Leaf area, chlorophyll content, and root dry mass in oil palms (*Elaeis guineensis* Jacq.) affected by the plumero disorder

Área foliar, contenido de clorofila, y masa seca de raíces en palmas de aceite (*Elaeis guineensis* Jacq.) afectadas por el disturbio del plumero

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ABSTRACT

The plumero disorder in oil palm is characterized by an abnormality in the development of the leaf area, yellowing of young leaves, and longitudinal chlorotic strips parallel to the central rib. In this research, the leaf area of leaf 17, the specific leaf area, chlorophyll contents, and root dry mass were evaluated in an oil palm (*Elaeis guineensis* Jacq.) plantation on the northern coast of Colombia to characterize the morphophysiological damage and quantify the severity of the disorder. For the statistical analysis, an ordinal regression model and analysis of variance tests were performed. The results indicated that the palm reduces its leaf area before the disorder is visually evident. Leaves became thicker and lower in chlorophyll content. There was also an increase in the tertiary and quaternary root dry mass in the initial grades. This variable decreased in the more severe grades of this disorder.

Key words: physiology, damage, disease severity, dry matter.

RESUMEN

El disturbio del plumero en palma de aceite se caracteriza por una anormalidad en el desarrollo del área foliar, el amarillamiento de las hojas jóvenes y el rayado clorótico longitudinal paralelo a la nervadura central. En esta investigación se determinó el área foliar de la hoja 17, el área foliar específica, los contenidos de clorofilas y la masa seca de raíces en una plantación de palma de aceite (*Elaeis guineensis* Jacq.) en la costa norte de Colombia con el objetivo de caracterizar los daños morfofisiológicos y cuantificar la severidad del disturbio. Para el análisis estadístico se realizó un modelo de regresión ordinal y pruebas de análisis de varianzas. Los resultados indicaron que la palma reduce su área foliar antes de que el disturbio sea evidente a nivel visual. Las hojas se vuelven más gruesas con menor contenido de clorofilas. También se presentó un aumento de la masa seca de raíces terciarias y cuaternarias en los grados iniciales. Esta variable disminuyó en los grados más severos del disturbio.

Palabras clave: fisiología, daño, severidad de enfermedad, materia seca.

Introduction

Since 2010, a disorder called plumero has been recognized in the oil palm (*Elaeis guineensis* Jacq.). This disorder is named after a Spanish word that refers to cleaning dusters because of the similarity between the leaf shape and this object. Symptoms of plumero are characterized by a visual reduction of the leaf area, yellowing of leaves, longitudinal chlorotic strips parallel to the central rib, drying of the tips of the leaflets in younger trees, and the presence of yellow strips usually located on one side of the central rib. The yellow strip may appear on one or several leaflets or one or several leaves (Arias *et al*., 2014). The causal agent or predisposing factors of this disorder are still unknown.

Plants show physiological responses to changes in environmental factors and the incidence of disease-causing organisms. In oil palms, research has been carried out to evaluate the physiological response of the plant to the attack of an abiotic agent. For example, the disease known as lethal wilt (*Candidatus* Phytoplasma asteris) generates increases in the internal temperature, decreasing the rate of photosynthesis and the ability to take nutrients from the oil palm that reduces the ability to produce sugars, the maintenance of leaves, and the production of new roots. During this process, the palm ends up decaying, preventing the leaves from opening their stomata to take up CO_2 and transpire (Cayón *et al*., 2007). The oil palm has a fasciculate radical system composed of primary, secondary, tertiary and quaternary roots (Reyes-Santamaría *et al*., 2000). Most of the absorption of nutrients is through the quaternary and absorbing tips of primary, secondary and tertiary roots (Tailliez, 1971). Ramírez *et al.* (2004) indicate that palms with lethal wilt produce very few roots, so they cannot

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take up the water required for nutrition and regulation of leaf temperature. In general, plants are often limited by several stressful factors that occur simultaneously, making it difficult to predict their geographical distribution based on physiological responses of an individual factor (Mittler, 2006; Fischer *et al*., 2016; Fischer and Melgarejo, 2020).

The negative impact of foliar diseases is directly expressed on yield components due to changes that pathogens induce in physiological processes responsible for crop productivity such as the increase in leaf area and the accumulation and distribution of dry mass in plant organs (Schierenbeck *et al*., 2014). The disease known as bud rot causes a decrease in photosynthesis, stomatal conductance, transpiration, efficient use of water and chlorophyll content, and increases the carotenoid content in oil palms.

