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Research Paper

Leaf spectral behavior of common bean (*Phaseolus vulgaris* L.) infected by anthracnose or Fusarium wilt at three levels of severity

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ABSTRACT: *This study aimed to identify wavelengths and spectral reflectance bands of common bean leaves, using principal component analysis to discriminate between healthy and infected leaves with the fungi Colletrichum lindemuthianum or Fusarium oxysporum f. sp. Phaseoli. The experiments were carried out in a greenhouse. Spectral reflectance measurements were taken daily. The spectral difference of the mean reflectance values for each bean cultivar and disease severity level was calculated to emphasize regions of interest in the Visible and Near Infrared spectral bands. The results show that Red Edge spectral band presented the wavelengths that better discriminated infected leaves for both diseases.*
KEYWORDS: *Principal components, reflectance, disease, precision farming*

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I. INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is attacked by several diseases, including the Fusarium wilt caused by the fungus *Fusarium oxysporum* f. sp. *Phaseoli*, and anthracnose caused by the fungus *Colletrichum lindemuthianum*, which occur practically throughout Brazil [13].

Fusarium wilt and anthracnose can cause productivity losses of 80% and 100%, respectively, when climatic conditions are favorable and plants are infected in their early stages of development [8] and [11].

Chemical control of these diseases may be possible. Ideally, fungicide application at varying rates with diseases at an early stage of infection. These measures can reduce application cost and environmental impact.

The plant-pathogen interaction can cause changes in leaf organelles and pigments [9], such as leaf necrosis and lesions (anthracnose) and wrinkling, wilting and yellowing of leaves (Fusarium wilt).

The reflectance of plant leaves is the result of multiple interactions between incident radiation and biophysical and biochemical characteristics of plants. Several studies used the reflectance of leaves in the Visible (400 – 700 nm) and Near Infrared (700 – 1000 nm) range [2], [7] and [10], to detect changes in plant vitality with an emphasis on fungal diseases. Deterioration of leaf organelles and pigments caused by pathogens creates stress on plants and changes in their spectral responses. Thus, the severity of a disease can be assessed through the application of remote sensing tools such as reflectance of hyperspectral and multispectral data.

Hyperspectral reflectances can be used to detect physiological changes in leaves. These data show hundreds of reflectance values at different wavelengths. Thus, there is the possibility of data with redundant values and there is the possibility of reducing the number of wavelengths without significant loss of information and without compromising the spectral characterization of the target [12].

A multivariate technique widely used in reducing the dimensionality of data and selecting variables is principal component analysis (PCA). [1] used PCA to reduce the amount of original variables and determine wavelength intervals in the electromagnetic spectrum, which allowed them to discriminate, before the first symptoms became visible, between healthy wheat plants and those infected with fusarium.

[6] discusses several variable selection methods using ACP and the B4 method was used, which consists of selecting the variables with the highest load (loading) of the first principal component (CP1). This method is based on the premise that the most important principal components explain most of the total variation of the data and that they are formed by linear combinations of variables related to each other. When selecting a

variable from CP1 that is strongly associated with it, you are taking a representative of the variables of this main component. The rest of the variables were discarded.

The objective of this work was to spectrally characterize leaves of three common bean cultivars, infected with the fungi *Colletrichum lindemuthianum* or *Fusarium oxysporum* f. sp. *Phaseoli*, in three levels of severity. Determine the most appropriate spectral bands and wavelength for discriminating infected leaves.

II. MATERIAL AND METHODS

The experiments were conducted in a greenhouse, with a controlled environment, located at the Minas Gerais Agricultural Research Company – EPAMIG and at the Federal University of Viçosa (UFV), in the city of Viçosa, in the state of Minas Gerais, Brazil.

To collect the spectral reflectances of the common bean leaves, experiments were conducted using three bean cultivars: Rudá, RBS Supremo and Vermelhinho, according to the economic importance of each cultivar and susceptibility to anthracnose and Fusarium wilt.

