

Analysis of Infestation of Nematode *Meloidogyne exigua* in a Rubber Tree Plantation in the Triângulo Mineiro Region

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ABSTRACT

This study analyzed the use of the water balance, precipitation, temperature and NDVI (Normalized Difference Vegetation Index) to determine the optimum moment for evaluating infestation by the nematode *Meloidogyne exigua* in a rubber tree plantation by remote sensing. Nineteen sampling points were defined, divided into three areas, with high and low defoliation degrees, on a property in the Triângulo Mineiro region, where soil and root samples were collected for nematological analysis. Monthly digital images (OLI – Landsat 8 sensor) were obtained from August 2015 to August 2016. Rainfall, temperature and water balance were analyzed. The latter had no correlation with the indices; in contrast, temperature and precipitation correlated directly with NDVI. The best months for nematode soil infestation analysis were December, January and February, while for root infection it was August.

Keywords: NDVI, plant parasites, water balance.

1. INTRODUCTION AND OBJECTIVES

The rubber tree belongs to the family Euphorbiaceae, including 11 species described in the genus, among which the world's most important is *Hevea brasiliensis*. *Hevea* species are naturally found in the Amazon basin and in parts of the adjacent plateau. Its importance is due to the fact that natural rubber is obtained from its latex (Costa et al., 2001; IAC, 2015). Thailand, Indonesia, Malaysia, India and Vietnam were the greatest producers of natural rubber in 2012, representing around 81% of all the natural rubber produced in the world, while Brazil produced only about 1.51% of the global demand. Considering the country's total production, the state of São Paulo alone contributed with around 54% (IAC, 2015).

However, until the beginning of the 20th century, Brazil was the largest producer of natural rubber and the factor that rendered homogenous plantations unviable was the South American leaf blight, caused by the fungus *Microcyclus ulei* (P. Henn.) v. Arx (Gasparotto et al., 1997; Vinod, 2003). Besides diseases and some pests, nematodes are pathogenic agents of major agricultural relevance and, through the years, have contributed to yield reductions in several crops around the world. The most damaging nematode species for the rubber tree in Brazil is *Meloidogyne exigua*, which affects seedling production and natural rubber tree development, due to the reduction of the root's ability to absorb water and nutrients, with a subsequent reduction in latex production and even plant death (Lordello et al., 1983; Santos et al., 1992).

Planting in regions with a well-defined dry season simultaneously favors avoidance of South American leaf blight and results in the need to obtain knowledge about rubber tree yields under conditions of limited water supply, since the decrease in water availability changes the phenological behavior of the crop, besides affecting many plant metabolic pathways and physiological processes (Ortolani et al., 1986; Tezara et al., 2002) that can be aggravated when associated with the presence of diseases.

Vegetation spectral indices have been used largely to monitor Earth's vegetation cover (Miura et al., 2001). Such indices are combinations of spectral data in two or more bands, selected with the objective of synthesizing and improving the relation of such data with biophysical parameters of vegetation. In order to minimize the variability caused by external factors,

spectral reflectance has been transformed and integrated into several vegetation indices (Ponzoni, 2001).

The most-used vegetation index is the Normalized Difference Vegetation Index (NDVI) (Cohen et al., 2003; Dorigo et al., 2007), which can be analyzed by interpreting images from remote sensors and, in particular, by values obtained on different dates, allowing the evaluation of the variation in a green area in a given period, with values ranging in the interval $[-1 +1]$. In this context, NDVI, besides being used to distinguish and classify primary and secondary forests in different regeneration states because of its direct relation with biomass (Ponzoni & Shimabukuro, 2009), it also allows plant growth to be measured and monitored (Tucker et al., 2001).