The detailed description of some morphological and physiological aspects of a diseased plant allows knowing the infection processes, severity and damage of the disease (Rakib *et al*., 2019). So, the objective of this study was to characterize the morpho-physiological damages and to quantify the severity of the plumero disorder.

Materials and methods

Location

The work was carried out in an oil palm plantation located on the north coast of Colombia (9°56' N and 73°16' W, and altitude of 95 m a.s.l.). According to the Holdridge classification system, the plantation is located in the ecological formation tropical dry forest (Espinal and Montenegro, 1963). An average temperature of 27.5°C and average precipitation of 1300 mm were recorded.

Diagrammatic scale of the disorder

The diagrammatic severity scale proposed by Arias *et al.* (2014), indicates that palms affected by plumero disorder can be found in five grades: yellow strip, grade 1, grade 2, grade 3 and grade 4. Yellow-strip palms are characterized by the presence of at least one yellow strip in the canopy without visual reduction of the width of the leaflets, while grades 1 to 4 show a reduction in the width of the leaflets and sharp insertion of the leaflets in the rachis. In grade 1, the effect can be seen at the level of the 1st leaf, grade 2 at the level of the 9th leaf, grade 3 at the level of the 17th leaf, and grade 4 when the affected leaves are seen underneath the level of the 17th leaf.

To manage a scale with a smaller number of grades to facilitate the analysis and the diagramming of tables and figures, we used the same scale proposed by Arias *et al.* (2014), but grades 1 and 2 were combined into grade 1-2 and grades 3 and 4 were combined into grade 3-4. The combination of grades was confirmed by a multiple correspondence analysis of an exploratory nature from which two dimensions were extracted from the leaf area (LA) and specific leaf area (SLA) data. The planting year, the number of the lot or farm, the genetic origins, and the degrees of severity were included as factors of plumero, yielding an explained variability of 70.72%. This can be observed in Figure 1; the proximity between the points correspond to grades 1 and 2, as well as grades 3 and 4.

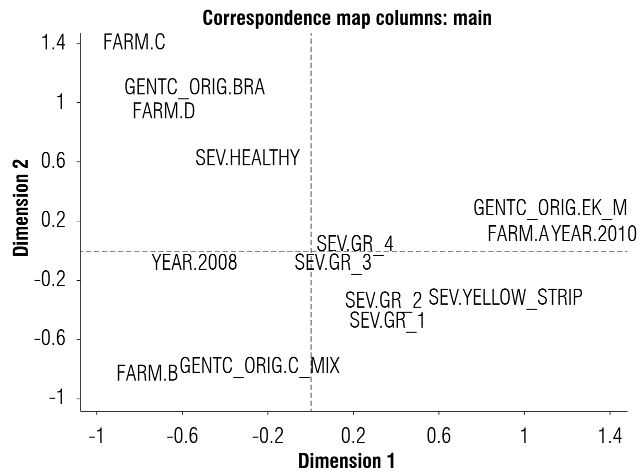

FIGURE 1. Multiple correspondence analysis of leaf area (LA) and specific leaf area (SLA). GENTC_ORIG.BRA: genetic origin Brabanta; GENTC_ORIG.C_MIX: genetic origin Congo Mixed; GENTC_ORIG.EK_M: genetic origin Ekona x Djongo * Mongana; YEAR.2008: planting year 2008; YEAR.2010: planting year 2010; SEV.HEALTHY: severity healthy; SEV.YELLOW STRIP: severity yellow strip; SEV.GR_1: severity grade 1; SEV.GR_2: severity grade 2; SEV.GR_3: severity grade 3; SEV.GR_4: severity grade 4.

Palm sampling

Samples were collected from 24 healthy palms and 24 palms from each of the severity grades of plumero, according to the multiple correspondence analysis listed in Table 1. The palms were randomly selected. The following variables were determined for each of the palms: leaf area (LA), specific leaf area (SLA), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl t), primary root dry mass (PRDM), secondary root dry mass (SRDM), tertiary and quaternary root dry mass (TQRDM).

