Multispectral aerial images for the evaluation of maize crops
Imagens aéreas multiespectrais para avaliação da cultura do milho

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ABSTRACT
The combination of multispectral aerial images and computational processing is emerging as one of the solutions used in precision agriculture to observe the nutritional status of plants. The objective of this study is therefore to associate the nitrogen content and dry weight of the aerial part of maize plants (DW) with the vegetation indices obtained by multispectral aerial images (NDVIA and NDREA), and with the SPAD index and the Greenseeker NDVI, in the vegetative stage V6. To this end, randomized blocks in a factorial scheme of 6x4 (six nitrogen doses at the base and four different flight altitudes) were used, with three replications. The collected data was submitted to ANOVA with the F test (p = 0,05) and subsequent regression analysis. The study showed that it is possible to estimate the dry weight of the aerial part of maize plants and the nitrogen content in the leaves through the processing of multispectral aerial images, using the NDVI and NDRE spectral vegetation indices. The portable chlorophyll meter SPAD (model SPAD-502) also had promising results in the estimation of nitrogen content, while the Greenseeker NDVI sensor accurately estimated nitrogen content and dry weight.

Index terms: Zea mays; precision agriculture; nitrogen; computational processing.

INTRODUCTION
The growing need for food at the global level reveals the need to intensify agricultural production systems and increase the efficiency of both fertilization and the nutrient uptake by plants (Hinsinger et al., 2011). Understanding how grain production is influenced by the limitations of nutrients will therefore contribute to a better management of nutritional deficiencies in crops (Felicity et al., 2014).

Technological advances have made it possible to employ computational techniques in conjunction with remotely piloted aircraft (RPA) to improve agricultural management. RPAs may be used to obtain aerial images, enabling greater precision in agriculture (Moretto et al., 2016). In conjunction with the images, spectral vegetation indices (VIs) can also be used, which can reveal different behaviors of the plant through calculations performed on sets of pixels in the image (Honda; Jorge, 2013).

Precision agriculture encompasses solutions to check the nutritional status of plants, streamlining the application of nutrients. Foliar analysis methods can be employed to assist in this process. The current methods used to establish the quantity of chlorophyll, dry weight...
and nitrogen (N) in plants are laborious, since they require the extraction of samples and laboratory analysis (Schefer et al., 2016). Another alternative is the use of portable equipment, such as the Greenseeker NDVI and SPAD-502, which can be used to quantify weight or for foliar analysis. These devices have shown good results for the maize crop (Vargas et al., 2012). However, since individual readings of the plants need to be carried out, this becomes a time-consuming process, in addition to being sensitive to equipment handling conditions. It is also worth noting that while N is one of the most influential nutrients in maize yields, its improper use has a great polluting potential (Magalhães et al., 2014).

Due to the limitations of the traditional methods, either because they are laborious or require the destruction of samples, the method proposed in this work seeks to demonstrate that it is possible to gather information to check the nutritional status of plants in an automated way. RPAs could be used to this end, which have undergone great technological advances regarding data communication speeds, load capacity and flight autonomy (Santana et al., 2019).

The technological advances of RPAs have been remarkable in recent years, both for applications relating to land use, as in issues inherent in the reduction of environmental damage, reducing the need for inputs and maximizing profits (Honda; Jorge, 2013). An increasingly efficient application of N during the crop cycle is also being pursued, since this nutrient promotes the development of the plant, while a low availability of this nutrient prevents higher yields in maize (Vian et al., 2018).

Considering the above, the objective of this study is to associate the nitrogen content and dry weight of the aerial part of maize plants with the vegetation indices obtained by multispectral aerial images gathered at different flight altitudes, and also with the SPAD index and the Greenseeker NDVI, in the vegetative stage V6.