Two common bean plants were grown in each vase in 0.415 L of substrate (Tropstrato HT©, Vida Verde, Mogi Mirim, SP, Brazil).

For anthracnose, an experiment was carried out for each bean cultivar. The experimental design used was completely randomized, with four levels of pathogen concentrations as treatment [zero (control), 1.2×10^4 (Low level), 1.2×10^5 (Medium level) and 1.2×10^6 (High level) conidia/mL], with six replicates. The arrangement of the vases on the bench was made by drawing lots. The experiment was repeated twice.

For Fusarium wilt, the same type of experiment was performed, however, the treatments were four levels of pathogen concentrations [zero (control), 1.0×10^4 (Low level), 1.0×10^5 (Medium level) and 1.0×10^6 (High level) conidia/mL].

2.1 Inoculation with *Colletotrichum lindemuthianum*

The inoculum of *C. lindemuthianum* was multiplied in test tubes containing sterilized pods and partially immersed in agar-agar. The tubes were kept for approximately ten days at 24 ^oC, for the production of conidia used in the inoculation.

In a greenhouse, 30 seeds of each bean cultivar were sown in styrofoam trays (68 x 35 cm) with 128 cells. Six days after planting, the bean plants were transplanted into plastic vases.

Inoculation was performed three days after transplanting the seedlings to plastic vases, atomizing the conidia suspension at concentrations of $1.2x10^6$, 1.2×10^5 and 1.2×10^4 conidia/mL, on both surfaces of the primary leaves, with the aid of a manual atomizer.

2.2 Inoculation with *Fusarium oxysporum* f. sp. *phaseolli*

In a greenhouse, 30 seeds of each bean cultivar were sown in styrofoam trays (68 x 35 cm) with 128 cells. Six days after planting, the bean plants were transplanted into plastic vases.

For the inoculation of *F. oxysporum* f. sp. *phaseoli*, the plants were removed from the styrofoam trays and the roots washed with water according to the methodology presented in [3].

After washing the plant roots, one third of the length of the roots was cut with the aid of scissors, then the cut roots were immersed in a suspension at a concentration of $1x10^4$ conidia/mL (macro and microconidia) for 5 minutes. This procedure was repeated at concentrations of $1x10^5$ and $1x10^6$ conidia/mL. Afterwards, the plants were transplanted in vases containing 2.5 L of substrate and taken to the greenhouse.

2.3 Measurement of the spectral reflectances of bean leaves

In each vase, four fully developed leaves were chosen to carry out the spectral reflectance measurements. Measurements were taken daily after inoculation until total leaf fall, always at the same time of day, between 10:00 and 14:00 h.

The leaf spectral reflectance was measured with the ASD FieldSpec Pro FR spectroradiometer (Analytic Spectral Devices, Boulder, USA), with the "plant probe" for measurements of contact with the leaf. This probe has an integrated 100 W halogen lamp, which was turned on 90 minutes before each data collection to stabilize it. The spectroradiometer has a spectral range between 350-1100 nm and the useful reading range was between 400 and 900 nm, discarding noisy spectral data at the extremes. Calibration of the spectroradiometer using a white reference, with a Spectralon plate (Labsphere, North Sutton, USA), was performed at the beginning of each data collection and then at regular intervals of 15 minutes. The measurement time of each reading was adjusted to 544 ms, and each reflectance collection, on each leave, was the average of 10 readings taken by the spectroradiometer.

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2.4 Selection of wavelength bands based on principal component analysis

The PCA is a multivariate technique that transforms the original data set, such that the transformed data are projected in a space that maximizes the sample variance and variance [6].

[5] and [6] present methods B1, B2, B3 and B4 for variable selection using PCA. In this work, the B4 method was used to select relevant wavelength intervals to discriminate between healthy bean leaves and leaves infected with *C. lindemuthianum* or *F. oxysporum* f. sp. *phaseoli*.