Leaf area and specific leaf area

The leaf area of leaf 17 (LA17) is an indicator of the total leaf area of the oil palm. LA17 has been used as a model for different measurements in the oil palm due to its average location within the total leaves of the palm and its stable nutrient content (Corley and Tinker, 2003). The formula proposed by Corley *et al*. (1971) was used to determine the leaf area of leaf 17 (LA) for the oil palms *Elaeis guineensis* (Eq. 1).

$$LA17 = 0.55 * [(LW) * n] \quad (1)$$

where LA17 is the leaf area of leaf 17(m^2), 0.55 is the correction factor for the palms *Elaeis guineensis*, L is the average length of the four largest central leaflets (m), W is the average width of the four longest central leaflets, and n is the total number of leaflets.

To determine the SLA the method of López *et al*. (2014) was used where a 30 cm long segment was taken from the middle part of two central leaflets of leaf 17. The width (w) and area of each segment was measured. Segments were then dried in an oven at 75°C for 72 h, and the dry weight was determined. Thus, the SLA ($cm^2\ g^{-1}$) was equal to the mean area of the leaf segments divided by their dry weight.

$$SLA = l\ w / p \quad (2)$$

where, l is the average length of the middle part of the leaflet that in this case takes the value of 30 cm, w is the mean mid-width of the leaflets, and p is the mean of the dry weight of the middle part of the leaflets.

Chlorophyll

The contents of Chl a, Chl b and Chl t were determined using the acetone extraction method (Flórez and Cruz, 2004). This consisted of collecting 10 leaf disks of 5 ml in diameter of non-veined plant tissue that were then preserved in Eppendorf tubes with 2 ml of 99% absolute alcohol. The tubes were labelled, covered with aluminum foil, and refrigerated. The leaf disks were macerated with 8 ml of a cold solution of 80% acetone and $CaCO_3$ (0.5 g L^{-1}). Absorbances of 645 nm and 663 nm were determined in the macerate using a spectrophotometer (BioMate 3, Madison, USA). The following equations were used to calculate the content of Chl a (Eq. 3), Chl b (Eq. 4) and Chl t (Eq. 5):

$$Chl\ a = \{[(12.7 \times D663) - (2.69\ D645)] \times V\} / (1000 \times W) \quad (3)$$

$$Chl\ b = \{[(22.8 \times D645) - (4.48\ D663)] \times V\} / (1000 \times W) \quad (4)$$

$$Chl\ t = \{[(20.2 \times D645) + (8.02\ D663)] \times V\} / (1000 \times W) \quad (5)$$

where D is the optical density, V the volume of the extract used in the determination of the optical density in ml, and W is the fresh mass in mg of the 10 leaf disks of plant tissue.

Root dry mass

Soil samples were collected at the four cardinal points of the base of the palm tree using a sharp-edged steel cylinder of known volume. The roots were then washed, separated into primary, secondary, tertiary and quaternary roots that were then dried in an oven at 75°C for 72 h. Since the weight of the roots was in grams and the volume of the soil sample was known, the data was extrapolated to dried roots per cubic meter of soil (kg m^{-3}).

Statistical analysis

An ordinal regression model with a degree of severity of *P* was applied as the response variable. The income variables were LA, SLA, Chl a, Chl b, Chl t, PRDM, SRDM, TQRDM in the statistical program Rstudio. Analysis of variance (ANOVA) and the test of minimum significant difference was performed with an alpha calculated using the Bonferroni correction (α_B) (Eq. 6).

$$\alpha_B = 1 - (1 - \alpha)^{1/n} \quad (6)$$

where n is the number of variables in this research; the number of significant variables in the model was n = 4, so the alpha with the Bonferroni correction used was $\alpha_B = 0.0127$ with $\alpha = 0.05$. A matrix of correlations between variables was performed to assess the relevance of using univariate analysis instead of multivariate analysis.

To facilitate the analysis, grade 1 was collapsed with grade 2 (labelled as grade 1-2) and grade 3 was collapsed with grade 4 (labelled as grade 3-4). This collapse was performed considering an analysis of multiple correspondences that was performed as data exploration. The proximity between the points corresponding to grades 1 and 2, as well as grades 3 and 4 descriptively validated the collapse of the categories. The veracity or falsity of atypical data was also determined using the box-plot graph of the RStudio software.