The characteristic spectral signature of green and healthy vegetation demonstrates an evident contrast between regions of the electromagnetic reflective spectrum region, specifically and more prominently in red and near-infrared. The greater this gradient, the greater the vegetation vigor in the imaged area – this is the principle on which vegetation indices are based, combining the spectral information in these two bands of the electromagnetic spectrum (Shimabukuro et al., 1999). Thus, higher values (near +1) of NDVI indicate the presence of healthy vegetation (strong chlorophyll vigor), while lower values can point to the presence of pathology, water stress or defoliation, or logging. Values near zero indicate the presence of exposed soils, and values near -1 point to the presence of objects, such as water bodies.

This study analyzed the use of water balance, rainfall, temperature and NDVI to determine the optimum moment to evaluate infestation by the nematode *Meloidogyne exigua* in a rubber tree plantation in the Triângulo Mineiro region, defining a methodology to remotely establish the need and time for local sampling to confirm the infestation by this important pathogen.

2. MATERIALS AND METHODS

The area analyzed is in the Triângulo Mineiro region, Minas Gerais (MG), in the sub-basin of the rivers Grande and Paranaíba, where 130 ha of rubber tree clone RRIM600 were planted in January 2008. Three areas of the plantation were selected, the first presenting high defoliation and infestation by *M. exigua*, while areas 2 and 3 had less defoliation and nematode infestation (Figure 1).

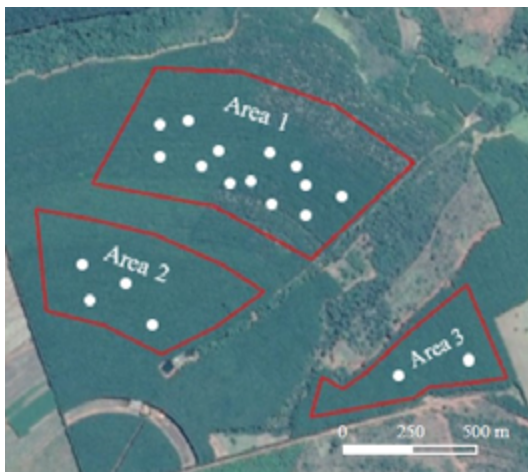


Figure 1. Location of experimental areas and sampled points taken from Google Earth – panoramic image and experimental areas marked.

The climate in the region is classified as Aw, with dry winters from June to August (Köppen, 1923). Since no weather station is registered with the Instituto Nacional de Meteorologia (Inmet) in Prata, MG, temperature, rainfall and the values used for estimating deficient and surplus soil moisture (water balance) were obtained from the station in Campina Verde, MG, which is the closest to the study area (Table 1).

Table 1. Climatic data of the meteorological station (Inmet) of Campina Verde, MG, and the humidity deficiency and surplus for calculating the water balance.

Month	Rainfall (mm)	Average Temperature (°C)	Water Balance
Aug	0.00	23.2	-81.0
Sept	13.00	24.9	-91.2
Oct	26.60	25.9	-100.7
Nov	227.80	26.6	0.0
Dec	106.60	26.7	-13.8
Jan	456.20	26.1	265.5
Feb	114.40	27.1	-3.2
Mar	120.00	26.5	-5.3
Apr	13.00	25.5	-59.4
May	168.20	22.4	2.8
June	52.40	20.7	-0.6
July	0.20	21.7	-26.0
Aug	20.20	23.3	-44.9

Nineteen sampling points were selected to represent the infestation by *M. exigua*, allocated in the three areas (Figure 1). Location coordinates were recorded for each sampling point to determine corresponding NDVI values. A statistical analysis between NDVI indices and the rainfall, temperature and water balance variables was performed using the Microsoft Office Excel Action Stat program. Nematode infestation was evaluated from soil and root samples collected at previously selected points.

Monthly images from the Landsat 8 OLI sensor were obtained from August 2015 to August 2016, in the orbit 221, point 073, available on the Instituto Nacional de Pesquisas Espaciais (Inpe, 2010) webpage. Bands 4 and 5 were used, corresponding to the red and near-infrared regions, with spatial resolution of 30 meters. These images were processed using Envi 4.8 software at the LASER Laboratory of the Federal University of Uberlândia (UFU), Monte Carmelo campus.

