Quantification of shedding of leaves in yerba mate and determination of associated hormonal levels

Quantificação da queda de folhas na erva-mate e determinação dos níveis hormonais associados

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ABSTRACT
In recent years, leaf shedding in the yerba mate crop has been a phenomenon of important economic incidence, the cause of which is still unknown but has been attributed to various factors. The objective of the present study was to establish a methodology to quantify leaves shedding in yerba mate and to determine whether the high leaf shedding is due to a biotic or abiotic effect. In both plantations, high shedding of leaves was significant only when the lowest values of heliophany and the highest values of relative humidity were recorded. Coincidentally, a high percentage of green leaf detachment was recorded with the characteristic symptomatology of the fungus Cylindrocladium sp. An accumulation of ABA, JA and SA hormones was detected in yerba mate plants with high shedding of leaves, which was associated with the attack of the necrotrophic fungus Cylindrocladium

sp. With predisposing climatic conditions for the manifestation of Cylindrocladium, an early harvest would reduce the loss of green leaves. The differences found among plants Cylindrocladium attack could be due to a genetic condition. The JA pathway may be the most important line of defense activated by Cylindrocladium infection. A high accumulation of JA and ABA has been detected in plants with high leaf shedding that could suppress the SA signaling pathway responsible for SAR establishment.

Keywords: ilex paraguariensis, cylindrocladium, hormone, pathogens, climatic conditions.

RESUMO
Nos últimos anos, o derrame de folhas na cultura da erva-mate tem sido um fenómeno de importante incidência económica, cuja causa ainda é desconhecida mas tem sido atribuída a vários factores. O objectivo do presente estudo era estabelecer uma metodologia para quantificar a queda de folhas na erva-mate e determinar se a alta queda de folhas se deve a um efeito biótico ou abiótico. Em ambas as plantações, o alto desprendimento de folhas só foi significativo quando foram registados os valores mais baixos de heliofania e os valores mais altos de humidade relativa. Coincidentemente, uma elevada percentagem de desprendimento de folhas verdes foi registada com a sintomatologia característica do fungo Cylindrocladium sp. Foi detectada uma acumulação de hormonas ABA, JA e SA em plantas de erva-mate com alto desprendimento de folhas, que foi associada ao ataque do fungo necrófilo Cylindrocladium sp. Com condições climáticas predisponentes para a manifestação do Cylindrocladium, uma colheita precoce reduziria a perda de folhas verdes. As diferenças encontradas entre as plantas ataque de Cylindrocladium poderia ser devido a uma condição genética. A via JA pode ser a linha de defesa mais importante activada pela infecção por Cylindrocladium. Foi detectada uma elevada acumulação de JA e ABA em plantas com elevado derrame de folhas que poderia suprimir a via de sinalização SA responsável pelo estabelecimento de SAR.

Palavras-chave: ilex paraguariensis, cylindrocladium, hormona, agentes patogénicos, condições climáticas.

1 INTRODUCTION

Argentina is the world's largest producer and second largest exporter of yerba mate. Its production takes place in the province of Misiones and in the northeast of the province of Corrientes. This activity is of great economic and social relevance for both provinces, especially for Misiones, which contributes 90% of the production.

The increase in demand necessarily leads to an increase in the cultivation area, or to work with a higher plant density. This situation often leads to problems such as diseases and excessive leaf drop.

Abscission is a naturally occurring process in all plant species and is genetically programmed in plant tissues and organs (Salisbury and Ross, 1992). In many cases, abscission is directly related to senescence, eliminating senescent or deteriorated organs. Sometimes, abscission is induced as a consequence of environmental stress signals, both abiotic and biotic (Quesada and Valpuesta, 2013). Many factors can simultaneously influence an organ to induce or not its abscission. At the hormonal level, auxins and ethylene play a fundamental role, integrating endogenous and environmental signals to which a plant is subjected (Quesada and Valpuesta, 2013).

Leaf senescence is a recycling process in which nutrients are transported from aging cells to young leaves, developing seeds and reserve tissues (Quesada and Valpuesta, 2013).

In recent years, shedding of leaves in the yerba mate crop has been a phenomenon of important economic incidence, the cause of which is still unknown but has been attributed to various factors.

According to some authors, the shelf life of yerba mate leaf is more than two years (Masloff et al., 2014). This situation can be accelerated, under certain circumstances, by adverse environmental conditions.

Some fungal genera have been associated with shedding of leaves in yerba mate. The fungus *Cylindrocladium spathulatum* causes a symptomatology characterized by a rounded spot on the leaves, until finally the leaves fall off the plant (Carpanezzi et al., 1985; Grigoletti Júnior and Garcia Auer, 1996; Rybak et al., 2014; Maidana Ojeda et al., 2016; Dummel et al., 2017).

On the other hand, the abscission of green leaves is generalized annually throughout the producing region, where its intensity is directly related to certain environmental conditions and plantation management systems. This shedding of leaves has been recorded in Argentina, Brazil (Rivera Flores, 1983; Carpanezzi et al., 1985; Grigoletti and Garcia Auer, 1996) and Paraguay (Masloff et al., 2014; Chamorro et al., 2015; Maidana Ojeda et al., 2016).

