International Journal of Advances in Engineering and Management (IJAEM)
Volume 4, Issue 3 Mar 2022, pp: 1356-1364 www.ijaem.net ISSN: 2395-5252

Early Detection of Fusarium Wilt in Common Bean, at Three Levels of Infestation, Using Leaf Spectral Information

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Submitted: 25-03-2022 Revised: 31-03-2022 Accepted: 03-04-2022

ABSTRACT: This study aimed to develop an artificial neural network classifier to discriminate between infected and healthy leaves with fusarium wilt, at an early stage of infection, using leaf reflectance data. Five spectral bands have been proposed, which were used as the neural network input variables and selected those that best discriminated fusarium wilt. Six vegetation indexes from hyperspectral and multispectral data were tested as vectors of characteristics of the neural network. Three cultivars of common bean and three levels of severity were used. The electromagnetic spectrum was divided into five spectral bands. In each band was made a principal component analysis and with the first principal component were generated the scores of each spectral band, which were used as input variables for the neural network. The vegetation indices used were NDVI, DVI, GNDVI, MCARI, RDVI and TCARI. The most efficient classifier used the DVI index obtained from multispectral data, as input variable and with two hidden layers. The result indicated that the use of hyperspectral data did not result in any significant improvement in classification, compared with the multispectral data. The classifier did not detect fusarium wilt prematurely. The classifier was efficient in discriminating infected and healthy leaves after the first symptoms become visible. The classifier got Kappa coefficient of 0.2105 in the classification a day after the appearance of the first symptoms characteristic of the disease.
KEYWORDS: Principal components, discriminate, selection, variables, root.

I. INTRODUCTION

Common bean (Phaseolus vulgaris L.) is attacked by several diseases, including fusarium yellowing or wilt, caused by the fungus Fusarium oxysporum f. sp. Phaseoli, and which occurs practically throughout Brazil.

Fusarium wilt is a root disease (root rot) and among its main effects include reduced stand and plant vigor. Productivity losses can reach up to 80% (PEREIRA et al., 2011), increasing their incidence in areas where cultivation is intense (TOLEDO-SOUZA et al., 2009). Crop monitoring becomes crucial to prevent the disease from spreading throughout the plantation.

Remote sensing techniques can be used to monitor changes in the spectral responses of vegetation, identifying stresses in crops. In this way, it becomes an important tool for estimating plant biophysical parameters. Several studies have shown the use of leaf reflectance in the Visible (400 - 700 nm) and Near Infrared (700 - 1,000 nm) spectral region to detect changes in plant vigor with emphasis on fungal diseases (MAHLEIN et al., 2010; RUMPF et al., 2010).

The interaction of the pathogen with the plant can cause changes in the pigments, water content and functionality of plant tissues. These factors cause changes in the spectral characteristics of plants (MAHLEIN et al., 2012).

Vegetation indices (IV) are used to estimate vegetation parameters. IVs are algebraic operations between reflectance values involving two or more spectral bands. Its objective is to extract and expand information about vegetation. Rumpf et al. (2010) used eight IVs to assist in the early detection of three fungal diseases in sugar beet plants. Yao et al. (2012) used IVs to determine early stress in soybean plants caused by the use of glyphosate.

The use of hyperspectral data involves a large number of variables and one of the problems related to the high dimensionality of the data is the imposition of requirements on the sample size (number of observations). Among the existing alternatives to reduce the dimensionality of data, principal components analysis (PCA) is one of the most popular.

This work aimed to: (1) select the VIs, from simulated hyperspectral and multispectral data, most representative of the total variance of the data, which will be used as input variables of the classifier; (2) to develop and compare classifiers by artificial neural networks to discriminate leaves infected with fusarium wilt at three levels of severity.

II. MATERIAL AND METHODS.

The experiments were carried out in a greenhouse, with a controlled environment, located on the premises of the Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG, Regional Unit Epamig Zona da Mata (UREZM) and on the premises of the Federal University of Viçosa (UFV), in the city of from Viçosa, Minas Gerais.

