VEGETATION INDICES FROM ACTIVE CROP CANOPY SENSOR AND THEIR POTENTIAL INTERFERENCE FACTORS ON SUGARCANE

L. Taubinger, L.R. Amaral, J.P. Molin

Biosystems Engineering Department, "Luiz de Queiroz" College of Agriculture
University of São Paulo
Piracicaba, São Paulo, Brazil

ABSTRACT

Among the inputs usually used in sugarcane production the nitrogen (N) is the most important due to its highly variable response. In sugarcane the use of canopy sensors to N management is a possibility as it is done in other crops. However, because the sugarcane is different from the crop that the sensors were initially developed, it is necessary to evaluate the efficiency of different vegetation indices (VI) as estimators of N nutrition, as well as to identify potential interference factors. Therefore, studies with canopy sensors have been developed in Brazil for sugarcane since 2007. The objective of this study was to evaluate the performance of seven VIs for sensing N status and to evaluate the effect of daytime and the conditions of substrate and wetness on leaves. It was not possible to identify a behavior pattern of VIs on measurements throughout the day. The index MCARI/OSAVI was not affected by the substrate and by the water on the leaves and it was able to identify the levels of N, although it had weak relationship with the chlorophyll content in leaves. NDVI, NDRE and Yara ALS were efficient in identifying the N rates, showing high correlation with chlorophyll content, but susceptible to interference factors.

Keywords: Proximal sensing, nitrogen application, multispectral sensor, sensor interferences

INTRODUCTION

Sugarcane (Saccharum spp.) is the most important crop in sugar and ethanol production in tropical and subtropical regions, accounting for approximately 80% of world sugar production and about 35% of global ethanol production (FAO, 2011). Brazil is the main producer, within 570 million tons produced in 7.1 million hectares (Agrianual, 2010), accounting for more than a third of world production (FAO, 2011), with great economic, social and environmental importance. The application of more efficient processes, which increase yield and reduce production costs, mainly by lowering inputs use, is crucial for the development of the sector.

Under Brazilian conditions generally soil analysis for nitrogen (N) recommendation is not used. The recommendations are made based on soil type, variety and age of the field (plant cane or stubble cane – number of ratoons), without taking into account the availability of N in the soil and its spatial variability. Thus, the use of canopy sensors is an alternative to the traditional recommendation of N (Amaral and Molin, 2011).

The use of canopy sensors has been effective in N fertilization in different crops (Raun et al., 2002; Kitchen et al., 2010; Vellidis et al., 2011). However, in sugarcane, this technique is still a challenge (Molin et al, 2010; Amaral and Molin, 2011; Lofton et al, 2012).

Bausch and Brodahl (2012) indicate that several vegetation indices (VI) have been evaluated and developed to enable the N management during the growing season in different crops. Among the many factors that affect the reflectance of crops and consequently the vegetation indices are the stress, climate, soil and plant factors.

Eitel et al. (2008), working with wheat, concluded that simple indices such as NDVI and CI are influenced by other factors such as the amount of biomass and the influence of the substrate, while the compost index MCARI/MTVI2, which takes into account the reflectance of specific wavelength bands of blue, green, red and near infrared, better correlates with the N status.

For corn, Wu et al. (2008) found the same result using the compost index MCARI/OSAVI, which was more appropriate for estimating the chlorophyll content in the leaves.

Thus it is necessary to conduct studies to understand the behavior of vegetation indices that better express the nutritional status of sugarcane and are less susceptible to the influence of other variables. Thus, the objective of this study was to verify the influence of different substrates, the effect of wetness on leaves during the measurements and the variation throughout the day in different vegetation indices calculated from canopy sensor, as well as to assess the effectiveness from the VIs in identifying N rates applied to the sugarcane.

MATERIALS AND METHODS

The study consisted of experiments in greenhouse and in field with sugarcane (*Saccharum spp*.). In the greenhouse (daytime experiment) the effect of measurements made with canopy sensor at different moments of the day was analyzed. In the field the effects of N rates, substrate types and wetness on leaves in the VIs obtained with canopy sensor were analyzed.

The measurements were performed with the canopy sensor (CropCircle, Model ACS-470, Holland Scientific, NE, USA), which provides the reflectance at wavelengths of 450, 550, 650, 670, 730 and 760 nm, by exchanging optical filters and sensor calibration and the vegetation indices used were obtained from those wavebands (Table 1).