Excess rainfall can cause waterlogging of soils, which can be aggravated in compacted soils or soils without green covers. Oxygen limitation originates numerous physical, chemical and biological changes in the soil, with production of toxic substances, as well as ethylene, and loss of soluble nitrogen compounds and

imbalances in other nutrients due to microbial anaerobic activity (Bonadeo et al., 2017). In fact there is evidence of macro- and micronutrient deficiencies in plant tissues due to excess water in the soil (Pardos, 2004).

In summary, the above factors may be responsible for high shedding of leaves in yerba mate. Excessive rainfall and unharvested plants can provide a suitable microenvironment for the presence of pathogens, accentuated by unfavorable soil and nutritional conditions.

So far, no estimates of yield losses have been made, making it difficult to correlate them with variables that could be affecting yield.

The aim of the present study was to establish a methodology for quantifying of shedding of leaves in yerba mate. The other objective was to determine if the high shedding of leaves is due to a biotic or abiotic effect.

2 MATERIALS AND METHODS

2.1 STUDY SITE

The study was conducted in Colonia El Chatón (Leandro N. Alem, Misiones) (27° 36' 50" S and 55° 15' 50" W), at 330 m.a.s.l. The average annual temperature, recorded at the EEA Cerro Azul Meteorological Station (INTA), is 20.8 °C and the average annual precipitation is 1998.3 mm, distributed mainly between October and April (Olinuck, 2017).

The soils of this region belong to the Naranjal Series, typical Gran Grupo Kandiudulte, of the Order Ultisol. They are characterized by wide undulating plains where well-defined hillocks stand out and with slopes that mostly do not exceed 3% (Godagnone and de la Fuente, 2013).

Two commercial plantations were selected with the same planting frame (2.5 m x 1.5 m), one with normal and the other with high shedding of leaves. Although the management of the plantations is similar, they differ from each other in terms of planting age. The yerba mate plantation with normal shedding of leaves is 40-year-old, while the yerba mate plantation with high shedding of leaves is 30 years old.

2.2 CHARACTERIZATION OF SOIL PHYSICAL PROPERTIES

The physical properties were characterized: penetration, structural stability,

apparent density and total pores, infiltration and gravimetric moisture (Soil Laboratory of the Cerro Azul Agricultural Experiment Station - INTA).

2.3 SHEDDING OF LEAVES QUANTIFICATION

Shedding of leaves was quantified through the number of leaves and dry weight per unit area. For this purpose, a methodology frequently used in forestry was used (Huber and Oyarzún, 1983; Prause et al., 2003; Mosquera et al., 2007; Zapata et al., 2007; Gutiérrez et al., 2012; Murcia and Ochoa, 2012; Pinos, 2015), which was adapted for yerba mate. It is based on the use of collectors with a certain dimension, distributed in the plantations.

Twenty collectors were randomly installed (10 per plantations), with a dimension of 0.50 m x 0.50 m on each side and a height of 10 cm from the ground, to prevent the accumulation of water. The material accumulated inside the collectors was collected weekly, during 12 months (from June 2018), in plastic bags to avoid moisture losses. In the laboratory, the material collected was classified (leaves with natural senescence and green leaves). In addition, the symptoms caused by different types of fungi were recorded. Finally, the material was dried, at 60 °C for 48 hours, until constant weight, and then the dry weight was recorded, as recommended by Anderson (1973).

Climatic variables (rainfall, relative humidity and heliophany) were registered in the meteorological station of the Cerro Azul Agricultural Experiment Station (INTA).

2.4 SAMPLING FOR HORMONAL ANALYSIS

Ten plants were selected, five with normal shedding of leaves and another five with high shedding of leaves. For hormone analysis, 10 leaves of each plant were sampled and placed on an aluminum foil. The samples were immersed in liquid nitrogen. Finally, the samples were lyophilized and stored at -20 ° C until analysis.

2.5 EXTRACTION AND PURIFICATION OF ENDOGENOUS HORMONES

JA, SA and ABA were extracted from 200 mg DW (dry weight) of 10 leaves of plants with high and normal shedding of leaves as described by Durgbanshi et

al. (2005), with some modifications. Plant material was homogenized in an Ultraturrax T25 basic homogenizer (IKA, Staufen; Germany) with 5 ml deionized water. D5-OPDA and D6-JA (Leibniz-Institute of Plant Biochemistry; Halle, Germany) were used as internal standards, and samples were added with 50 ng of each. Samples were centrifuged at 1540 × g for 15 min, supernatant was adjusted to pH 2.8 with 15% (v/v) acetic acid and extracted twice with diethyl ether, and organic fraction was evaporated under vacuum. Dried extracts were dissolved in 1 ml methanol and filtered on a vacuum manifold at flow rate < 1 ml min^{-1}, and eluate was evaporated at 35 °C under vacuum in a SpeedVac SC110 (Savant Instruments; New York, NY, USA). Four biological replicates were used for assays.