To collect the spectral responses of bean leaves, experiments were carried out using three bean cultivars representing three groups: carioca (cv. Rudá), preto (cv. Supremo) and red (cv. Vermelhinho), given the economic importance of each group and the susceptibility to fusarium wilt.

In each pot, two plants of the same bean cultivar were grown in 0.415 L of substrate (Tropstrato HT©, Vida Verde, Mogi Mirim, SP, Brazil).

An experiment was carried out for each bean cultivar. The experimental design used was completely randomized (DIC), with four treatments (control and three levels of pathogen concentrations: 1.0 x 10^4 (low level), 1.0 x 10^5 (medium level) and 1.0 x 10^6 (high level) conidia/mL), with six repetitions: each repetition being a pot containing two bean plants inoculated with a level of pathogen concentration. The arrangement of the vases on the bench was made by drawing lots. The experiment was repeated twice.

Inoculation With Fusariumoxysporum F. Sp. Phaseolli

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

For inoculation with F. oxysporum f. sp. phaseoli, the plants were removed from the styrofoam trays, and the roots washed with running water according to the methodology presented by Dongo and Müller (1969).

After washing the roots of the plants, a third of the length of the roots was cut with the aid of scissors, then they were immersed in a suspension at a concentration of 1x10^4 (low level) conidia/mL (macro and microconidia) for 5 min. This procedure was repeated at concentrations of 1x10^5 (medium level) and 1x10^6 (high level) conidia/mL, to study the three levels of infection severity of the plants. Then the plants were transplanted into pots containing 2.5 L of substrate and taken to the greenhouse.

Plant evaluation was performed daily after inoculation, based on the scale described by Pastor-Corrales and Abawi (1987), in which: 1: no foliar or vascular symptoms; 3: 1 to 10% symptomatic leaves, mild plant wilting and vascular discoloration of the hypocotyl; 5: 11 to 25% symptomatic leaves, moderate plant wilting, extensive vascular discoloration up to the first node; 7: 26 to 50% symptomatic leaves, severe plant wilting and vascular discoloration throughout the stem and petiole and 9: dead plant.

Measurement Of Spectral Responses Of Bean Leaves

In each pot, four fully developed leaves were chosen for the spectral reflectance measurements that were made daily after inoculation, at the same time of day, between 10:00 and 14:00 hours.

During the two experiments, for the three severity levels, 4,874 reflectance measurements were performed, 1,106 of which were in healthy plants and 3,768 in infected plants.

Leaf spectral reflectance was measured with an ASD FieldSpec Pro FR spectroradiometer (Analytic Spectral Devices, Boulder, USA), with a plant probe for leaf contact measurements. 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 350-1,100 nm and the useful reading range was between 400 and 900 nm, ruling out noisy spectral data at the extremes. The calibration of the spectroradiometer using the blank 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, in each leaf, was the average of 10 readings performed by the spectroradiometer.

With the original reflectance data from the leaves, Hotelling's T^2 average test was performed in order to verify if the separation between the classes of interest was significant, at a 1% significance level.

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Volume 4, Issue 3 Mar 2022, pp: 1356-1364 www.ijaem.net ISSN: 2395-5252

CLASSIFIER DEVELOPMENT

The original data contained 751 variables (751 wavelengths) and 4,874 repetitions, the number of variables being high in relation to the number of repetitions, which must be at least 20 repetitions per variable measured (HAIR et al., 2010; SIDDIQUI, 2013).

To reduce the number of variables, the originaldataset was divided into five spectral bands, according to the spectral bands of the RapidEye system satellites.

Spectral bands	Wavelength range (nm)	Number of variables
Blue	440 – 510	70
Green	520 – 590	70
Red	630 – 685	55
RedEdge	690 – 730	40
Near infrared	760 – 850	90

The division of each sample into these five spectral bands was also due to the direct relationship that each spectral band has with constituent parts of the bean leaves. In the visible spectrum (380 to 760 nm), the change in the spectral response of the leaf is due to the variation in the content of organelles, such as carotenoids and chlorophyll. In the near infrared (760 – 1200 nm), variations in spectral responses are due to variations in the physical structure of the sheet (BAURIEGEL et al., 2011).