Daytime experiment

The experiment was conducted in a greenhouse of the Biosystems Engineering Department, ESALQ/USP, Piracicaba, SP, Brazil (22° 42' S - 47° 37' W). Three varieties (CTC 9, SP 90-3414 and RB 855156) were planted in January 2009 in pots of 0.5 m^3 with medium textured soil. Pots received water via drip irrigation, keeping the soil moisture at field capacity.

To check the influence of the moment of the day on the different VIs, due to possible plants physiological changes along the day, the measurements were taken every two hours, between 6:00 h and 20:00 h. Three measurements were obtained (around 100 values per reading) at different points in each pot in each daytime reading.

At the same time readings with portable chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Sakai, Osaka, Japan) also performed. Two diagnostic leaves were adopted for comparison purposes, the TVD (top visual diulep - leaf +1) and the other leaf was two expanded leaves below (leaf +3 - oldest leaf). A measurement in the middle of the leaf blades on five distinct leaves was realized per pot.

on all evaluations the reflectance measurements were realized adapting the sensor to a support leg, so all the measurements captured the reflectance from the same plant site. Similarly, the leaves measured with the chlorophyll meter were marked, performing the readings always on the same leaves.

Due to lack of true replicates (one pot for each variety), even working with readings in different places in the pots, it is a concern that using analysis of variance and mean comparison tests would be inconsistent. Thus, the mean values for each daytime was calculated and the confidence interval for the mean (95%) was estimated and the graphs were plotted for visual analysis.

Table 1. Vegetation indices used with indication of their respective authors; due to the available optical filters, some changes were made in this work: between wavelength 760 and 800 nm was used 760 nm, between 550 and 590 nm was used 550 nm and between 700 and 730 nm was used 730 nm.

Vegetation Index	Equation		Reference
NDVI	$(R_{760} - R_{670})/(R_{760} + R_{670})$		Rouse et al. (1974)
CI	$(R_{760}/R_{590}) - 1$		Gitelson et al. (2005)
GNDVI	$(R_{780}-R_{550})/(R_{780}+R_{550})$		Gitelson and Merzlyak (1996)
Yara ALS	$100(\ln(R_{760}) - \ln (R_{730}))$		Jasper et al. (2009)
NDRE	$(R_{760} - R_{730})/(R_{760} + R_{730})$		Barnes et al. (2000)
MCARI/MTVI2	MCARI	$[(R_{700}-R_{670}) - 0.2(R_{700}-R_{550})](R_{700}/R_{670})$	Eitel et al. (2008)
	MTVI2	$\{1.5[1.2(R_{800}-R_{550}) - 2.5(R_{670}-R_{550})]\} / \{\sqrt{[(2R_{800}+1)^2 - (6R_{800}-5\sqrt{R_{670}}) - 0.5]}\}$	
MCARI/OSAVI	MCARI	$[(R_{700}-R_{670}) - 0.2(R_{700}-R_{550})](R_{700}/R_{670})$	Wu et al. (2008)
	OSAVI	$(1+0.16)(R_{800}-R_{670}) / (R_{800}+R_{670}+0.16)$	

Field experiments

The objectives of the field experiments were to identify potential factors that affect the different VIs obtained by the canopy sensor. The experiments were divided into: effects of N rates, influence of the substrate and from the wetness of the leaves.

Effect of nitrogen rates

Canopy sensors have been designed for identifying N nutrition of crops like wheat and corn, which have leaf architecture and development behavior different from sugarcane. Because of that it is necessary to examine if some VI could be more efficient in identifying sugarcane N response.

In order to reach that, an experiment with N rates conducted by São Paulo Agency of Agribusiness Technology (APTA) and Agronomic Institute of Campinas (IAC), Piracicaba-SP, Brazil (22°41´ S – 47°38´ W) was evaluated. The plots consisted of five 10 m long sugarcane rows, with four replications in randomized blocks. Treatments were the application of four N rates (0, 50, 100 and 150 kg N ha^{-1}), and the variety grown was IAC 87-3396. The evaluation was made in the second ratoon (first stubble) of the crop with the application of such treatments for two consecutive growing seasons.

The assessment with portable chlorophyll meter (SPAD-502) was also done in the same time following the procedure described previously (Daytime experiment) but with 20 readings per plot.