Hormone identification and quantification by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS-MS)

JA, SA and ABA were separated from plant tissues by reversed-phase HPLC, using an Alliance 2695 separation module (Waters; Milford, MA, USA) equipped with a Restek Ultra C18 3 μm column (100 × 3.0 mm). Fractions were separated using a gradient of increasing methanol concentration, constant glacial acetic acid concentration (0.2% in water), and initial flow rate 0.2 ml min^{-1}. The gradient was increased linearly from 40% methanol/60% water-acetic acid at 25 min, to 80% methanol/20% water acetic acid. Initial conditions were restored after 1 min, and the system was allowed to equilibrate for 7 min. The identification and quantification of phytohormones was performed with a quadrupole tandem mass spectrometer (MS/MS) (Quattro pt Ultima, Micromass, Manchester, UK) fitted with an electrospray ion (ESI-) source, in multiple reactions monitoring mode (MRM) using precursor ions and their transitions (m/z) to ABA (m/z 263/153), D_6-ABA (m/z 269/159), JA (m/z 209/59), D_6-JA (m/z 215/59), SA (m/z 137/93), D_3-SA (m/z 141/97). The cone energie used was 35 V. The spectrometry software used for data analysis was MassLynx version 4.1 (Waters).

3 RESULTS

The results of the soil physical analysis are presented in Table 1.

Table 1. Average values of soil physical properties

Parameters	Normal shedding of leaves	High shedding of leaves
Mechanical resistance to penetration (MPa)	1,51	1,50
Structural stability (mm)	1,72	1,78
Apparent density (Kg m^{-3})	1,08	0,95
Infiltration (min.)	1,96	1,77
Gravimetric moisture (g g^{-1})	33,27	33,01
Total pores (%)	59,46	65,12

The data of the climatic variables recorded are presented in Figure 1.

Figure 1. Registered climatic variables: average rainfall (mm), relative humidity (%) and heliophany (hours).
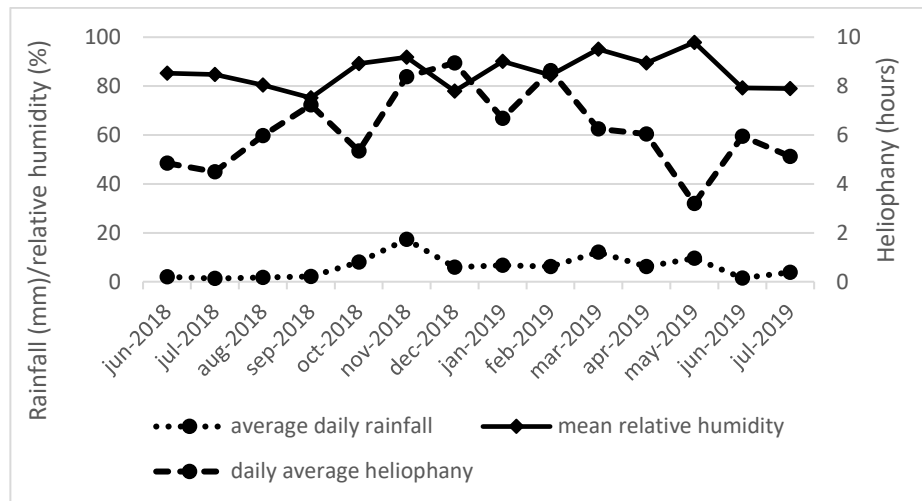

In winter, there is a greater amplitude between relative humidity (high) and heliophany (low) (Fig. 1).

Shedding of leaves behavior was similar in both plantations evaluated. Throughout the year, shedding of green leaves exceeded the leaves with natural senescence, but in the plantation with high shedding of leaves this proportion was higher. In autumn there was an increase in the percentage of leaves with natural senescence (Fig. 2).