An ACP was applied in each spectral band to reduce the number of variables, considering the correlation between the variables in each band. In this work, the first principal component (CP1) of each spectral band explained more than 88% of the variance of the original data, and with this CP1, the scores were calculated for each sample of the dataset of each band. These score values were the characteristics used as ANN input vectors. Before performing the principal component analysis, all data were centered on the mean. Thus, each variable has a mean of zero, that is, the coordinates are moved to the center of the data, allowing differences in the relative intensities of the variables to be easier to perceive (SOUZA and POPPI, 2012).

VEGETATION INDICES

Six vegetation indices common to hyperspectral and multispectral spectrometry were calculated: Normalized Difference Vegetation Index – NDVI (Rouse et al., 1974); Green Normalized Difference Vegetation Index – GNDVI (Yang et al., 2007); Difference Vegetation Index – DVI (Tucker, 1979); Modified Chlorophyll Absorption Reflectance Index – MCARI (Daughtry et al., 2000); Transformed Chlorophyll Absorption Reflectance Index – TCARI (Haboudane et al., 2002); Relative Difference Vegetation Index – RDVI (Roujean and Breon, 1995).

The blue (B), green (G), red (R), red-edge (RE) and near-infrared (NIR) spectral bands of multispectral data were simulated by averaging the reflectance readings in the RapidEye system satellite bands.

Training And Architecture Of Artificial Neural Networks

In this work, Multi-Layer Perceptron artificial neural networks (ANN) were trained to classify bean leaves using reflectance data. The ANN was trained using the ANN toolkit of the Matlab computer program (MathWorks, Natick, USA).

Different neural network architectures were tested. The five proposed spectral bands were tested, individually and in combination, as feature vectors used as input to the ANN. The six hyperspectral and multispectral IVs used in this work were also tested, individually and in combination, as input variables of the classifier.

ANNs with architectures were developed using two intermediate layers with different numbers of neurons (n1 and n2) and two neurons in the output layer (two classes: healthy and infected). The numbers of neurons tested, both in the first and second intermediate layers, were: 2, 4, 6, 8 and 10. Thus, 25 ANNs were trained for each set of input variables, with different neurons in the intermediate layers.

The dataset had 4,874 samples, of which 2,924 were used for training, 975 for validation and 975 for testing. All datasets were chosen so that reflectance measurements of healthy and infected leaves would be present. The “early stop” method, described by Haykin (2000), was used to stop ANN training. This method uses the validation set to stop

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Volume 4, Issue 3 Mar 2022, pp: 1356-1364 www.ijaem.net ISSN: 2395-5252

updating the ANN free parameters during training and thus avoid overfitting the data. During training, the mean square error (MSE) is calculated with the training vectors and with the validation vectors. Training stops when the validation NDE starts to increase (HAYKIN, 2000). The number of iterations used to confirm the trend of increasing NDE is dependent on the problem (PRECHELT, 1998), and ten iterations were used in the present work.

Indexes	Hyperspectral	Multispectral
NDVI	$(R_{800} - R_{670}) / (R_{800} + R_{670})$	NIR – R / NIR + R
DVI	$R_{800} - R_{680}$	NIR – R
GNDVI	$(R_{800} - R_{550}) / (R_{800} + R_{550})$	IR – G / IR + G
MCARI	$[(R_{700} - R_{670}) - 0{,}2.(R_{700} - R_{550})].R_{700} / R_{670}$	[(RE - R) – 0,2*(RE - G)]*RE/R
RDVI	$\sqrt{NDVI.DVI}$	$\sqrt{NDVI.DVI}$
TCARI	$3.[(R_{700} - R_{670}) - 0{,}2.(R_{700} - R_{550})].R_{700} /R_{670}$	3*[(RE - R) – 0,2*(RE - G)*(RE/R)]

CLASSIFIER EVALUATION

The classification of the classifier was made from the test sample. From this test set it was possible to build the classification confusion matrix (CONGALTON, 1991; SOUSA et al., 2010).

Knowing that, using the Matlab ANN toolkit (MathWorks, Natick, USA), at the beginning of the training some ANN parameters are randomly generated and that these values can influence the final result of the training, each architecture was trained ten times. Among these ten trained ANNs, the one with the highest Kappa index with the test sample was chosen.