Results

All ordinal regression models evaluated reported the variables LA and SLA as significant ($P<0.05$) and indicated comparisons of degrees of severity: healthy with yellow strip, yellow strip with grade 1-2, and grade 1-2 with grade 3-4. However, the best fit model was model 4, where the variables LA, SLA, Chl t, TQRDM and the severity degree comparisons were reported as significant (healthy with yellow strip, yellow strip with grade 1-2; and grade 1-2 with grade 3-4). This model also reported an Akaike information criterion value of 279.38, the lowest among the various adjusted models (Tab. 1). The parameters of the best fit model are shown in Table 2. This model yielded 53.6% correct classifications; that is 53.6% of the palms observed at a certain degree of severity were correctly classified in that same degree of severity. The correlation matrix (Tab. 3) indicates that the variables LA, SLA, Chl t, and TQRDM were not correlated, so it was not applicable for performing multiple variance analysis.

TABLE 1. Adjusted ordinal models.

Variable	Model 1	Model 2	Model 3	Model 4
	Probability			
LA	<0.0001	<0.0001	<0.0001	<0.0001
SLA	0.0069	0.0058	0.0078	0.0063
Chl t	0.0450	0.0464	0.0601	0.0021
Chl b	0.1318	0.1324	0.1741	
PRDM	0.6483			
SRDM	0.3539	0.4075		
TQRDM	0.0010	0.0011	0.0004	0.0010
Healthy | Yellow strip	<0.0001	<0.0001	<0.0001	<0.0001
Yellow strip | grade 1-2	<0.0001	<0.0001	<0.0001	<0.0001
Grade 1-2 | grade 3-4	<0.0001	<0.0001	<0.0001	<0.0001
Ordinal statistical models				
Statistics	Model 1	Model 2	Model 3	Model 4
Akaike information criterion	271.17	269.38	268.08	267.94

LA - leaf area, SLA - specific leaf area, Chl b - chlorophyll b, Chl t - total chlorophyll, PRDM - primary root dry mass, SRDM - secondary root dry mass, TQRDM - tertiary and quaternary root dry mass.

TABLE 2. Estimated parameters for the reduced model 4.

Variable	Estimator	Standard error	t value	Probability
LA	-1.025	0.180	-5.684	<0.0001
SLA	-0.055	0.020	-2.730	0.0063
Chl t	-0.810	0.264	-3.069	0.0021
TQRDM	-0.699	0.213	-3.282	0.0010
Healthy | yellow strip	-14.948	2.239	-6.675	<0.0001
Yellow strip | grade 1-2	-13.764	2.180	-6.314	<0.0001
Grade 1-2 | grade 3-4	-11.925	2.087	-5.714	<0.0001

LA - leaf area, SLA - specific leaf area, Chl t - total chlorophyll, TQRDM - tertiary and quaternary root dry mass.

TABLE 3. Correlation matrix between physiological variables.

	SLA	Chl t	LA	TQRDM
SLA		-0.1113	0.2348	0.1421
Chl t	-0.1113		0.0742	-0.0791
LA	0.2348	0.0742		0.0443
TQRDM	0.1421	-0.0791	0.0443	

SLA - specific leaf area, Chl t - total chlorophyll, LA - leaf area, TQRDM - tertiary and quaternary root dry mass.

Univariate analyses indicated that the variables LA, SLA, Chl t and TQRDM were significant (P<0.05) which means that LA, SLA, Chl t and tertiary and quaternary root dry mass were different in healthy palms from those affected by plumero (Tab. 4).

TABLE 4. Anova of the variables leaf area (LA), specific leaf area (SLA), total chlorophyll (Chl t) and tertiary and quaternary root dry mass (TQRDM).

Source	DOF	Sum of squares	Mean square	F value	Probability
LA					
Severity	3	59.3596	19.7865	17.89	<0.0001
Error	120	132.7284	1.106		
Total	123	192.088			
SLA					
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	1368.9987	456.3329	4.99	0.0027
Error	120	10978.4834	91.4873		
Total	123	12347.4821			
Chl t					
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	4.5253	1.5084	3.04	0.0316
Error	120	59.4762	0.4956		
Total	123	64.0016			
TQRDM					
Source	**DOF**	**Sum of squares**	**Mean square**	**F value**	**Probability**
Severity	3	14.8904	4.9634	8.58	<0.0001
Error	117	67.666	0.5783		
Total	120	82.5565			

DOF - Degrees of freedom

Leaf area, specific leaf area

The leaves of palms affected by plumero (yellow strip, grade 1-2, and grade 3-4) had lower LA, SLA and Chl t than healthy palms (Fig. 2A-C). The roots of affected palms increased TQRDM at the initial stages (yellow strip and grade 1-2) but then decreased significantly in grade 3-4 (Fig. 2D).