Figure 2. Type of leaf collected (%) in the two commercial plantations: (a) normal and (b) high shedding of leaves.
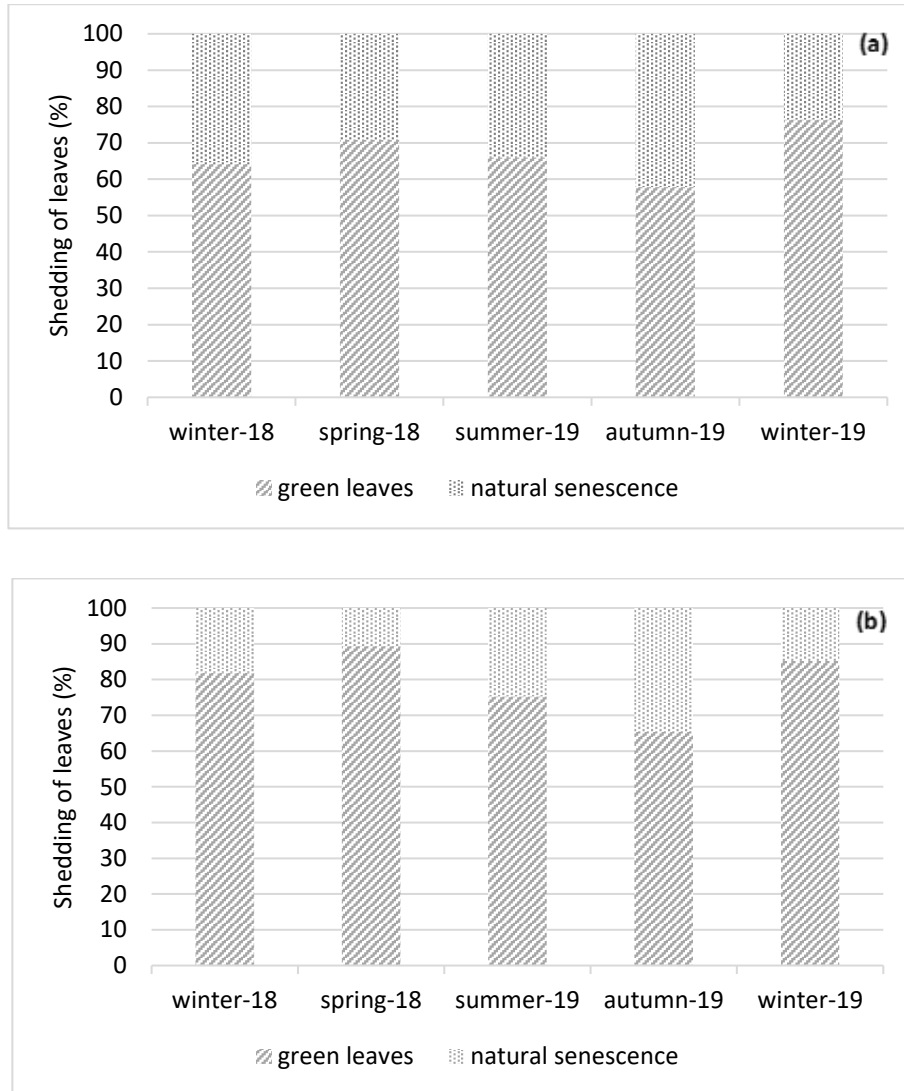
The dry weight of shedding of green leaves, in general, was low during the period analyzed and no significant differences were recorded between the different months, except in June 2019. That month the shedding of green leaves was 56.9 g per plant and 87.57 g per plant, in plantations with normal and high shedding of leaves, respectively (Fig. 3 a and b).

Figure 3. Average dry weight (g) of shedding of green leaves per plant, in plantations with normal (a) and high (b) shedding of leaves. Means values (± S.E.) followed by different letters above bars are significantly different at $P < 0.05$ (n = 10).
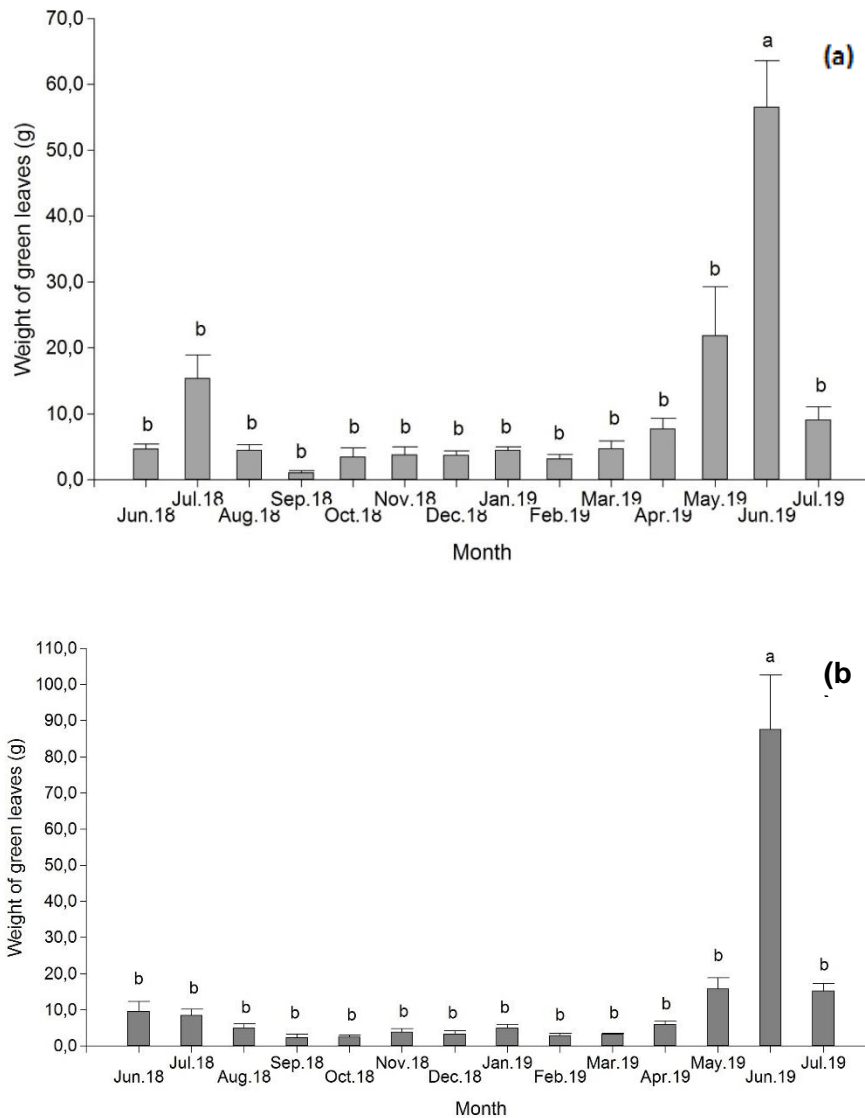
In terms of yield, there was a decrease of 1,745 and 2,829 kg of green leaf per ha, in plantations with normal and high shedding of leaves, respectively. Based on the average yield of the plantations, these values represent a decrease of between 14.5 and 18.9%.