At the end of the training process, for each combination of neurons in the two intermediate layers, the test sample was used to generate the confusion matrix. From the confusion matrix, the Kappa index was calculated. It was considered as the best architecture of the ANN the one that presented the highest value of Kappa index.

To assess the difference between two Kappa indices, the Z test was used, according to Congalton and Green (1998), with a significance level of 5%.

EARLY DETECTION OF FUSARIUM

For the detection of fusarium, before its first symptoms became visible, the RNA that presented, statistically, the highest Kappa index was used.

A dataset was used with 14 samples of healthy plants, 24 samples of infected plants for each level of infestation, totaling 38 samples, for each level of disease severity. Daily, this dataset was separated for the early detection of the disease. The samples from this dataset were not part of the ANN training, validation or testing set.

III. RESULTS AND DISCUSSION

DISEASE DEVELOPMENT

The bean plants not inoculated with the pathogen, which served as a control for the experiment, remained healthy throughout the data collection period.

The inoculated plants remained without showing any symptoms of fusarium wilt during the latency period of the disease. After the latency period the typical symptoms of the disease appeared. for cv. Rudá the first symptoms appeared on the eleventh day after inoculation of the pathogens (DAI); for cv. Red, the first symptoms appeared on the twelfth DAI and for cv. Supreme the first symptoms appeared in the fourth DAI.

The most visible symptoms of fusarium wilt were observed in the aerial part of the plant, where few leaves withered, yellowed and fell from the base of the plant. The most noticeable symptom was the irregular development of infected plants, which were smaller than the control plants.

For cultivars Rudá and Vermelhinhothe most expressive symptom was observed in the growth of infected plants. Infected plants had their growth impaired by the infestation of pathogens. These plants were smaller in size than the control plants. This fact is related to the colonization of plant roots by the pathogen. The infection, caused by pathogens in the sap-conducting vessels, impairs the transport of nutrients and water to other parts of the plant, hindering its development.

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Volume 4, Issue 3 Mar 2022, pp: 1356-1364 www.ijaem.net ISSN: 2395-5252

		DAI										
Cultivar	**Levels**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Rudá	Low	1	1	1	1	1	1	1	1	1	1	1
	Medium	1	1	1	1	1	1	1	1	1	1	1
	High	1	1	1	1	1	1	1	1	1	1	1,1

		DAI									
Cultivar	**Levels**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Rudá	Low	1	1,1	1,2	1,4	2,0	2,1	2,2	2,6	2,7	3,1
	Medium	1,1	1,1	1,2	1,6	2,2	2,4	2,7	3,2	3,5	3,7
	High	1,2	1,2	1,6	1,9	2,7	3,1	3,3	3,6	3,9	4,1

		DAI										
Cultivar	**Levels**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Vermelhinho	Low	1	1	1	1	1	1	1	1	1	1	1
	Medium	1	1	1	1	1	1	1	1	1	1	1
	High	1	1	1	1	1	1	1	1	1	1	1

		DAI									
Cultivar	**Levels**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Vermelhinho	Low	1	1	1,1	1,2	1,3	1,9	2,0	2,6	2,7	3,1
	Medium	1	1,1	1,4	1,8	1,9	2,3	2,6	3,2	3,5	4,1
	High	1,1	1,4	1,7	1,9	2,2	2,8	3,1	3,7	4,2	4,8

The cv. Supremo presented the most aggressive symptoms of the disease for the high level of severity. The first characteristic symptoms appeared in the fourth DAI and in the ninth DAI the plants were completely dead. Symptoms were primarily expressed in reduced plant growth, chlorosis and leaf drop, wilting and plant death.

For low and medium levels of severity, for cv. Supreme, the symptoms observed were little loss of turgidity and reduction in plant growth.