Method B4, described in [5], requires only one PCA applied to the *k* x *n* matrix of original data. This method retains the *p* variables of the first principal component, which retains most of the data variance, with the highest load values. The remaining *k* - *p* variables are rejected. The criterion for determining the value of *p* is based on the load factor λo. The sum of the charges of the *p* variables retained must be equal to or greater than λo. In this work, a load factor equal to 0.80 was used.

III. RESULTS AND DISCUSSION

3.1 Disease development

The bean plants not inoculated with the fungi, which served as control for the experiment, remained healthy throughout the data collection period. The inoculated plants remained without any symptoms of anthracnose or Fusarium wilt during the latency period of the disease. After the latency period, typical disease symptoms were visualized, on average, four days after inoculation (DAI) for anthracnose and 10 DAI for Fusarium wilt.

The reflectance spectra of healthy leaves and infected leaves with the *C. lindemuthianum* in three levels of severity for RBS Supreme are shown in Figure 1A and 1B. Changes in reflectance values along the spectrum characterize the occurrence of different levels of disease severity.

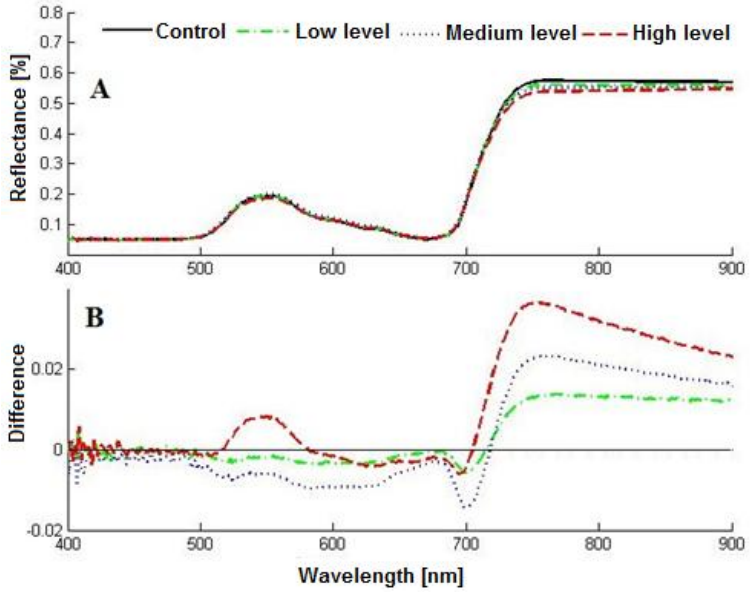

Figure 1: (A) Spectral curves for different severity levels to anthracnose for Supreme RBS. (B) Spectral reflectance difference between healthy and infected leaves.

For the Supreme RBS (Figure 1A), the spectral curve for the high level of severity showed lower reflectance in the Green band (520 to 590 nm) and in the NIR (760 to 850 nm), in relation to healthy leaves. The reduction in reflectance in the Visible band, specifically in the Green region, may be associated with the presence of the first symptoms of the disease. The lower reflectance of infected leaves, in relation to healthy leaves in the NIR, is associated with the intense degradation of the internal physical structure of the leaf.

For the low and medium levels of severity, a behaviour is similar to that of the high level was observed, characterizing the initial stages of anthracnose. At the medium level, it was possible to notice greater reflectance in the Green and Red band (630 to 685 nm), which may be associated with greater degradation, by the fungus, of the organelles that contain the pigments involved in the photosynthesis process.

The spectral differences between healthy and infected leaves (Figure 1B) were perceived along the spectrum as in the Green band, however, they were better characterized in the NIR. This indicates that there was deterioration of organelles that constitute the leaf structure, allowing greater transmittance of incident light and decreasing the reflectance of infected leaves. A significant difference was also noticed in the Red Edge band (690 to 730 nm), a characteristic region of stress in vegetation.