Spots caused by *Cylindrocladium* sp., *Colletotrichum* sp. and *Guignardia* sp. were found on shedding of green leaves and leaves with natural senescence (Fig. 4). The symptoms of the different genera of fungi found were more frequent on green leaves, and less frequently on chlorotic leaves.

Figure 4. Symptoms of the fungal genera found in yerba mate plantations: (a) *Colletotrichum* sp., (b) *Guignardia* sp. and (c and d) *Cylindrocladium* sp.
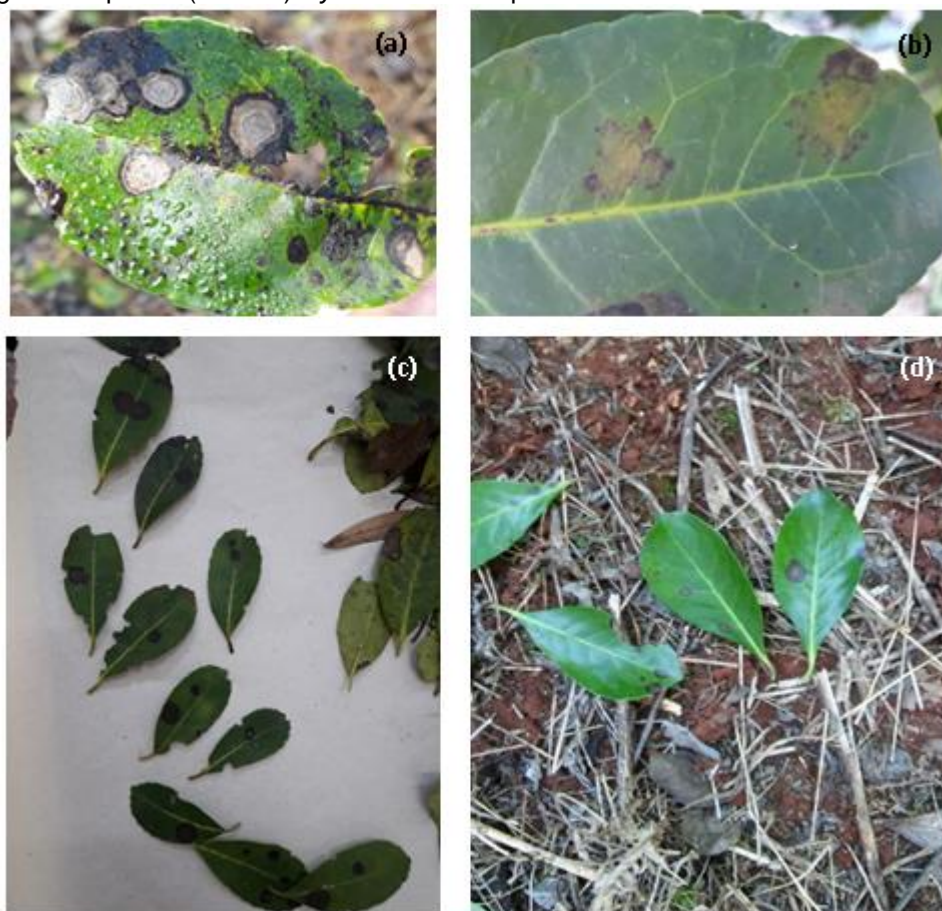
Of the three fungal genera, *Cylindrocladium* sp. was the most important. The presence of *Cylindrocladium* sp. spots on green leaves collected in the boxes varied among the different months evaluated. The highest values of green leaves with black spot symptoms were recorded in the winter months. In July 2018, 92% of the green leaves collected in the boxes had *Cylindrocladium* sp. symptoms, and in June 2019 that value reached 97% (Fig. 5).

The higher presence of *Cylindrocladium* sp. coincided with records of low daily heliophany (high negative correlation) (Tabla 2). No differences were found between the plantations in the percentage of leaves with *Cylindrocladium* sp. The average data for both plantations are shown in Figure 5.

Table 2. Pearson's correlation coefficient between heliophany and presence of green leaves with cylindrocladium (p < 0.05).

