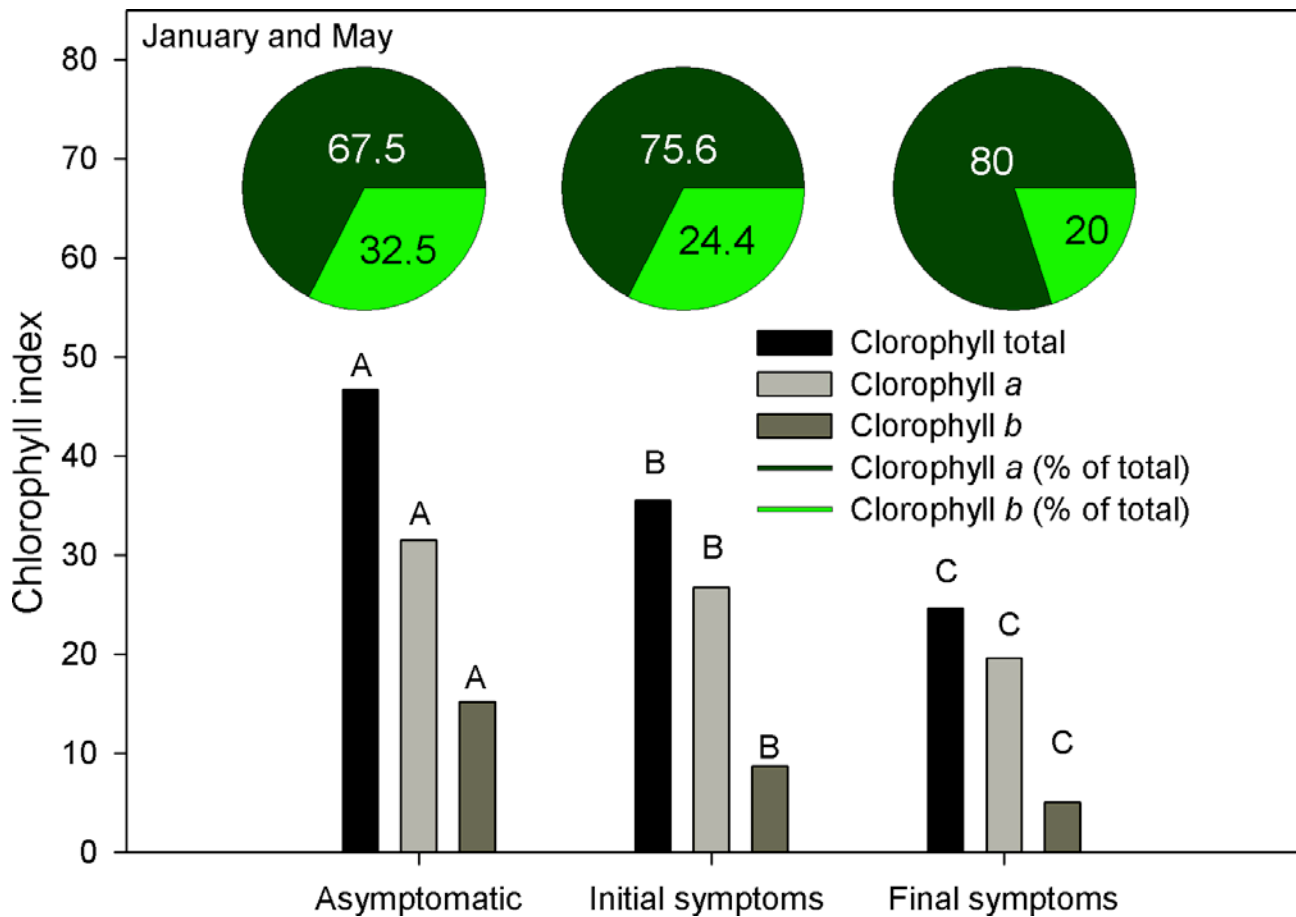
Figure 3. Chlorophyll indices from asymptomatic grapevine leaves and those with initial and final symptoms of grapevine leaf stripe disease (GLSD). Uppercase letters in columns indicate no significant differences between treatments in total chlorophyll, chlorophyll A and chlorophyll B by Tukey test ($P = 0.05$).