The evaluation occurred when the plants were 0.5 m average stem height (Amaral and Molin, 2011; Portz et al., 2012). The sensor was maintained at an average distance of 0.8 m from the canopy, driven manually with a collection frequency of 10 Hz. The data were analyzed by analysis of variance and when significant, comparison means test was proceed (Scott-Knott at 5%), regression analysis and linear correlation by SISVAR statistical software (Ferreira, 2011).

Substrate influence

The area measured by the sensors is variable in function of the height and biomass of the crop, so not always the emitted light beam hits only the plant canopy, also capturing reflectance from soil and residue, which could cause noise in the measured values. Seeking to verify this influence, readings were taken on different substrates in a 10 m long sugarcane row of variety CTC2 in the fourth ratoon (third stubble), with average stem height of 0.5 m.

The substrate conditions were: sugarcane straw originated from mechanized harvesting deposited on the ground (14 Mg ha^{-1}); manual removal of straw, exposing the clay soil (dark red); deposition of sand on the soil surface, to simulate the surface reflectance of a sandy soil. Six dynamic measurements were realized (six replications) on each substrate condition, with the sensor kept at an average distance of 0.8 m of the canopy, driven manually with a sampling frequency of 10 Hz.

The data were submitted to analysis of variance and comparison means test (Scott-Knott at 5%) by SISVAR statistical software (Ferreira, 2011).

Influence of water on the leaves

Sugarcane producers need to fertilize large areas, working 24 hours a day. Thus, even under conditions of light rainfall or in the presence of dew, the operation cannot be interrupted. Therefore, we must examine if there is influence of the wetness on leaves in the measurements with canopy sensors, and if there are some VI that reduces this effect.

We used a backpack sprayer equipped with a large drop diameter nozzle generator to simulate rainfall (32.4 mm h^{-1}). Readings with the sensor were performed before (dry), during (rain) and after (dew) the rainfall simulation (Fig. 1). For each condition four static measurements were taken (about 600 values) in four distinct spots of a field planted with the variety CTC2 in the fourth ratoon.

The data were submitted to analysis of variance and comparison means test (Scott-Knott at 5%) by SISVAR statistical software (Ferreira, 2011).

Fig. 1. Collecting data with the sensor before (A) and during (B) the rainfall simulation; wet leaves after the rain simulation (C).

RESULTS AND DISCUSSION

Daytime experiment

In the measurements throughout the day (Fig. 2) it was not possible to find a behavior pattern that could be explained by plant physiology and/or remote sensing. If the behavior was analyzed for each variety, a behavior pattern as function of daytime could be inferred. However, analyzing the behavior of more than one variety this false assumption was avoided.

About the VIs, the large variation observed between daytime must have occurred mainly by small, however, the existing change in position and angle of the sensor in relation to the leaves of plants in each measurement. For the SPAD values the wide variation occurred due to the large variability in the readings taken on the same leaf.

It could be observed that when the wavelengths were the same (eg. CI and GNDVI or NDRE and Yara ALS) the response was very similar. Moreover, using the same equation with different wavelengths, the behavior was different (eg.

NDVI, GNDVI and NDRE). These findings concerns when considering the possibility of adapting the original VIs in function of optical filters available in a