Nematode analyses of root and soil samples were executed according to the methods proposed by Coolen and D’Herde (1972) and Jenkins (1964), respectively. Root infection was evaluated considering the number of individuals per gram of roots, while soil infestation was determined in 250 ml of soil and adjusted to a soil volume of 150 cm³. Nematodes were counted in a Peters chamber using a photonic microscope.

NDVI was computed in every image during the 13-month period using the method developed by Rouse (1974), where NDVI is the ratio of the difference of reflectance measures in the channels of near-infrared (0.70 μm – 1.30 μm) and red (0.55 μm – 0.70 μm) to the sum of these channels, according to Equation 1:

$$NDVI = \frac{(p2 - p1)}{(p2 + p1)} \tag{1}$$

p1: reflectance value in the red region of the electromagnetic spectrum of objects on the earth’s surface; p2: reflectance value in the near-infrared region of the electromagnetic spectrum of objects on the earth’s surface.

Water balance was computed by the method of Rolim et al. (1998), modified, using evapotranspiration for rubber trees. Pearson’s correlation was calculated for the values of water balance (WB) and nematode analyses using the NDVI index for each month for Area 1 (higher infestation) and Areas 2 and 3 (lower infestation).

Finally, linear regressions were determined for the most correlated months for soil and root nematode populations as functions of NDVI, showing the behavior of the NDVI in relation to the presence of *M. exigua* in the roots of rubber trees and in the soil of the planted area.

3. RESULTS AND DISCUSSION

Nematode sampling confirmed field observations, occurring in greater number in Area 1, with approximately 2,437 nematodes per 150 cm³ soil and 3,780 nematodes per gram of roots. This plantation has greater infestation than that reported by Wilcken et al. (2015) in plantations in the state of São Paulo, which was 320 nematodes per 250 ml soil and 256 nematodes per 10 g roots. According to those authors, 87.5% of the soil samples containing *M. exigua* presented population levels between 8 and 100 nematodes, while the other 12.5% was between 100 and 320 nematodes per 250 ml soil; also, 94.7% of the root samples presented 16 to 100 nematodes, while 5.3% presented 100 to 256 nematodes per 10 g roots.

There was no correlation between water balance and NDVI for any of the areas. In contrast, temperature and rainfall correlated directly with NDVI, with values of 45% and 85%, respectively. This probably occurred due to variations in temperature and rainfall during the different seasons of the year, interfering directly in leaf senescence, in such a way that a reduction in NDVI values is observed in the colder and drier months of the year and, probably, lower nematode infestation, since temperature is the main factor affecting egg hatching and, for most species, it is not dependent on root exudate stimulus from the plants (Karszen & Moens 2006). The life cycle of nematodes of the *Meloidogyne* genus is commonly completed in three to four weeks at 27 °C. However, every species reduces or even completely paralyzes vital activities at temperatures above 40 °C or below 5 °C (Ferraz, 2001).

These physiological variations can be detected, according to Shimabukuro et al. (1999), due to the characteristic spectral signature of green healthy vegetation, demonstrating an evident contrast between the visible regions, specifically in red and near-infrared (Figure 2B). The greater the contrast, the greater the vegetation vigor in the imaged area (Figure 2D). This is the principle on which vegetation indices are based,

combining the spectral information in these two bands of the electromagnetic spectrum. Reflectance in the red region (around 0.6 µm to 0.7 µm) is low due to the absorbance by leaf pigments, especially by chlorophyll. However, in the near-infrared region (around 0.8 µm to 0.9 µm), there is high reflectance due to spreading by leaf cell structure.