	Leaves with *Cylindrocladium* (%)	Heliophany
Leaves with *Cylindrocladium* (%)	1,00	4,3 E-03
Heliophany	-0,71	1,00

Figure 5. Shedding of green leaves with spots caused by *Cylindrocladium* sp. Means values (± S.E.) followed by different letters above bars are significantly different at P < 0.05 (n = 10).
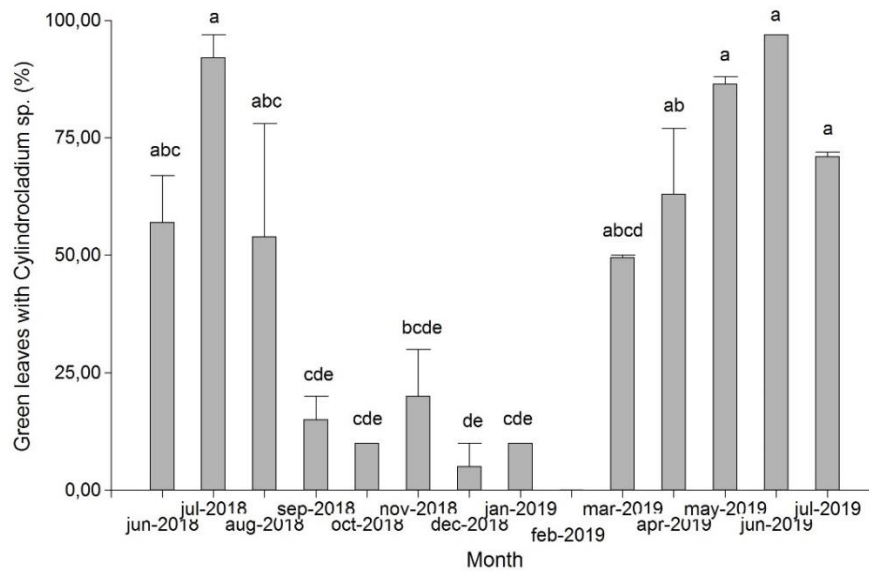

Figure 6. Plant with high shedding of leaves.

Although it was not quantified in this work, a differential behavior between plants was observed in both plantations. Under the same management conditions, there were plants with normal or high shedding of leaves. In plants with high shedding of leaves, spots of *Cylindrocladium* were observed (Fig. 6).

4 CHANGES IN THE LEVELS OF ENDOGENOUS HORMONES IN LEAVES OF *I. PARAGUARIENSIS*

To study the relationship between endogenous hormone content and foliar abscission triggered by fungi attack, an experiment on plants with differential level of susceptibility to *Cylindrocladium* sp attack was performed to get a profile of JA, ABA and SA hormone accumulation.

In plants with normal shedding of leaves (control), the hormonal concentrations of all hormones remained at lower levels compared to plants with leaf abscission. Among the quantified hormones, the ABA level was the highest compared to the other two hormones in both control plants as well as plants with high shedding of leaves (Fig. 7). On the contrary, the lowest level was recorded for SA (Fig. 9).

The ABA level showed an increase of 1.33 times in plants with high shedding of leaves compared to those with normal shedding of leaves (Fig. 7) In a similar fashion, the JA level increased significantly (2-fold) in plants with high shedding of leaves (Fig. 8). On the contrary, no statistical differences between plants with normal and high leaf shedding in SA level were detected (Fig. 9).

Figure 7. Endogenous ABA content in plants with normal or high shedding of leaves. Means values (± S.E.) followed by different letters above bars are significantly different at $P < 0.05$ (n = 4).

Figure 8. Endogenous JA content in plants with normal or high shedding of leaves. Means values (± S.E.) followed by different letters above bars are significantly different at $P < 0.05$ (n = 4).

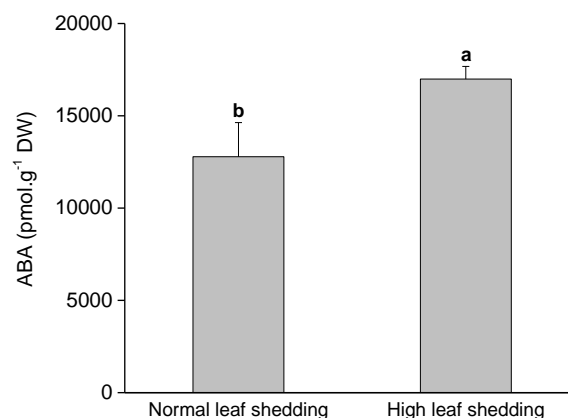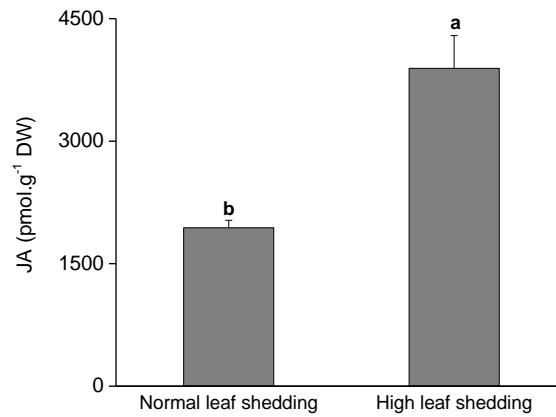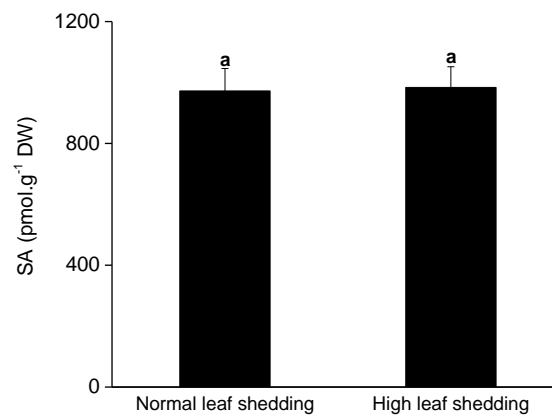Figure 9. Endogenous SA content in plants with normal or high shedding of leaves. Means values (± S.E.) followed by different letters above bars are significantly different at $P < 0.05$ (n = 4).