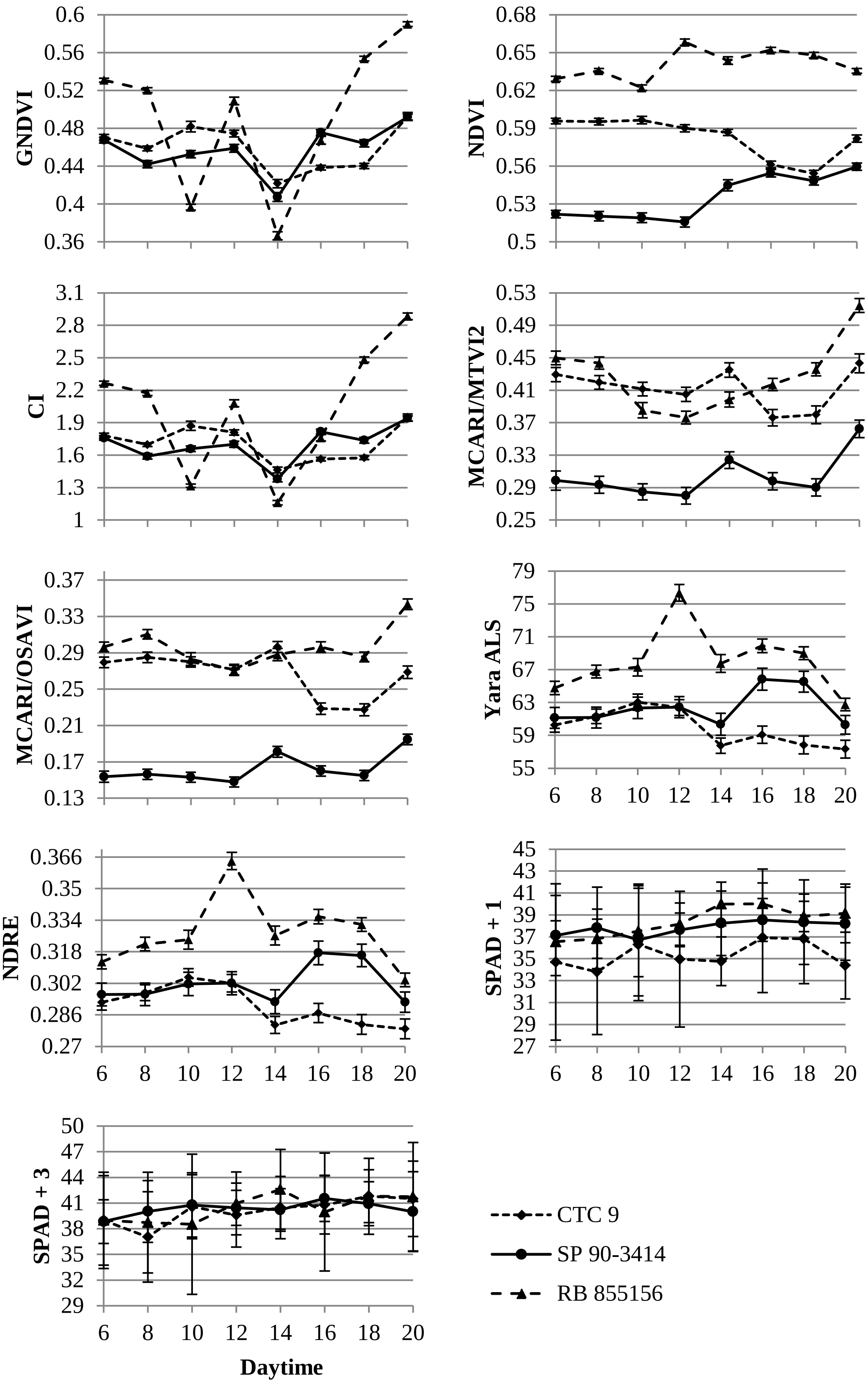

Fig. 2. Vegetation indices and SPAD values obtained throughout the day (from 6:00 to 20:00 h) and their confidence intervals in the three sugarcane varieties studied.

given study. In this kind of study the results must take into account the wavelengths actually used, not only the name of the studied VI. Such identification was efficiently realized, for example, in the work by Wu et al. (2008) and Shiratsuchi et al. (2010), who differentiated the VIs in their initial configurations from VIs that had some adaptation on spectral bands, like $NDVI_{red}$ and $NDVI_{red\text{-}edge}$.

In the conditions of this study it was not possible to identify a standard behavior of VIs measured throughout the day. However it is not possible to say that daytime interference does not exist. In conditions of reduced water availability the plants tend to reduce their metabolism during the hottest hours of the day, as also rolling the leaves to reduce water loss by transpiration (Lisson et al., 2005). Because of this change in leaf architecture the reflectance may be changed. This mechanism can vary considerably between varieties and can be correlated with water stress tolerance (Inman-Bamber, 2004), hence the importance of studying different varieties. Thus, more studies should be conducted to really dispose this interference factor.

Field experiments

Effect of nitrogen rates

The VIs were distinct in their capability in identifying the N rates (Table 2). CI, GNDVI and MCARI/MTVI2 were inefficient in capturing the different N rates ($p > 0.05$). Eitel et al. (2008) stated that the MCARI/MTVI2 was a good estimator of chlorophyll and N leaf for wheat. However, in the conditions of this study the VI was not able to differentiate N rates.