It can be seen in Figure 2C that, due to the drought period, there is little chlorophyll vigor, which causes a low NDVI value, thus, making the resulting image darker. In contrast, in Figure 2D, corresponding to the NDVI image for November, in the rainy season, the area of interest is more whitish, indicating the presence of vegetation with strong chlorophyll content. This can be seen in the original images shown in Figures 2A and 2B where, in the first one, the magenta color indicates the presence of soil due to defoliation; while in Figure 2B, a greener area can be seen, indicating the return of healthy foliage.

Table 2 shows the analysis of variance for the regression of different NDVI values in relation to soil infestation by nematodes and, as illustrated, significant at a 5% probability, that is, one variable directly affects the other. The same can be observed for the roots, insofar as that the variation in root infection by nematodes affects the NDVI values. The standard error for both analyses was close to 0.06, demonstrating the reliability of the data.

According to Figure 3A, representing the regression analysis of NDVI for soil nematode infestation, in the best month for sampling, the correlations are confirmed, demonstrating that as soil nematode infestation increases, there is a corresponding reduction in NDVI, similar to that of the roots, which was less severe (Figure 3B). Possibly this was because there is greater water availability and the plant was less stressed, supporting nematode infestation and infection.

The correlation value of NDVI in Area 1 with data of nematode population in soil and roots, in the different months evaluated, showed the same behavior (Figure 3), which means that both soil and roots had negative correlation with NDVI. In August 2016, for example, there was a higher value for root samples, reaching 70%, meaning that there was greater nematode infection in what can be considered as the best month for sampling, followed by the months of February, March and April.

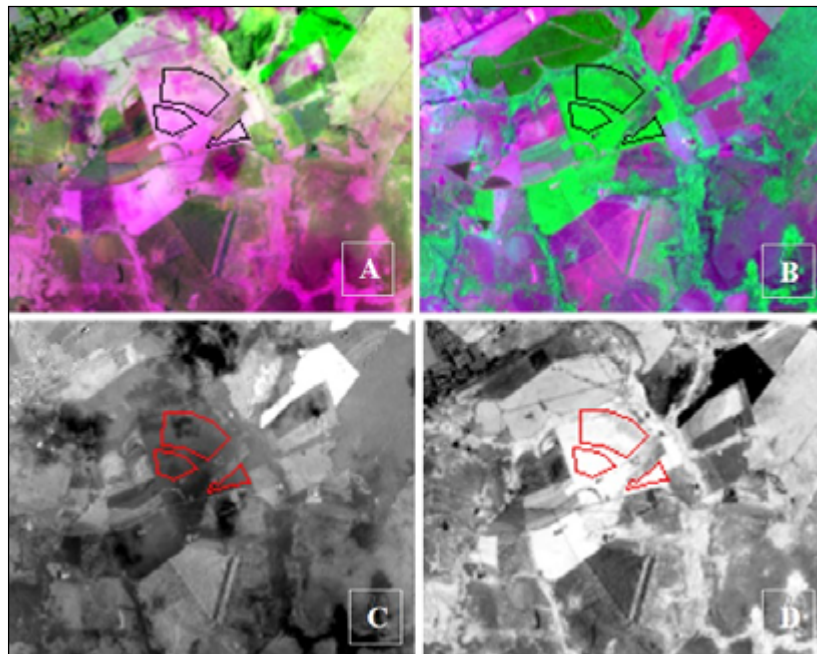


Figure 2. Original images: A) Landsat453 – July 21st, 2015 and B) Landsat453 – November 8th, 2016; NDVI images: C) Landsat453 – July 21st, 2015 and D) Landsat453 – November 8th, 2016.

Table 2. Analysis of variance for NDVI in relation to soil infestation and root infection.

Anova NDVI – Soil					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F_c</i>	<i>P</i>
Regression	1	0.028144481	0.028144	6.422587	0.027761*
Error	11	0.048203211	0.004382		
Total	12	0.076347692			
Anova NDVI – Roots					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F_c</i>	<i>P</i>
Regression	1	0.038038482	0.038038	6.422587	0.027761*
Error	11	0.043295826	0.003936		
Total	12	0.081334308			

df: degrees of freedom; *SS*: sum of squares; *MS*: Mean Square; *F_c*: F calculated; *p*: probability.