		DAI										
Cultivar	**Levels**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Supremo	Low	1	1	1	1	1	1	1,1	1,6	2,1	2,8	2,9
	Medium	1	1	1	1	1	1	1,2	1,6	2,4	3,0	3,1
	High	1	1	1	1,2	1,6	4,3	7,1	8,4	-	-	-

		DAI									
Cultivar	**Levels**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Supremo	Low	3,2	3,3	3,7	4,0	4,2	4,9	5,2	5,5	5,8	6,2
	Medium	3,4	3,9	4,2	4,4	5,1	5,7	5,9	6,2	6,4	6,6
	High	-	-	-	-	-	-	-	-	-	-

The Fc values, calculated for Hotelling's T^2 test of the vectors of mean reflectances of the classes of healthy leaves and infected leaves are shown below. The difference between the two classes was significant at the 5% probability level for the three disease severity levels. Therefore, the development of a classifier to discriminate between the two classes may be feasible.

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Levels	Valuesof Fc
Low	9,53*
Medium	11,28*
Highe	8,57*

* = significant at the 5% probability level by Hotelling's T^2 test.

ANALYSIS BY PRINCIPAL COMPONENTS

Reducing the dimensionality of the data is a crucial point when working with large volumes of data, the use of ACP allows retaining much of the variance of large volumes of data in other variables called principal components, which is expected to be in a very large number. smaller than the original variables. Nest, it is shown how much each CP1, from each band, retained the total variance of the data.

	Blue band	Green band	Red band	RedEdge band	Infrared band
CP1	94,59	99,24	96,47	91,35	99,72

For each spectral band, the CP1 retained at least 91.35% of the total variance of the data, therefore, the CP1 of each band was used to generate the values of the scores used as the ANN input vector, without significant loss of information.

In this way, there was a reduction from 751 original variables to five new independent variables, retaining more than 91% of the total variance of the original data. Combinations of these new variables were used as input vectors for the artificial neural network.

Classifier By Artificial Neural Networks

The classifiers, which used the scores generated by the CP1 of the five spectral bands proposed in this work as vectors of characteristics, had Kappa coefficients statistically equal to zero. All classifiers performed equal to a random classification. Spectral band scores alone or in combination did not discriminate healthy plants from plants inoculated with fusarium wilt pathogens.

As for the classifiers that used IVs as the input variable, those that presented the best results in the discrimination of diseased and healthy leaves used the DVI as a vector of characteristics. The column with the Zc values (calculated Z) are greater than the tabulated Z value (1.96), indicating that the classification of both ANNs is significantly better than a random classification.

Data	n1	n2	Kappa coefficient	Variance	Zc
Hyperspectral	2	2	0,2912	0,0081	3,24*
Multispectral	2	2	0,2475	0,0092	2,58*

There was no significant difference between the values of the Kappa coefficients for the ANN using the DVI obtained from hyperspectral and multispectral data.

The classifiers that used NDVI, GNDVI, MCARI, TCARI and RDVI, alone or a combination of them, as input variable, had Kappa coefficients statistically equal to zero. These classifiers performed equal to a random classification.

It was possible to verify that the use of hyperspectral data did not imply a significant improvement in the classification when compared with multispectral data.

The DVI was more sensitive, as an input variable, in the discrimination of diseased and healthy leaves. This can be justified by the fact that the DVI obtained from multispectral data is the difference between the IR and Red bands. The colonization of common bean roots by pathogens directly affects the transport of nutrients and water to the photosynthetic organelles located in the leaves, causing a change in the spectral response of the same. The Red band is part of the visible spectrum, being sensitive to variations in the content of organelles such as chloroplasts, which were harmed by the decrease in the rate of nutrients reaching the leaves. The IR band is sensitive to variations in the physical structure of the plant, such as water content. This content was also impaired by the colonization of pathogens in the conducting vessels of the bean roots and stem.

However, it is worth mentioning that the object of study of the classification was a root

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disease that interfered little in the aerial part of the plant, causing small variations in the spectral responses of the leaves.

The class that presented the most confusion was healthy leaves, where 40.74% of the leaves that should be classified for this class were classified as infected. This confusion can be attributed to the fact that the pathogen had little effect on the bean leaves, which remained healthy throughout the data collection period.