Moreover, Wu et al. (2008), also working with wheat and some VIs, found that MCARI/OSAVI was the best one for determination of chlorophyll in the leaves by satellite images. This VI was efficient in identifying the N rates in the present study, similar to the NDVI, Yara ALS and NDRE.

It was possible to observe high difference between the treatments with N and no N treatment, explained by the low response to N that occurs in many Brazilian situations (Cantarella et al., 2007).

Emphasis should be given to the CI, where previous studies have found great similarity with NDVI, both calculated from the wavelengths of the 590 nm (amber) and 880 nm (Solari et al., 2008; Shiratsuchi et al., 2010; Amaral and Molin, 2011). However, when working with wavelengths in the visible region nearest to the originals, respectively 560 and 670 nm for CI (Gitelson et al., 2003) and NDVI (Rouse et al., 1974) this high similarity did not happen, corroborating what was observed also in the daytime experiment. Thus, the setting to the CI used in this study (green) made it unable to identify plant N nutrition, as also was highly variable with CV higher than 13%.

SPAD measurements for both the leaf +1 and leaf +3 were able to identify difference between N rates better than the VIs from the canopy sensor (Table 2). Analyzing the relationship of VIs with leaf chlorophyll content (SPAD values) we observed that MCARI/OSAVI had a reduced effectiveness in relation to the others VIs (Table 3). These data show that the correct estimation of sugarcane N demand based on canopy sensors is still a challenge.

Table 2. Mean values for the vegetation indices and SPAD measured in the leaves +1 and +3 in the different N rates; analysis of variance (ANOVA), linear and quadratic regression (p<F)

N rate (kg ha^{-1})	Vegetation indices							SPAD	
	NDVI	CI	GNDVI	MCARI/ MTVI2	MCARI/ OSAVI	Yara ALS	NDRE	Leaf + 1	Leaf + 3
0	0.475 a	1.783	0.462	0.342	0.194 a	53.02 a	0.259 a	45.278 a	45.015 a
50	0.557 b	1.859	0.480	0.362	0.239 b	62.38 b	0.302 b	46.173 a	47.780 b
100	0.584 b	2.051	0.503	0.372	0.252 b	65.84 b	0.318 b	47.058 b	49.688 c
150	0.599 b	2.235	0.525	0.370	0.251 b	68.56 b	0.330 b	48.508 c	50.038 c
ANOVA	0.008	0.130	0.179	0.229	0.038	0.004	0.003	0.001	0.001
Linear regression	0.002	-	-	-	0.012	0.001	0.001	< 0.001	< 0.001
Quadratic regression	0.127	-	-	-	0.116	0.160	0.150	0.436	0.090
CV (%)	7.25	13.07	7.75	5.76	11.28	6.95	6.56	1.45	2.65

(1) Different letters indicate difference between the means of treatments by Scott-Knott test at 5%

There is not a local consensus yet about the leaves to be taken for evaluation. In this study, the leaf +3 showed a better adjustment of the quadratic function as well as lower RMSE and higher R^2, which may be an indication that this leaf is most suitable for estimation of the sugarcane nutrition status. Because this element is movable in the plant, older leaves must be able to identify the lack of N earlier, so the leaf +3 may be preferred. The relationship between VIs and SPAD was better than that observed by Eitel et al. (2008) in wheat. However, it is necessary to develop other studies to prove the effectiveness of the VIs in estimation of N status and the best leaf to be sampled in the sugarcane.

Substrate influence

It was observed variable influence of substrates in the VIs (Table 4). The results corroborate Wu et al. (2008) and Eitel et al. (2008) to whom the indices MCARI/OSAVI and MCARI/MTVI2 were not influenced by the substrate. Also, the NDRE was the VI that presented the best results, evidenced by low values changes observed between treatments. The index Yara ALS was also able to minimize the influence of substrate. CI again showed inconsistence of data and a high coefficient of variation (CV). Greater variation in the CV to *amber* CI was also observed by Amaral and Molin (2011) in comparison with *amber* NDVI.

However, even showing high CV, this VI configuration was able to identify N rates applied.

NDVI and GNDVI were susceptible to substrate interference, probably due to the strong influence of wavelengths in the visible region of these indices. Thus, changing the color of the substrate, also changes the reflectance values from canopy/substrate and consequently the VI values. Huete (1989) reports that the ground contributions of vegetation spectral response vary within the quantity exposed, surface condition and their intrinsic properties, such as the mineralogical, organic and water absorption characteristics.