**MATERIAL AND METHODS**

**Study location**

The data collection was carried out from August 2018 to January 2019 in an experimental area located in the northwestern region of the state of Rio Grande do Sul, in the municipality of Rio dos Indios, in an area with the following geographical coordinates: Latitude 79°14’42.3”; - Longitude 52°49’16.6” and an altitude of 528 m (Google Earth, 2018).

**Treatments and experimental designs**

Different nitrogen fertilizations were applied to the maize crop to produce variability in the plants’ nitrogen content and dry mass and to evaluate the vegetation indices to detect these variations. The fertilizations consisted of six doses of N (0, 10, 20, 30, 40 and 50 kg ha⁻¹).

A randomized complete block design in a factorial 6x4 scheme (six doses of N at the base and four different flight altitudes: 30, 60, 90 and 120 meters from the ground) were used, with three replications.

Each plot consisted of 14 rows with a spacing of 0.45 cm between rows, five meters in length and 6.3 m in width, totaling an area of 31.5 m² per plot. In addition, only the central region of each plot was considered as useful area, i.e., the three central meters of the eight central lines, totaling 10.8 m² of useful area.

Nitrogen fertilization was applied manually and only in the useful area so as to avoid contamination between treatments due to the high mobility of N in the soil. The N source used was urea, containing 45% of N in its composition. N was applied according to the instructions of the fertilization and liming manual for the states of Rio Grande do Sul and Santa Catarina (Sociedade Brasileira de Ciência do Solo – SBCS, 2016). The yield expectation of the experimental area was 10 tons per hectare. The maize hybrid used in the experimental area was DKB 230 PRO 3.

**Response variables**

The plants’ chlorophyll contents were determined by the readings of a chlorophyll meter of the Konica-Minolta brand, model SPAD-502 (SPAD). The readings were made in the vegetative stage V6. Readings were performed in 15 plants per plot. In addition, the readings were made on the last leaf fully expanded, in the points in the middle third, next to the central vein. The readings were made in the morning, between 10 and 12 o’clock.

Fifteen readings were also performed in each plot in the vegetative stage V6 with the portable meter Greenseeker NDVI, which estimates the health and vigor of crops by reading the NDVI index. The index used by Greenseeker NDVI can vary from 0 (zero) to 0.99, measuring the response of the plant to certain light stimuli. In this regard, the portable meter was positioned between 0.80 meter and 1 meter above plants. Furthermore, all readings were made under natural lighting conditions, also in the morning, between 10 and 12 o’clock.

The multispectral images were obtained in the vegetative stage V6, with a DJI Matrice 200 RPA with an embedded multispectral camera of the brand Micasense model RedEdge-M. This camera has a focal distance of...
6 mm, and a Ground Sample Distance (GSD) of 8 cm per pixel for flights at an altitude of 120 meters from the ground. The images were captured in the overlap mode at 80% (front and side) and in five spectral bands: Red (R: 668nm–678nm), Green (G: 560nm–580nm), Blue (B: 475nm–495nm), Near Infrared (NIR: 840nm–880nm) and RedEdge (717nm–727nm). The image resolution was 1280x960 pixels, and they were stored in the TIFF format.

The images were captured at four different flight altitudes with the RPA, namely: 30, 60, 90 and 120 meters from the ground, between 11h30min and 14h30min, in days with few clouds and under natural lighting. The flight altitudes used in this study were established considering Brazilian regulation of flights to remotely piloted aircraft, as well as their security measures (Agência Nacional de Aviação Civil – ANAC, 2017). It is important to remember that Brazilian law authorizes flights up to 120 meters from the ground in rural areas, as long as safety standards are met. In addition, calibrations of the camera were performed before and after the flight with a calibration panel that accompanies the Micasense RedEdge-M multispectral camera. The multispectral images were processed with the Pix4DMapper software.

