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Original article

Phenological evaluation of *Ilex paraguariensis* as a basis for a green leaf predictive model

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ABSTRACT

The objective of this work was to describe the vegetative phenology of yerba mate (Ilex paraguariensis St. Hil.) and the correlation with environmental variables: precipitation, relative humidity, PAR radiation, and gravimetric water, also photoperiod, mean, minimum and maximum temperatures. The work was carried out in a commercial orchard located in Gobernador Virasoro, Corrientes, Argentina (28° 02.48'' S and 56° 00' 34'' W, elevation 151 m a.s.l). Thermal requirements (degree days) and the relationship between a phenophase and each individual environmental variable were calculated using Spearman's correlation coefficient (rs). From 2015 and during 36 consecutive months, five vegetative phases were identified: swollen bud, incipient leaves, leaves attached at the apex, unfolded leaves and mature leaves. The presence of different sprouting rhythms was corroborated, with different intensities in spring, summer and autumn. The results conclude that thermal times vary according to the rhythms presented and stages of crop development.

Key words: development stages, degree days, climate

INTRODUCTION

The yerba mate plant (*Ilex paraguariensis* St. Hil) belongs to the Aquifoliacea family. It is represented, approximately, by 660 species, most of the genus *Ilex*, being recognized in South America about 60 species. It has leathery leaves, simple and alternate, straight trunk and smooth bark. Its roots are axonomorphic, with secondary roots that are located in the first centimeters of the soil and allow the absorption of nutrients [1].

With leaves and fine stems of yerba mate, a stimulating drink known as mate, chimarrao or terere is prepared. The ingestion of yerba mate has important biological properties, such as antioxidant activity, protective effects against DNA damage and vasodilation [2]. Currently, according to the National Institute of Yerba Mate [3], there are more than 174 820 ha^{-1} cultivated in Argentina, distributed in the province of Misiones and northern Corrientes. It is considered a crop of cultural and economic interest for thousands of families that live from its production, and it is also grown in Brazil, Paraguay, and Uruguay.

Since the final product of the crop is the consequence of a process derived from the agricultural interventions carried out during the whole cycle, it is necessary for researchers and producers to know the agricultural phenology and the duration of the different phenological stages. Despite this, research in this field and for this species is scarce. Knowing the visible changes that occur throughout the cycle of a plant species, considering the effect of meteorological factors, would allow growers to efficiently manage the cultural work of the crop.

Phenophase is defined as a distinct event in the annual life cycle of a plant or animal in relation to seasonal and climatic changes [4].

From plant behavior in relation to environmental factors arises the need to evaluate the phenology of the yerba mate plant as a basis for a predictive model of green leaf. Therefore, the aim of this work was to describe the vegetative phenology of *Ilex paraguariensis* St. Hill and to correlate the environmental variables, temperature, relative humidity, photoperiod, PAR radiation, precipitation and gravimetric water, with the vegetative phenophases.

MATERIALS AND METHODS

The evaluations were carried out in a commercial plot located in Gobernador Virasoro locality, (28° 02,48″ S and 56° 00′34″ W, elevation 151 m a.s.l), Corrientes, Argentina. These soils present good physical conditions for root and water penetration; the superficial horizon presents high contents of organic matter. They are moderately fertile, with low cation exchange capacity, due to the type of low activity clays (kaolinites), although with good moisture retention capacity. The most fertile soils for cultivation are represented by Alfisols and Ultisols, with slopes of less than 5 %, good fertility and low aluminum content [5]. The region climate, in the Köppen classification system, is considered humid subtropical (Cf w'a), which expresses a mesothermal climate, warm temperate, without a dry season, with rainfall distributed regularly throughout the year and average annual temperatures of 25 °C. Rainfall is abundant with an annual average of 1923 mm in the last ten years. The months with the highest and lowest rainfall are April and October and July and August, respectively.

Precipitation was measured with a seesaw type rain collector and the photoperiod was calculated using tables (astronomical theoretical heliophany). To obtain the micrometeorological data, an eight-channel datalogger was used, located at the study site, with temperature, PAR radiation and relative humidity sensors in two canopy strata, above the canopy and in the middle stratum. Averages were obtained for each fortnight [(6)] and month evaluated (Table 1). Soil samples were taken weekly from the first 30 cm to determine the gravimetric water, subjected to a temperature of 105 ºC, until constant mass, expressed as a percentage of water per mass of dry soil.