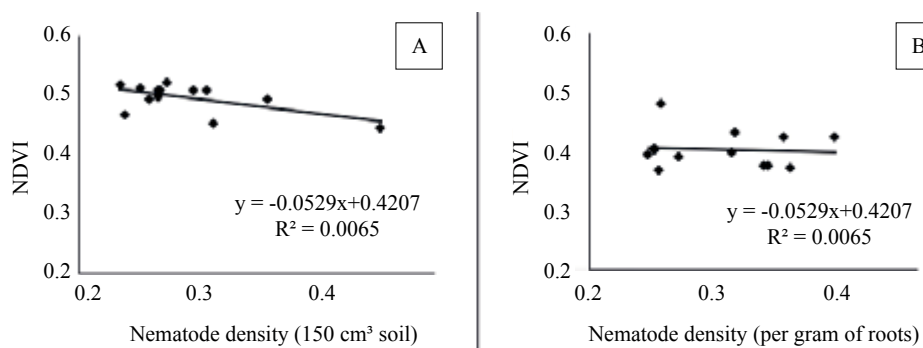


Figure 3. Regression analysis of NDVI in January for soil nematode infestation (A) and in August for root infection (B), in Area 1. *y*: normalized difference vegetation index; *x*: nematode density; *R*²: coefficient of determination.

The months of December to February presented greater correlation for soil infestation with nematodes. This contrasts to what was found for coffee plants by Almeida et al. (1987), in which greater population densities of *M. exigua* occurred from April to July, and by Souza et al. (2000), who observed greater infestation and root galls in the rainy season, while the population of juveniles diminished in the soils.

Thus, it seems that soil infestation has no correlation with root infestation, which was also observed by Pezzoni Filho (2014), during the analysis of the space-temporal dynamics of rubber tree nematodes, with different soil infestation from that found in the roots, which was greater in the latter, with about 60,000 per 10 g of root.

A further observation is that the big population of *M. exigua* in the roots is due to its sedentary habit, with oviposition in a gel mass attached to its body, and the determination of population density considers both eggs and juveniles. Plant cell changes are observed in the early colonization stages, such as increased concentration of amino acids and proteins, increased root exudates of minerals, lipids, and growth hormones, with subsequent formation of giant cells and galls that disrupt conducting vessels, resulting in wilting, nutrient deficiency and poor plant development (Barbosa, 2013). However, these reflex symptoms are visible only when the plant suffers some abiotic stress, such as drought and high temperatures, increasing its evapotranspiration.

There was no strong correlation for Areas 2 and 3, possibly because the stress was not as severe as in Area 1, since there was less nematode infestation and the plants were able to withstand climatic stresses.

These quantifications of NDVI demonstrate an important use in monitoring plantations since, according to Karnieli et al. (2002), when remote sensing multi temporal images are converted to vegetation indices, they become powerful tools for monitoring changes occurring in vegetation cover and biomass production.

4. CONCLUSIONS

The infestation values evaluated presented negative correlation with NDVI, demonstrating the efficacy of using satellite images to analyze nematodes in rubber tree plantations. Therefore, the values of NDVI enabled the observance of the effect of temperature

and rainfall on increasing or decreasing nematode populations, establishing a better segregation of healthy and diseased areas in a pre-evaluation of the plantations and directing field sampling, which results in labor optimization and lower costs associated with traveling and sampling. Thus, the best sampling time for confirming root infection by *M. exigua* is August, while the months with better correlation for soil infestation were December to February.

ACKNOWLEDGEMENTS

The authors are thankful to Universidade Federal de Uberlândia (UFU) for the support.

SUBMISSION STATUS

Received: 24 Mar., 2017

Accepted: 24 Nov., 2018

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