The spectral vegetation indices used in processing of multispectral aerial images were Normalized Difference Vegetation Index (NDVI) and Normalized Difference Red Edge Index (NDRE). The NDVI index is a vegetation index obtained through mathematical equation between the sum between infrared and red bands values, divided by the sum between infrared and red bands values. Meanwhile, NDRE is obtained through mathematical equation between the difference of infrared and rededge bands, divided by the sum between infrared and red edge bands values. In addition, in both indices, values can vary between -1 and 1, with negative values are associated to clouds, snow and soils, while positive values are related to vigor (biomass), chlorophyll contente and health of the culture analyzed.

In order to perform the laboratory analysis of the nitrogen content, the last completely expanded leaf was collected from three random plants of each plot, in the vegetative stage V6. All samples were dried in an oven at a temperature of 65 °C and subsequently analyzed in laboratory to determine total nitrogen through the Kjeldahl method.

The dry weight of the plants was determined in the vegetative stage V6. To this end, the aerial part of three plants were randomly collected from each plot. These plants were dried in an oven at a temperature of 65 °C. The three dried samples from each plot were weighed in a precision scale. The collected data was submitted to analysis of variance with the F test (p = 0.05). The significant results of the analysis of variance were evaluated by the regression test. The statistical software Statística and the SISVAR software (Ferreira, 2011) were used for these analyses.

**RESULTS AND DISCUSSION**

The first analysis refers to the dry weight (DW) and nitrogen content responses in relation to different nitrogen doses (N) applied during sowing. Subsequently, the existence of statistically significant differences between the data collected at different flight altitudes was verified. To this end, the data obtained in the V6 stage were submitted to analysis of variance by the F test (p ≤ 0.05), with the significant results being evaluated through a regression analysis.

As expected, the DW (R² = 0.87) and the N content (R² = 0.92) reacted positively to increasing N doses, demonstrating the appropriate effect of treatment, since the maize hybrid used is a simple hybrid that is responsive to nitrogen fertilization. It is worth noting that the goal of the N doses was to create variability in the N concentrations of the leaves and in the amount of DW. The different quantities of DW and N content were therefore used as treatments for the statistical analyses in this subsection. As such, it was possible to analyze the spectral vegetation indices (VIs) and the different flight altitudes.

The analysis of variance showed a significant effect (p ≤ 0.05) of the factors DW and flight altitude for the variable NDVI of the multispectral aerial images (NDVIA), with the interaction between factors not being significant (p > 0.05). The analysis of variance also showed a significant effect (p ≤ 0.05) of the factors DW and flight altitude for the variable NDRE of the multispectral aerial images (NDREA), with the interaction between the factors not being significant (p > 0.05). The analysis of variance showed a significant effect (p ≤ 0.05) of the factors N content and flight altitude for the variable NDVIA, with the interaction between the factors not being significant (p > 0.05). The analysis of variance also showed a significant effect (p ≤ 0.05) of the factors N content and flight altitude for the variable NDREA, with the interaction between the factors not being significant (p > 0.05).

As it can be observed in Table 1, the higher altitudes showed greater accuracy in the estimates. It was therefore necessary to ascertain whether there was a statistical difference between them. As such, the Tukey test was performed to evaluate NDVIA and NDREA at the four flight altitudes, for both DW and N content, which revealed no difference between the two higher flights altitudes, enabling the choice between either of the two.
Considering the results obtained until now, one can see that the higher flight altitudes (90 and 120 meters) had a higher coefficient of determination in the analyses. This is due to the fact that images captured at greater heights have lower noise levels, i.e., lower exposure to dust, shadows and other variables that might cause interference in the images. The images captured at higher altitudes from the areas of interest (field) also have greater homogeneity, and it is not possible to differentiate soil, shadows and maize plants at the V6 stage. This is in agreement with Reips and Gubert (2019) and Moretto et al. (2016), who argue that in the processing of images for the extraction of information, unnecessary elements and aspects that are not part of the analysis should be removed. In addition, Shafian et al. (2018) obtained good results estimating the nutritional information of sorghum crop with multispectral images obtained at an altitude of 120 meters relative to the ground.