Observations: In order to characterize the phenological phases of the species, an adaptation of the semiquantitative scale of Fournier 1974 was used, which establishes the observation of five to ten individuals for plants in the wild. Considering that they are cultivated plants, 20 plants were taken for the study. This scale arbitrarily establishes four intervals (0-4) of 25 % intensity, where 0 = absence of the phenophase, 1 = 25 %, 2 = 50 %, 3 = 75 % and 4 = more than 75 % of the crown.

The duration of each phenophase was expressed as cumulative growing degree days (CGD), for which a base temperature of 5 °C was considered [(7)]. Four experimental blocks were established within a homogeneous frame, in terms of origin, age (four years) and general condition of the plant (mature branch cut), soil type and location, and crop management. In each block, five adult plants spaced at 1 x 2.5 m were randomly selected for phenological observations. The evaluations were carried out from September 2015 to July 2018, every two weeks during sprouting and monthly during growth pauses. Considering year 1, the 2015-2016 campaign (September-July), year 2, 2016-2017 (August-July) and year 3, 2017-2018 (August-July). For the differentiation of the different sprouting rhythms that occurred throughout the period evaluated, observations of internode length were made.

Due to the lack of background in phenology studies in yerba mate and considering that the phenological cycles of tropical plants are complex, the phenological method was calibrated during the first months. This methodology allowed familiarization with the behavior of the plant to identify the different phenological phases. Plants were harvested in August of each year evaluated, according to the annual harvest plan, table cutting. This type of harvest consisted of cutting branches 3 cm in diameter, at the same height, 10 cm above the previous cut, leaving the young branches uncut and others cut at the apex about 10 cm above the bifurcation.

Phenological stages: the phenological evaluation scale used was based on macroscopic observation of the external plant morphology. Five vegetative phenological events (phenophases) were studied from the beginning of sprouting to harvest. The vegetative phenophases studied were differentiated according to the sprouting rhythms presented throughout the year. Clearly recognizable external characteristics were used for each stage of development.

Vegetative phenophases: bud swelling (V1): the phenological stage V1, considered as bud swelling, was established in each sprouting rhythm. Incipient leaves (V2): incipient size, 1.5 to 2 cm in length, of the first leaves to develop, after bud swelling and before they fold. Leaves attached at the apex. (V3): considers the behavior of leaves near the apex, which remain attached. Fully unfolded leaves (V4): this stage allowed to highlight a stage prior to maturation. Mature leaves (V5): this phase marks the beginning of the plant's maturation stage and the end of a sprouting rhythm. Leaves begin to turn dark green.

Statistical analysis: the relationship between a phenophase and each individual environmental variable was analyzed using Spearman's correlation coefficient (rs), using InfoStat statistical software [8]. The evaluated variables, PAR radiation, relative humidity, mean, minimum and maximum temperatures, were calculated as an average of the sensors located in the crop, above and below the canopy. Environmental variables were correlated with phenological variables throughout the study period (36 months). To analyze the monthly relative frequency of the species in each phenophase, the Rayleigh (Z) test for circular distribution was used and the results were plotted using circular frequency histograms [9]. The ORIANA 3 software [10] was used to make these graphs, in which the months were converted into angles, with 15° intervals for each observation (0° first fortnight of August, 15° second fortnight of August, 345° second fortnight of December). Arrows represent significant mean angles (Rayleigh test $p<0.001$), while the length of the vector r (0-1) indicates the concentration around the mean angle.

RESULTS AND DISCUSSION

The accumulated precipitation values for year 1, 2 and 3 showed large differences, 1606.4 mm, 2324 mm and 2147 mm, respectively. Through the evaluated time series, it can be observed that different precipitation values were recorded from other years, February 2016 (12 mm), April and May (509 mm, 479 mm), April 2018 (31 mm). The relative humidity evaluated in the yerba mate plantations, showed an annual variation. Probably the management system used by the company, mainly pruning and harvesting, partly modifies the values recorded. The values show a variation in a range from 75 % (July 2017) to 95 % (July 2018).