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Figure 2A shows the reflectance spectra in healthy leaves infected with *F. oxysporum* f. sp. *phaseoli* in the three severity levels for RBS Supreme. The spectral curves for the low and medium levels of severity showed higher reflectance values in the Green band. The high level had lower reflectance values in the Verde region. This reduction in reflectance values in the Verde region is associated with the onset of infection, which, as a root disease, interferes with the plant's nutrient and water transport system. This reduction in the Green band may be associated with a lack of sap for the leaf structure organelles.

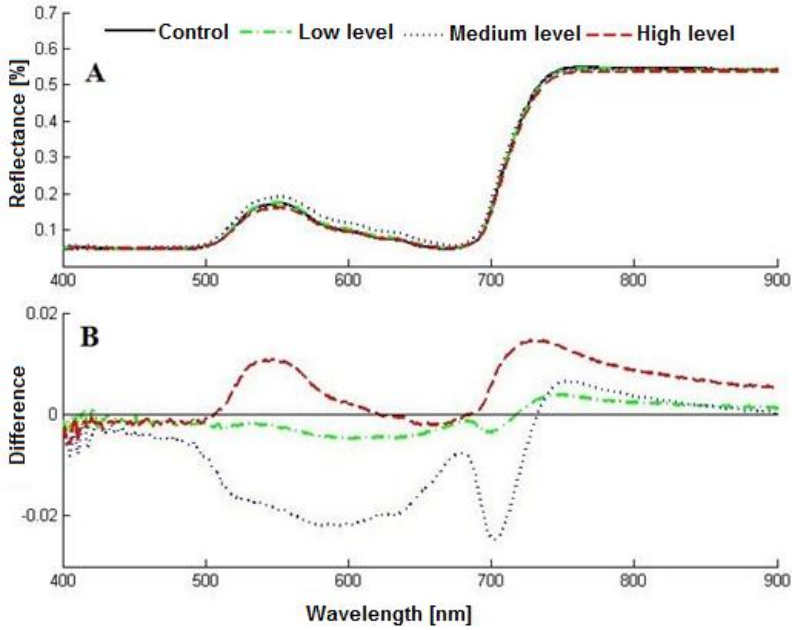

Figure 2: (A) Spectral curves for different severity levels of Fusarium wilt for Supreme RBS. (B) Spectral reflectance difference between healthy and infected leaves.

The medium level spectral curve (Figure 2A) characterized degradation of photosynthetic pigments, as observed by the greater reflectance in the Red region.

The spectral differences between healthy and *F. oxysporum* f. sp. *phaseoli* (Figure 2B) were analyzed along the spectrum. There is a significant difference in the Green and Red Edge bands. The spectral band of Green is directly related to the photosynthetic pigments, indicating a decrease in the leaf organelles responsible for these pigments. The Red Edge region characterizes a stress situation in the plant. This stress may be associated with the installation of pathogens in the plant's root system, degrading the plant's nutrient transport system.

The other cultivars studied in this work obtained results similar to those of RBS Supremo for both diseases, however, they were less expressive.

3.2 Determination of relevant wavelength bands for discrimination of anthracnose and Fusarium wilt

The data were submitted to a PCA to evaluate the most relevant wavelengths for discriminating leaves infected with *C. lindemuthianum* or *F. oxysporum* f. sp. *phaseoli*. A load factor of 0.8 of the first principal component was used to select the most representative wavelengths to discriminate between healthy and infected leaves [5] and [1].

The Table 1 shows the bands of wavelengths selected by PCA for Rudá cultivar in the three levels of severity for both diseases.

Table 1. Group of wavelengths selected by principal component analysis for the Rudá cultivar

Diseases	Severity levels	NWL (nm)	Groups of wavelengths (nm)
Anthracnose	Low	10	703 – 712
	Medium	9	703 – 711
	High	9	702 – 710
Fusarium wilt	Low	11	761 – 770; 774
	Medium	11	764 – 765; 768 – 769; 784; 801; 814; 817; 822; 831; 851
	High	11	759; 764 – 766; 768 – 770; 874 – 875; 881; 900

NWL = number of wavelengths selected by PCA.