Based on the results presented so far, the data obtained in flights at an altitude of 120 meters was chosen to perform the remaining comparisons. After choosing the flight altitude, the relationship of DW with the NDVIA and NDREA values and the NDVI variable obtained with the Greenseeker sensor (NDVIG) was analyzed, as can be seen Figure 1. The analysis of variance also showed a significant effect (p ≤ 0.05) of the DW factor on the NDVIG variable. The Figure 1 also reveals an increasing linear trend of the effect of DW variability on the NDVIA, NDREA and NDVIG variables. One can also see that the coefficients of determination of NDVIA (R² = 0.91) and NDREA (R² = 0.79) were greater than the coefficient for NDVIG (R² = 0.68), revealing that there is greater accuracy in the DW data when estimated via multispectral aerial imaging. The results presented in Figure 1 were similar to the results obtained by Schlemmer et al. (2013), where the authors applied five different doses of N (0, 50, 100, 150 and 200 kg ha⁻¹) to a maize hybrid to evaluate the NDVI index obtained by aerial images in relation to the concentration of N in the plants’ leaves, also in the vegetative stage V6, obtaining a coefficient of determination of R² = 0.75.

Still, the different doses of N were efficient in creating variability in the DW so that a positive relationship could be identified between NDVIA, NDREA, NDVIG and the N doses applied at the sowing of the maize (Figure 1). In addition, no N saturation was found. As such, the availability of N could be seen to increase the concentration of N in plants and the chlorophyll content in the leaves, raising their photosynthetic rate and consequently their mass, causing a difference in the spectral reflectance. This information could be captured with the aid of remote sensors, which together with image processing was able to identify an increase in the weight of the aerial part of the plants.

### Table 1: The coefficients of determination of the dry weight and nitrogen content factors for the NDVIA and NDREA spectral indices obtained through the multispectral images collected in four different flight altitudes.

| Flight altitude (meters) | Coefficient of determination (R²) |
|-------------------------|----------------------------------|
|                         | Dry Weight | Nitrogen Content |
|                         | NDVIA      | NDREA | NDVIA | NDREA |
| 30                      | 0.87*      | 0.78* | 0.88* | 0.80* |
| 60                      | 0.87*      | 0.78* | 0.89* | 0.82* |
| 90                      | 0.98*      | 0.96* | 0.96* | 0.96* |
| 120                     | 0.92*      | 0.79* | 0.91* | 0.77* |

*Significant through the F test (p ≤ 0.05).

It is worth remembering that the amount of photosynthetically active mass results in increases in the reflectance of the near infrared band and a high absorption in the red band, explaining the increase in NDVI values, as has already been observed by Maresma et al. (2016).

In relation to the N content, the analysis of variance showed a significant effect (p < 0.05) on the SPAD variable (Figure 2) obtained by the Konica SPAD-502 portable chlorophyll meter, and also on the NDVI variable (Figure 3) obtained with the Greenseeker sensor (NDVIG). The analysis of variance also showed a significant effect (p
between the factors N content and NDVIA and NDREA obtained through multispectral aerial images at 120 meters. As such, it was possible to obtain the coefficients of determination through regression analysis (Figure 3).

$y = 0.2561x + 34.572$
$R^2 = 0.64 (p = 0.05)$

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Figure 2: Relationship of nitrogen content (N) with the SPAD index obtained with the SPAD-502 portable chlorophyll meter.

$NDVIA \quad y = 0.0039x + 0.2524 \quad R^2 = 0.77 \quad (p \leq 0.05)$
$NDREA \quad y = 0.0007x + 0.5612 \quad R^2 = 0.91 \quad (p \leq 0.05)$
$NDVIG \quad y = 0.0075x + 0.7373 \quad R^2 = 0.82 \quad (p \leq 0.05)$

Figure 3: Relationship of the N content with the NDVIA and NDREA values obtained from multispectral aerial images at a height of 120 meters and NDVI obtained with the Greenseeker sensor (NDVIG).