5 DISCUSSION

Leaf senescence is part of the development process of plants and its occurrence depends largely on the growth habitat to which each species is adapted (Munné-Bosch and Alegre, 2004).

In yerba mate, leaf shedding is an event that commonly occurs in the autumn-winter season, coinciding with periods of high rainfall and unharvested plantations (Burtnik, 2006).

In this work, the values of the soil physical variables indicate that the condition of the soil is fair to poor (Bárbaro et al., 2017). Both plantations have high compaction, with poor infiltration levels, which could generate waterlogging conditions. This situation could be affecting yerba mate plants, which require well-drained soils.

Species more sensitive to flooding show greater growth reduction, because they experience reductions in the rate of formation and expansion of new leaves (Angelov et al., 1996). Thus, the photosynthetically active leaf area may also be reduced by accelerated leaf senescence and abscission (Kozlowski, 1997). The data obtained from the present work reinforce this theory. In the winter months, a higher proportion of green leaf fall was observed (Fig. 2). This could be due to the fact that during those months there was higher relative humidity, associated with higher cloudiness, and therefore, low heliophany (Fig. 1). Thus, leaf abscission is part of an "escape" strategy by which plants seek to reduce the size of their canopy in response to stress (Striker, 2012).

In both plantations, high shedding of leaves was significant only in June 2019, where the lowest values of heliophany and the highest values of relative humidity were recorded in the previous weeks. This situation caused the delay of harvest in the plantation with normal shedding of leaves. Meanwhile, in the plantation with high shedding of leaves, harvesting was not carried out because it was not economically convenient for the grower. This situation created an environment with less lighting and ventilation due to the unharvested foliage. Similar situations occurred prior to this study. During 2017, there was an exceptional period of excess rainfall, which caused a delay in the harvest and, as a consequence, higher shedding of leaves in yerba mate plants. That same year there was a decrease in heliophany (20% below the historical average) (Olinuck, 2017).

According to Crous et al. (2006), the genus *Cylindrocladium* represents an important group of pathogens associated with diverse hosts in tropical and subtropical regions of the world. It is usually associated with high humidity and rainfall conditions (Rhodes et al., 2005; Akonda et al., 2014; Rybak et al., 2014). In addition, it has been reported for several species of *Ilex* sp. as a cause of numerous foliar diseases (Gill et al., 1971; Rivera Flores, 1983; Lau, 1997; Lechat et al., 2010).

The predisposition to attack by *Cylindrocladium* sp. is high when stress situations are generated in plants, the most important of which are the decrease in soil pH, the presence of low luminosity, suboptimal temperatures and prolonged exposure to nutritional deficit (Blanco Vieira and Romero Cardozo, 2017). In this

context, the climatic conditions during the autumn-winter months have favored the presence of *Cylindrocladium* sp., where a high percentage of shedding of green leaves with the characteristic symptomatology of the fungus was recorded.

In addition, different plant responses to *Cylindrocladium* attack were found. This could be indicating a condition of greater susceptibility of some plants to the fungus, as already mentioned by some authors in other species (Flores et al., 2017).

Shedding of plant leaves or other organs such as petals and flowers are characteristics of abscission. This separation of plant organs from the main plant body occurs when either there is an injury due to biotic/abiotic stress or as a regular process in plant growth and development. Currently, drought is known to trigger cauline leaf abscission; however, biotic stimuli may also initiate it (Patharkar and Walker, 2016). Thus, pathogen-triggered leaf abscission appears to be an effector mechanism eliminating disease leaves, which reduces the possibility of infection spreading to healthy tissue (Patharkar et al., 2017). For example, necrotrophic pathogens -such as *Cylindrocladium* sp- kill host plant cells using toxic metabolites and then feed on the remains. Therefore, these fungi cause devastating diseases in both horticultural and agronomic crops, but our understanding of plant defense responses against these pathogens is still limited.

Among the factors that regulate the complex process of senescence and abscission, plant hormones have pivotal importance based on their interaction with each other, their transport, concentrations in different tissues, and affinities of their receptors (Ahmad et al., 2010; Peleg and Blumwald, 2011; Estornell et al., 2013). Therefore, a number of more classical plant hormones exert influence over abscission, among them SA, JA and ABA (Patharkar and Walker, 2018; 2019).