Chlorophyll

The leaves of palms affected by plumero (yellow strip, grade 1-2, and grade 3-4) showed lower Chl t content than healthy palms (Fig. 2C).

Root dry mass

The dry matter of tertiary and quaternary roots increased in the initial degrees of severity (yellow strip and grade 1-2) compared to healthy palms, and it was significantly reduced in the last degrees (grade 3-4) (Fig. 2D).

Discussion

Leaf area and specific leaf area

LA progressively decreased as the degree of severity increased (Fig. 2A). This occurs long before a reduction in the width of the leaflet, as described by Arias *et al.* (2014) in the plumero severity scale. The decrease in LA caused by some pathogens drastically affects the interception of solar radiation and reduces the generation and distribution of biomass (Carretero *et al.*, 2009) because they accelerate the necrosis and senescence of leaves, causing fewer photoassimilates to be used in the synthesis of dry matter (Schierenbeck *et al.*, 2014).

The palms affected in grade 3-4 had thicker leaf laminae. The SLA indicated the variation in the relative thickness of the leaves as a consequence of leaf structural alterations, which make the leaf very sensitive to environmental and external factors (Reyes-Santamaría *et al.*, 2000), and a good indicator of crop productivity (Poorter and de Jong, 1999). The reduction of SLA indicated that the leaves have a thicker mesophyll due to the higher number and size of layers of palisade cells (Rodríguez and Cayón, 2008). This suggests that the number of photoassimilates in these leaves is greater (Ayala and Gómez, 2000) or photoassimilates are not efficiently transported from source to sink in response to nutritional deficiencies of elements such as boron (Wimmer and Eichert, 2013), magnesium (Verbruggen and Hermans, 2013) or potassium (Gerardeaux *et al.*,