As expected, the SPAD variable responded positively to the different N content levels of the maize plants. The obtained results are similar to those found by Segatto et al. (2017) and Schiavinatti et al. (2011) for the same vegetative stage V6, who found effects of the N dose on the SPAD index, with a linear increase in readings for the maize crop. In addition, Hurtado et al. (2011) also observed that the SPAD readings were positively correlated with the levels of N in maize plants. Furthermore, Xiong et al. (2015) observed a relationship between the SPAD index and N content, with the values of the SPAD readings ($R^2 = 0.79, p = 0.007$) being higher in plants with higher levels of N. As such, the presented results confirm the sensitivity of the equipment to detect different N contents, since the highest SPAD readings were obtained in plants with an intense green color of the leaves, with a greater reflectance at the 650nm spectral range where light is absorbed by chlorophyll, as observed by Pörto et al. (2014).

For Huang et al. (2014), the use of the SPAD chlorophyll meter dispenses the use of chemicals to test for N, and there is no need to remove leaves from plants as the SPAD value can be obtained at the moment of reading. On the other hand, the collection of aerial images eliminates direct human interference in agriculture, in addition to obtaining data from a wide area and not just from sampling points. Still regarding the N content, the regression analysis for the NDVIA and NDREA variables had greater coefficients of determination than the SPAD variable, as can be seen in Figure 3.

The Figure 3 also shows that the coefficients of determination of NDVIA, NDVIA and NDREA were greater than the coefficient for SPAD, demonstrating that there is greater accuracy in the N content data when they are obtained with a multispectral aerial camera. In addition, the NDVIA variable stands out with a coefficient of determination ($R^2 = 0.91$), higher than the NDVIG, NDREA and SPAD variables.

The results obtained in the NDVIA and NDREA readings were similar to the results found by Raeva, Šedina and Dlesk (2018). These authors observed that plants with higher accumulation of chlorophyll in the initial stages of development had a more intense green color, in addition to greater reflectance in the near infrared and Red-Edge bands. According to Formaggio and Sanches (2017), both the NDVIA and NDREA index use the infrared band. The behavior reported by Raeva, Šedina and Dlesk (2018) could therefore be observed, with values varying between 0.50 and 0.60 for NDVIA and between 0.24 and 0.33 for NDREA, which are similar to the findings of this study (Figure 3).

In addition, the results are also similar to those presented by Zaman-Allah et al. (2015), who obtained a coefficient of determination of $R^2 = 0.85 (p \leq 0.01)$ for the NDVIA variable when evaluating the response of the N content of maize plants in an experimental
area with the application of six different N doses (0, 10, 20, 40, 80 and 160 kg ha⁻¹). Zaman-Allah et al. (2015) also found that multispectral images captured with RPAs were efficient in detecting differences in the N content of maize hybrids. Similarly, it was possible to detect differences in the N content of plants through the computational processing of images, using the NDVI index. For the authors, the results found in the study “Unmanned aerial platform‑based multi‑spectral imaging for field phenotyping of maize” show that the use of multispectral cameras with RPAs performing flights between 100 and 150 meters have a good level of image resolution, in addition to enabling rapid data collection, which means nutritional analyses can be carried out for both small and large maize fields.

**CONCLUSIONS**

It is possible to estimate dry weight of the aerial part of maize plants and the nitrogen content in the leaves through the processing of multispectral aerial images obtained at an altitude of 30, 60, 90 and 120m, using the NDVI and NDRE spectral vegetation indices in the vegetative stage V6. It is also observed, that the two highest altitudes (90 and 120m) obtained the bests results when compared to lower altitudes (30 and 60m). It is emphasized that the Greenseeker NDVI sensor accurately estimated nitrogen content foliar (R² = 0.82, p ≤ 0.05) and dry weight (R² = 0.68, p ≤ 0.05) for the same vegetative stage V6.

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