It is likely that the canopy volume, throughout the growing period, retains water vapor inside the crop and higher values are obtained inside than outside, as well as before and after harvest. Similar values have been obtained for the influence of development on the microclimatic conditions of the crop [11]. Regarding gravimetric humidity for the study period, they show a minimum of 18.7 % (August 2017) and a maximum of 30 % (May 2016). Studies conducted in the producing region show average values of 28.59 % and 22.58 % [12] (Figure 1).

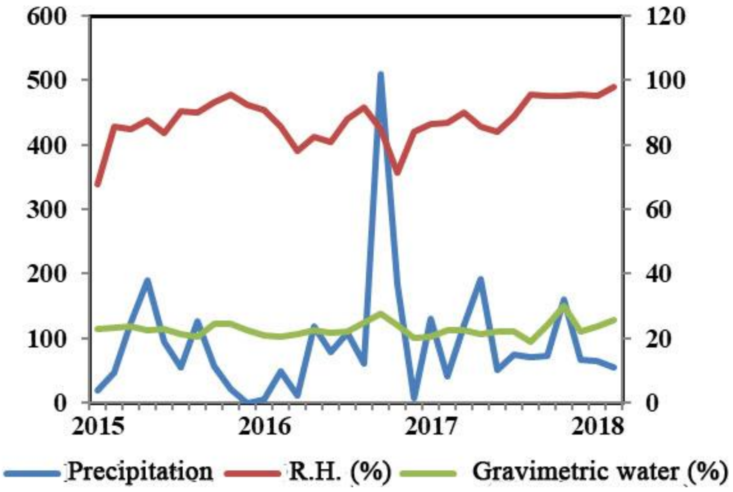

Figure 1. Precipitation values recorded for the study period (2015-2018)

It is necessary to consider that the gravimetric water content is influenced by the annual plan of cultural work that the company carries out, specifically by compost incorporation. In this sense, different authors have found that the compost incorporation modifies soil physical-chemical properties, associated with an increase in carbon content and water retention capacity [13].

When analyzing the photosynthetically active radiation (PAR) reaching the yerba mate stratum, large differences were recorded during the year and among the years evaluated. The highest mean radiation values were found during November, December and January months, for the first two years evaluated and October, November and December for year 3 (2017-2018). In a cultivated system, PAR radiation is determined by geographical, temporal and structural factors, such as: latitude, species, stand density, leaf area index, biomass and canopy structure, both vertical and horizontal [14] (Figure 2).

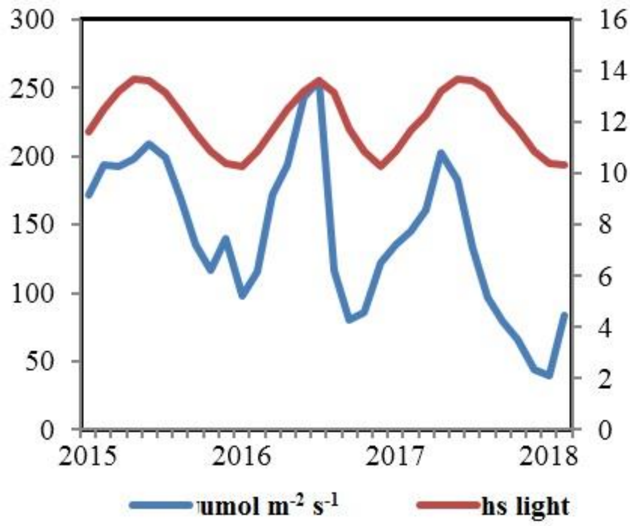

Figura 2. Photoperiod (hs) and photosynthetically active radiation values for the study period 2015-2018

The monthly mean, maximum and minimum temperature records are shown in Figure 3. The mean temperatures ranged from 0.5 to 3 °C. Minimums were recorded for the month of June 2016 (5.4 °C) and July 2017 (4.8 °C) and maximums for the month of January 2015 (29.9 °C), February 2016 (29.5 °C) and December 2017 (29.09 °C).