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The Red Edge spectral band was the most representative for anthracnose, as it was present at all levels of disease severity. This can be explained by the fact that pathogens cause a reduction in the chlorophyll content in the plant due to necrosis and chlorotic lesions that affect the reflectance in this spectral region [4]. For Fusarium wilt, the most representative spectral band was the NIR (760 – 850 nm), which is an indicative region of stress on vegetation, which can be associated with a deficiency of nutrients and water that reach the leaf structures.

The Table 2 shows the PCA selected wavelengths for Supreme RBS cultivar infected with *C. lindemuthianum* or *F. oxysporum* f. sp. *phaseoli*. The most representative spectral band of anthracnose was the range from 740 to 760 nm, for the three levels of disease severity. This spectral band is close to the NIR, which can be associated with the degradation of leaf structure organelles, which affects the reflectance of the leaves.

Table 2. Group of wavelengths selected by principal component analysis for RBS Supremo cultivar

Diseases	Severity levels	NWL (nm)	Groups of wavelengths (nm)
Anthracnose	Low	11	740 – 750
	Medium	9	751 – 759
	High	10	750 – 759
Fusarium wilt	Low	10	704 – 713
	Medium	10	699 – 708
	High	11	712 – 722

NWL = number of wavelengths selected by PCA.

For *F. oxysporum* f. sp. *phaseoli*, the most representative spectral band was the Red Edge (Table 2). The relevance of Red Edge to Fusarium wilt may be associated with the fact that pathogens colonize the vessels of the nutrient transport system, causing nutritional deficiency, reducing the chlorophyll content in leaf organelles responsible for photosynthesis, affecting the reflectance of infected leaves.

For Vermelhinho cultivar, the most representative spectral band for anthracnose and Fusarium wilt was the Red Edge (Table 3). For anthracnose, these wavelength intervals are justified by the fact that the pathogen causes degradation in the leaf's photosynthetic organelles through necrosis and chlorotic lesions, reducing the chlorophyll content.

Table 3. Group of wavelengths selected by principal component analysis for Vermelhinho cultivar

Diseases	Severity levels	NWL (nm)	Groups of wavelengths (nm)
Anthracnose	Low	11	714 – 724
	Medium	12	720 – 731
	High	11	714 – 724
Fusarium wilt	Low	10	708 – 717
	Medium	10	703 – 712
	High	11	711 – 721

NWL = number of wavelengths selected by PCA.

For *F. oxysporum* f. sp. *phaseoli*, in the three levels of disease severity, the relevance of the Red Edge spectral band may be associated with the fact that pathogens colonize the plant's root structures and damage the vessels responsible for the nutrient transport system, causing nutritional deficiency in the leaves, affecting the reflectance of the infected leaves.

According to the results obtained in this work, it was possible to verify that the most expressive region of the electromagnetic spectrum for the detection of anthracnose and Fusarium wilt was the Red Edge spectral band. Thus, the Red Edge band is recommended to use the reflectance values of this spectral region to discriminate between healthy leaves and those infected with *C. lindemuthianum* or *F. oxysporum* f. sp. *phaseoli*.

IV. CONCLUSION

The spectral reflectance of common bean can be used to detect anthracnose and Fusarium wilt, aiding in the monitoring and integrated management of these diseases.

The spectral difference of the mean reflectance values showed that the regions of the Visible and Near Infrared are the spectral bands that can be used to discriminate each severity level to both diseases.

According to the results obtained in this work, the wavelengths of the spectral band of the Red Edge were the most representative for the differentiation between healthy leaves and those infected with *C. lindemuthianum* or *F. oxysporum* f. sp. *phaseoli*.

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