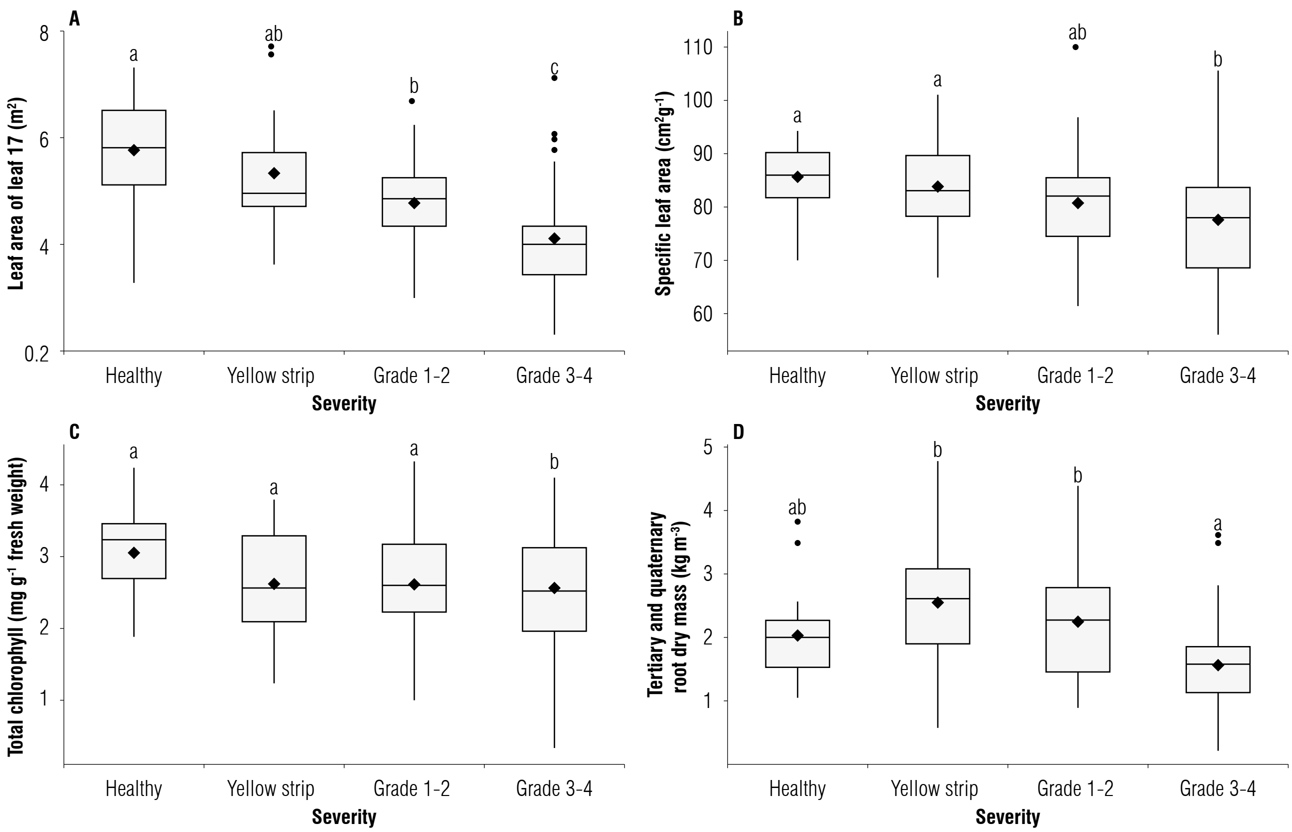

FIGURE 2. A) Leaf area of leaf 17 (m^2), B) specific leaf area, C) total chlorophyll and D) tertiary and quaternary root dry mass in healthy and affected palms by plumero. Different letters indicate differences in means with $P = 0.0127$.

2010). According to Nenova (2006), SLA is also increased due to iron deficiency and decreases due to excess iron, as observed in plants affected by plumero. However, palms affected by this disorder have a lower SLA because the leaf does not expand as the leaflets of healthy plants do. The expansion of the cells can be affected by stressful conditions such as temperature, evaporation, or water content of the soil, although the plant recovers quickly once the stress is over (Sadok *et al*., 2007). However, the elongation and final leaf area are affected in some cases when stress occurs in the last phase of leaf elongation (Granier and Tardieu, 2009).

Chlorophyll

Palms affected by plumero significantly reduced the chlorophyll content and root growth. A decrease in the chlorophyll content in response to abiotic and biotic stress is manifested by foliar yellowing, followed by wilting, affecting photosynthesis (Munné-Bosch, 2008) and consequently, reducing plant biomass (Casierra-Posada and Cutler, 2017; Sánchez-Reinoso *et al*., 2019). Mandal *et al*. (2009) stated that the chlorophyll content in diseased leaves of *Plantago ovata* affected by downy mildew was reduced by 24.39% in slightly chlorotic and 44.90% in severely chlorotic leaves as compared to healthy leaves, which appears to be one of the causes for the reduction of the photosynthesis process.

There are several alternatives to evaluate the establishment and development of a disease, such as estimating the chlorophyll content in the leaf (Uddling *et al*., 2007). Such estimation could provide a better alternative to evaluate disease severity in a plant (Chang *et al*., 2015) and be a good indicator for the degree of disease or infection and changes during pathogenesis (Rakib *et al*., 2019). The chlorophyll content in Ganoderma-infected oil palm seedlings declines as the infection progresses (Goh *et al*., 2016). Chang *et al*. (2015) also report that the chlorophyll content decreases as the disease progresses in different stages of cucumber growth. Chlorophyll content also decreases due to deficiencies of other nutrients including molybdenum (Agarwala *et al*., 1978) and boron. The latter generates oxidative damage in chloroplasts (Wimmer and Eichert, 2013). The reduction in chlorophyll content can also be caused by an attack of pathogens. In deciduous leaves attacked by phytoplasmas, chlorosis occurs as a form of cell death at the pathogen's entry points (Mittelberger *et al*., 2017).

The chlorophyll content is used to determine disease severity in some crops. In the case of fir decline, Oren *et al*. (1993) propose chlorophyll content and foliar nutrient analysis as a method to represent a range of severity for chlorosis to quickly identify the processes that occur in the soil of some areas where a decline occurs.

Root dry mass

In palms affected by plumero in the initial stages (yellow strip and grade 1-2) an increase in the development of tertiary and quaternary roots has been observed. This type of response could be associated with the response system of the plants to a sulfur deficiency with the slow initial growth of lateral roots and then with the rapid root growth (Hoefgen and Nikiforova, 2008; Gruber *et al*., 2013).

Conclusions

The plumero disorder compromises the physiological and productive performance of the affected palms by affecting the expansion of the leaf area and the development of roots. This characterization contributes to knowledge of the physiological damages caused by the plumero and is a fundamental tool for managing the disorder in the affected plantations.

Acknowledgments

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