In this study, we detected an accumulation of ABA, JA and SA hormones in plants of yerba mate with high shedding of leaves, which was associated with the attack of the *Cylindrocladium* sp necrotrophic fungus. Upon invasion of pathogens, JA is synthesized and mediates a signaling cascade starting numerous defense responses. In this sense, it has been documented that JA is involved in plant defense against necrotrophic fungi infection through the JA-receptor, COI_1 (Gimenez-Ibanez et al., 2016; Zhang et al., 2017). According to our results, the increase of approximately two-fold in JA level of plants with high shedding of leaves

would allow us to suggest that the JA pathway may the most important defense line triggered by *Cylindrocladium* infection. Chen et al. (2015) reported in Eucalyptus leaves (E. urophylla × E. tereticornis M1) infected with *Calonectria pseudoreteaudii*, teleomorph genus of *Cylindrocladium*, an up-regulation of key enzymes in JA biosynthesis (LOX and AOS) and high transcript abundance of LOX (Eucgr.J00821.1) in the susceptible clone. Therefore, those work demonstrated that this pathogen infection could enhance the biosynthesis of JA and activate the JA pathway. It is well documented JA acts synergistically with ethylene (ET) upon necrotrophic pathogen attack, and both signaling pathways are required for the production of defense proteins such as PLANT DEFENSIN 1.2 (PDF1.2), via the APETALA2/ERF (AP2/ERF) TF family, such as ERF1 and OCTADECANOID-RESPONSIVE ARABIDOPSIS 59 (ORA59) (Zarei et al., 2011; Pieterse et al., 2012; Wasternack and Hause, 2013). Recently, Chen et al. (2021) reported that the transcription factor WRKY75 positively modulates JA-mediated plant defense to necrotrophic fungal pathogens, which may occur through its function as a positive regulator in JA/ ET-mediated signaling pathways. Therefore, further studies will be necessary to investigate the role of other hormones (especially ethylene) in the regulation of leaf abscission-induced by *Cylindrocladium* infection in yerba mate.

On the other hand, SA is a major regulator of defense against biotrophic pathogens, which feed on living host tissues (Dong, 2004; Vlot et al., 2009; Ding and Ding, 2020). In this study, the activation of JA signaling has been shown to suppress, at least in part, the SA signaling pathway because this hormone had the lowest content in plants with high shedding of leaves. Thus, increasing evidence indicates that the SA- and ET/JA-mediated defense response pathways are mutually antagonistic (Li et al., 2019).

Regarding ABA, their levels and transcriptomic profling studies have that this hormone participates in important regulation during abscission in diferent species or organs (Giulia et al. 2013; Wilmowicz et al. 2016; Li et al. 2019). Moreover, the effect of ABA on abscission seems to depend on its interaction with auxin (such as indole-3-acetic acid, IAA) or ET, rather than being directly involved on its own. Thus, ABA could have an intermediary role in organ abscission (Aurelio et al., 2000; Agustí et al., 2007). On the other hand, ABA ABA biosynthesis is

required for efficient disease resistance against necrotrophic fungal pathogens (Ton and Mauch-Mani, 2004; García-Andrade et al., 2011); in particular its role in plant defense responses to necrotrophs can be positive or negative as well as dependent of the specific plant-pathogen interactions (Ghozlan et al., 2020). In this sense, ABA seems to play positive roles during the early events of infection by affecting stomatal closure and deposition of callose and the negative roles usually at later stages that include suppressing ROS accumulation and SA induction (Asselbergh et al., 2008; Ton et al., 2009). It has been suggested that the role of ABA inducing resistance and susceptibility may be due to the differential action of upstream and downstream components in ABA signaling pathway (Alazem et al., 2014). In this study, the increased ABA content quantified in plants with high shedding of leaves was associated with presence of necrotic spots caused by *Cylindrocladium* sp. detected in them. Likewise, Jelenska et al. (2007) reported that ABA was rapidly synthesized in plants following inoculation with other necrotrophic fungal pathogens; e.g., its concentration increased in sugar beet (*Beta vulgaris*) leaves as response to *Cercospora beticola* infection. Moreover, the effect of ABA appears to be complex and requires cross-interaction with SA, JA, and ET signaling transduction pathway (Koornneef and Pieterse, 2008; Verhage et al., 2010; AbuQamar et al., 2017).

6 CONCLUSIONS

Firstly, it was possible to use collection boxes for the quantification and classification of shedding of leaves of yerba mate plants. It allowed periodic monitoring to characterize the behavior of leaf fall.

Cylindrocladium sp. is a fungus that is present in the yerba mate plantations, and has its peak of appearance in autumn-winter. Predisposing climatic conditions such as humidity and low heliophany, caused by excessive rainfall and cloudy days, increase the incidence of the fungus, causing excessive shedding of leaves. This situation is aggravated when harvesting is delayed, with the consequent decrease in yield.

In general terms, it could be thought that, with predisposing climatic conditions, an early harvest would reduce the loss of green leaves.

The JA pathway may the most important defense line triggered by

Cylindrocladium infection.

High accumulation of JA and ABA detected in plants with high shedding of leaves which might suppress the SA signaling pathway responsible for the establishment of SAR.

It is necessary to continue monitoring the plants that have shown differences to *Cylindrocladium* attack. In this way, it could be assured that there is a genetic predisposing condition of some plants to the attack of the fungus.

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