Table 3. The RMSE and R^2 for ANOVA significant vegetative indices used as regression estimators of N rates and SPAD values in leaf +1 and +3

	N rate		SPAD +1		SPAD +3	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
NDVI	46.2	0.346	2.045	0.262	2.331	0.339
MCARI/ OSAVI	52.2	0.163	2.365	0.013	2.780	0.060
Yara ALS	48.0	0.293	1.984	0.305	2.307	0.353
NDRE	47.4	0.311	1.965	0.318	2.273	0.372
SPAD +1	48.5	0.277	-	-	-	-
SPAD +3	38.3	0.550	-	-	-	-

Grohs et al. (2009), working with *red* NDVI identified that there was interference when substrate conditions were corn or soybean straw. This shows that changes in substrate must be carefully observed by the users of canopy sensor technology for nitrogen fertilization, especially in crop stages with significant visible substrate.

Influence of water on the leaves

Variable influence from water conditions on the leaves was observed within the different VIs (Table 5). This may be a concern in field conditions, because the operation of fertilizer application has a short period for implementation and must not be interrupted. Thus, N application based on canopy sensors can be susceptible to errors in dosage when rain events occur, both during the rain or while the leaves are not completely dry.

Table 4. Vegetation indices values observed in different substrate conditions: clay soil exposed, straw and sand on the surface

	NDVI	CI	GNDVI	MCARI/MTVI2	MCARI/OSAVI	Yara ALS	NDRE
Straw	0.633 a	2.46 a	0.549 b	0.444 a	0.329 a	68.61 a	0.330 a
Sand	0.643 a	1.79 a	0.471 a	0.450 a	0.358 a	67.94 a	0.327 a
Clay soil	0.673 b	2.67 a	0.558 b	0.458 a	0.348 a	73.95 a	0.353 a
CV %	3.000	27.8	9.890	7.820	9.520	9.84	8.660

		0					
standard error	0.008	0.26	0.021	0.014	0.013	2.82	0.012

(1) Different letters indicate difference between the means of treatments by Scott-Knott test at 5%

NDVI, Yara ALS and NDRE were influenced by treatments, indicating that water conditions at the time of evaluation with canopy sensors is a source of error and should be carefully managed. For the remaining VIs no significant interferences were observed, probably due to the high CV, which represent an inconsistency of the data.

This influence of water on the canopy reflectance of the plants was also observed by Madeira et al. (2001), that working with grasslands found that the dew and the presence of water on the plant canopy increased reflectance in the visible (VIS) and decreased in the mid-infrared (MIR) and near infrared (NIR).

Table 5. Vegetation indices for rainfall conditions, dew and dry

	NDVI	CI	GNDVI	MCARI/MTVI2	MCARI/OSAVI	Yara ALS	NDRE
Rain	0.554 a	1.02 a	0.330 a	0.458 a	0.439 a	55.98 a	0,272 a
Dew	0.580 b	1.00 a	0.303 a	0.445 a	0.435 a	58.17 b	0.283 b
Dry	0.613 c	1.41 a	0.379 a	0.481 a	0.437 a	61.12 c	0.296 c
CV %	2.240	31.79	24.340	12.960	11.570	2.07	1.920
Standard error	0.007	0.18	0.041	0.030	0.025	0.61	0.003

(1) Different letters indicate difference between the means of treatments by Scott-Knott test at 5%

Moreover a drizzle decreased the reflectance in the near infrared (NIR). These changes influence the values obtained by the VIs and could be a problem to the user of this technology.

CONCLUSIONS

In the conditions of this study it was not possible to identify behavior patterns of VIs in the measurements throughout the day.

Among the studied VIs, MCARI/OSAVI showed interesting results. It did not show interference of the substrate and the wetness on leaves, and was effective in identifying the N rates. However, its relationship with leaf chlorophyll content was low.

NDRE and Yara ALS were similar in all analyzes performed, being efficient in identifying the N rates, with high correlation to the chlorophyll leaf content, but they were sensitive to wetness on leaves. The NDVI showed the same characteristics, but unlike the two previous VIs, it was also sensitive to variations in substrate.

CI, GNDVI and MCARI/MTVI2 showed variable sensitivity to substrate conditions and wetness on leaves while they were not efficient in identifying the N rates, therefore they should not be used for nitrogen status.

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