wavelength, especially for leaves with advanced symptoms (Figure 2e) due to markedly reduced absorption by chlorophyll *b*. In this spectral range, leaf reflectance was, respectively, 41 and 128% greater in the initial and final symptom categories, compared to the asymptomatic leaves. Leaf reflectance increased for wavelengths near 700 nm in the presence of GLSD symptoms (Figure 2e). The range near 700 nm are in the red edge, which is a unique feature of green vegetation that results from two optical properties of asymptomatic leaves: the high absorption of chlorophyll *a* in the 670 nm and, at longer wavelengths (near infrared), the increased reflectance due to the internal leaf scattering (Gitelson and Merzlyak, 1996). At 670 nm, leaf reflectance increased by, respectively, 22 and 102% for the initial and final symptom catego-

ries (Figure 2e).,These differences increased further to 53 and 126% at 700 nm for the same categories.

In the near-infrared region results indicated reflectance differences between asymptomatic and symptomatic GLSD leaves (Figures 2c and 2d). In January, when the grapevine phenological stage corresponds to mature leaves, the near-infrared reflectance decreased in leaves with final GLSD symptoms, suggesting advanced stages of cell disorganization and necrosis induced by the pathogens (Figure 2c). However, in May, which corresponds to a phenological stage nearing senescence, the near-infrared reflectance decreased in leaves with initial and final GLSD symptoms (Figure 2d), suggesting that spectral behaviour changes can be associated with either disease decline or leaf senescence. For example, mean leaf reflectance

in the presence of early and advanced symptoms was, respectively, 5 and 7% less than that of asymptomatic leaves in the 770 nm wavelength (Figure 2e).

The chlorophyll indices from the symptomatic and asymptomatic leaves examined in this study showed that asymptomatic leaves had greater amounts of total chlorophyll and of chlorophylls *a* and *b* (Figure 3). All indices decreased as the disease symptoms progressed, and as the disease advanced, the chlorophyll *a* to *b* ratio increased from 2:1 in asymptomatic leaves to 3:1 in the initial GLSD leaves and 4:1 in the final stage leaves (Figure 3).

Discussion

Asymptomatic leaves displayed the typical spectral behaviour of green and photosynthetically active vegetation. The low reflectance of asymptomatic leaves in green wavelengths (Figures 2a and 2b) coincides with the presence of pigments such as carotenoids, chlorophyll *a* and *b*, which have been associated with absorptions near the 520 nm, the green edge of the reflectance spectrum (Gitelson and Merzlyak, 1996). The reflectance increases observed in leaves with GLSD symptoms at the green edge were associated especially with reduced total chlorophyll, and, to a lesser extent, with carotenoids, since the carotenoid content is reportedly stable during an Esca infection (Petit *et al.*, 2006; Magnin-Robert *et al.*, 2011). In addition, Gitelson and Merzlyak (1996) showed that traces of chlorophylls *a* and *b* and significant content of carotenoids did not change leaf reflectance at 550 nm, suggesting a lesser spectral contribution of carotenoids compared to chlorophylls.

In the red region of the spectrum, the increased reflectance of GLSD leaves is also associated with pigments. One of these pigments is chlorophyll *b*, with reportedly maximum *in vivo* absorption at 650 nm (Gitelson and Merzlyak, 1996). Results have indicated sensitivity to chlorophyll concentrations and to metabolic disturbances at near the 700 nm wavelengths (Carter and Knapp, 2001). This important spectral feature can identify GLSD, since high reflectance values near 700 nm express the blue shift of the red edge reported in stressed vegetation (Jensen, 2007). Martinelli *et al.* (2015) also indicated that pathogens reduce the leaf chlorophyll contents due to necrotic or chlorotic lesions that increase reflectance at visible wavelengths, inducing a red edge shift. The results from the present study are in agreement those of with Carter and Knapp (2001), who reported that frequently in preliminary analysis the steep slope of reflectance curves in the far-red spectrum can produce the illusion that stress-induced differences are negligible near 700 nm, and that the spectral responses to stress near 700 nm have often been ignored. However, the simple subtraction of control curves from curves representing the stressed condition clearly shows the far-red response.