The greatest influence of the pathogen infection was on the size of the plants. Due to the infection of the pathogen in the nutrient transport system, the infected plants were shorter than the control plants. Indicating greater influence of fusarium wilt on the physical structure of the plant, which was not detected in the foliar measurements. Perhaps a measurement of the spectral response of the canopy would show greater variation as a function of the disease.

Classes	Infested leaves	Healthy leaves	Overall accuracy
Infected leaves	**170**	33	67,08%
Healthy leaves	74	**48**	
Producer accuracy	69,67%	59,26%	
Kappa coefficient	0,2475		

EARLY DETECTION OF FUSARIUM WILT

The best classifiers, for each bean cultivar, presented significant Kappa coefficient values, showing that they were better than a random classification are shown below.

Cultivar	n1	n2	Kappa coefficient	Variance	Zc
Rudá	10	4	0,2912	0,0081	3,24*
Vermelhinho	8	8	0,2401	0,0075	2,77*
Supremo	2	2	0,2475	0,0092	2,58*

The best results obtained by the classifier in the detection of fusarium wilt were for cv. Supreme, for the medium level of severity.

The classifier was unable to discriminate fusarium wilt early. Only on the eighth DAI, one day after the first characteristic symptoms became visible, did the ANN score better than chance, with a Kappa coefficient equal to 0.2105.

The best results in the classification occurred in the fifteenth DAI, with a Kappa of 0.4932.

There was an increase in the value of the Kappa coefficient with the passing of the days after the inoculation of the pathogens. Indicating that there was variation in the spectral response of infected leaves when compared with the responses of healthy leaves. However, this variation was not enough to obtain results similar to the discrimination of foliar diseases as obtained by Rumpf et al. (2010), Bauriegel et al. (2011) and Mahlein et al. (2012).

	DAI										
Classes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Infected	33,33	37,50	33,33	41,67	41,67	45,83	54,17	58,33	62,50	83,33	79,17
Healthy	42,86	50,00	57,14	50,00	57,14	42,86	42,86	64,29	69,23	64,29	50,00
Overall accuracy	36,84	42,11	42,11	44,74	47,37	44,74	50,00	60,53	65,79	76,32	68,42
Kappa coefficient	-21,28	-11,17	-14,70	-7,55	-1,06	-10,53	-2,85	21,05	31,58	48,34	30,06

	DAI									
Classes	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
Infected	72,00	65,22	69,57	73,91	60,87	65,22	63,64	77,27	68,18	59,09
Healthy	53,85	61,54	69,23	76,92	69,23	69,23	61,54	53,85	61,54	78,57
Overall accuracy	65,79	63,89	71,05	75,68	64,71	66,67	61,76	69,70	64,71	65,65

Kappa coefficient	27,57	25,28	40,46	49,32	30,61	32,97	20,79	31,82	25,55	35,29

From the first to the seventh DAI, the Kappa coefficient values indicated a worse classification than a random classification. This may be related to the fact that the pathogen penetrates the plant through the root. As the pathogen infected the young plant, the only symptom detected was a reduction in its development. Little loss of turgor was detected during the experiment.

Based on the results obtained in this work, it was possible to verify the possibility of discrimination between leaves infected with fusarium wilt pathogens and healthy leaves, after the first symptoms became visible.

For the early stages of the disease, RNA was not efficient in the classification. Compared with results obtained by other studies (RUMPF et al., 2010; BAURIEGEL et al., 2011; MAHLEIN et al., 2012), which used foliar diseases, it is clear the need to implement new methodologies that help remote sensing techniques in the early detection of root diseases in bean plants.

IV. CONCLUSION

The best classifier was the one that used as input variable the DVI vegetation index obtained from simulated multispectral data.

There was no significant improvement in classification when using IVs obtained from hyperspectral data, when compared with IVs obtained from multispectral data.

The classifier was able to discriminate between infected and healthy leaves, with a Kappa coefficient of 0.2105, after the first symptoms of the disease became visible, for cv. Supreme at medium severity level.

The classifier did not show efficiency in the early discrimination of fusarium wilt.

Future works proposing new methodologies together with remote sensing techniques may be carried out in order to increase the accuracy in the early detection of root diseases in bean plants.

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