These reflectance changes observed at wavelengths related to photosynthetic pigments were corroborated by the chlorophyll index results. The decreasing levels of total chlorophyll and chlorophyll *b* (Figure 3) are in agreement with our spectral observations (Figure 2), and with those of Petit *et al.* (2006) and Magnin-Robert *et al.* (2011). They reported strong decreases both in maximum fluorescence yield and in effective Photosystem II quantum yield due to GLSD pathogens.

The spectral behaviour of symptomatic leaves was different in the green (520-550 nm) and red edge (near 700 nm), in leaves with either initial or final symptoms. These results indicate that it was possible to detect changes in leaf reflectance in the early stages of disease using the features with maximal sensitivity to chlorophyll concentration (Gitelson and Merzlyak, 1996). Fontaine *et al.* (2016) reported that GLSD pathogens affect host photosynthetic rates, while photosynthesis and stomatal conductance decrease in leaves with chlorosis symptoms. Foliar symptoms are associated with stomatal closure and changing photosynthetic apparatus (Petit *et al.*, 2006; Magnin-Robert *et al.*, 2011). Net photosynthesis rate also decreased gradually in asymptomatic leaves of symptomatic Esca canes (Petit *et al.*, 2006; Magnin-Robert *et al.*, 2011). Chlorophyll content and fluorescence also decreased in symptomatic leaves of *in vitro* plants infected with either *P. chlamydospora* or *Phaeoacremonium angustius* (Santos *et al.*, 2005).

In the wavelength range shorter than 700 nm, reflectance from leaves is determined by chlorophyll absorption whereas it is modulated less by absorption and much more by scattering beyond 700 nm. The proportion of radiation reflected by leaves is greater at near infra-red (700 to 1,200 nm) than in the visible region, due to the internal scattering occurring at the interfaces between the cell walls and intercellular air spaces typical of spongy mesophyll tissues (Jensen, 2007). In this part of the spectrum, spectral properties may change due to either senescence or leaf stress.

Even if less than those observed in visible light, the depressions at near-infrared reflectance are also indicators of changing tissues and toxin accumulation in leaves with GLSD symptoms. Valtaud *et al.* (2009) reported that the intracellular structures were damaged more extensively in the chlorotic parts of symptomatic GLSD leaves than in healthy leaves, as the tonoplasts were disrupted. In addition to the changing photosynthetic apparatus, host defense mechanisms were also affected, especially the glutathione pool, defense proteins such as PR-proteins, and phenolic compounds. The occurrence of Esca symptoms in grapevine leaves is associated to exopolysaccharides produced by *P. chlamydospora* (Andolfi *et al.*, 2009), which may also induce reflectance changes.

Alterations in the spectral behaviour of leaves with GLSD symptoms at mean infrared wavelengths (greater than 1,200 nm) were also observed. However, this part of the spectrum is associated with leaf water content, and no pattern of spectral modification was recognized which could be associated to the diseases considered in this study.

Conclusions

Our results indicate that the main alterations in the spectral behaviour of grapevine leaves with GLSD symptoms take place mainly in visible light, as a result of reduced photosynthesis pigments (especially chlorophyll *b*), and at near infrared due to cell structure losses and toxin accumulation induced by pathogens. Even at different intensities, leaf reflectance changed in the presence of initial and final GLSD symptoms and at different stages of the host phenological cycle (before and after harvest). These results show that hyperspectral sensing technologies could be useful to detect changes in the spectral behaviour of grapevine leaves with GLSD, including when the symptoms are not easily perceived by human vision. The definition of the spectral behaviour of grapevines leaves using detailed hyperspectral information allows to characterize the GLSD symptoms to be characterized. Our results, based on laboratory-acquired spectral features, also expand knowledge of the dynamics of plant disease spatiotemporal heterogeneity in vineyards. Defining the spectral signatures of grapevine leaves with and without GLSD symptoms is fundamental for early disease identification and detection, and provides understanding of the host-pathogen interaction since these diseases are important for grape production systems. Further studies will be conducted in the Serra Gaúcha region, Brazil, to characterize the spectral behaviour of grapevine canopies with GLSD symptoms, and to promote the quantitative and qualitative analysis of spatiotemporal distribution of GLSD-affected plants, in the context of precision viticulture and regional disease monitoring and mapping by remote sensors.

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