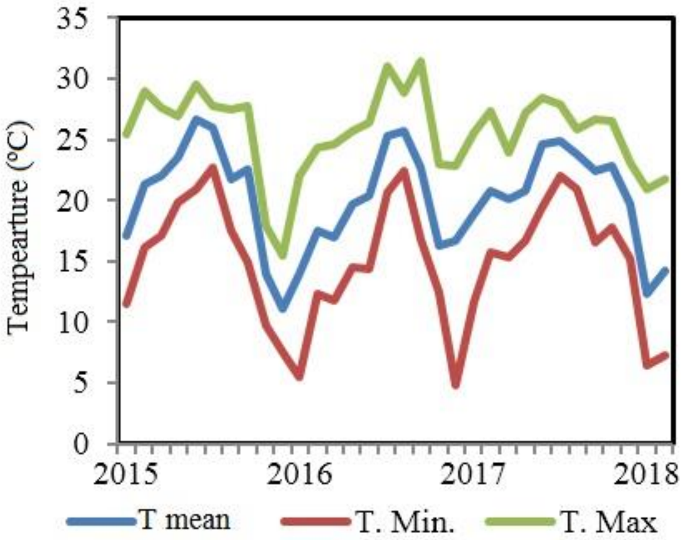

Figura 3. Temperature °C values (maximum, mean and minimum) for the study period 2015-2018.

Vegetative Phenophases

Bud swelling (V1)

During the periods evaluated, the first sprouting rhythm began after a period of biological dormancy and after harvest.

Figure 4 shows that for years one and two, the onset of budburst was recorded in September, lasting approximately 13 and 25 days, respectively. However, for year three the onset of sprouting was recorded on August 12, 2017, to end on August 31, 2017, with an approximate duration of 19 days. This difference can be attributed to the higher rainfall occurred in the month of August, 2017-2018 (262 mm) compared to the previous years evaluated (69 mm and 97 mm).

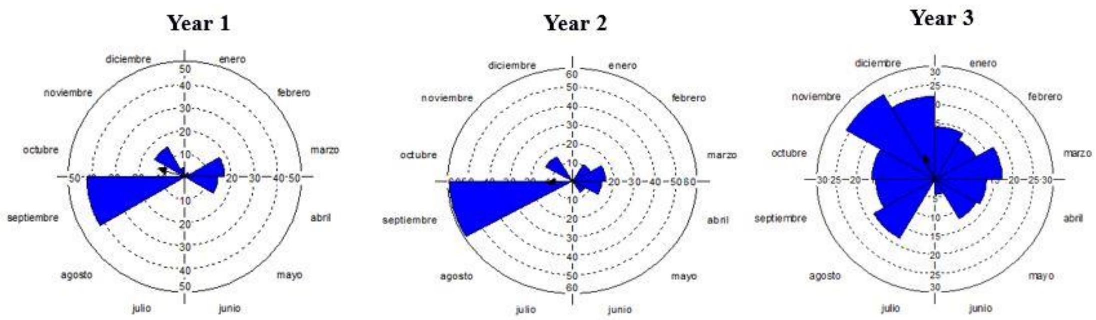

Figure 4. Circular histograms of monthly frequency for vegetative phenophase V1 (bud swelling) of Ilex paraguariensis for years, 1 (2015-2016), 2 (2016-2017) and 3 (2017-2018)

In the years evaluated, three rhythms of this phenophase could be identified. Similar results were found by other authors, who defined that there are alternating periods of yerba mate growth in three different periods: spring, summer and autumn [(15)]. Although three rhythms were observed during the observation period, with the observation methodology described, the change in the frequency, intensity and duration of the bud swelling phenophase is highlighted for years with the highest rainfall.

During year one, where rainfall was lower compared to years two and three, both the intensity and duration in the three rhythms are considerably reduced, presenting the shortest duration of this phenophase in the three years evaluated.

The growth pattern of the species was characterized using segmentation models during two years of evaluation, in this study it was verified that there is a systematic resting phase corresponding to a period with relatively cold temperatures, while the resting phases in the middle of the year were frequent, but not obligatory [(16)]. Depending on when it happens, at the end or at the beginning of a phenophase, the lack of water can accelerate crop development by raising the temperature of the plants and, therefore, reducing the number of days required to reach a given thermal integral [(17)].

The TT5 requirement calculated to initiate the first sprouting rhythm, considered after winter dormancy and after pruning, was 951.30 (GD). Once the phenophase started, the duration was 149 GD (year 1), 351.25 GD (year 2) and 259.65 GD (year 3). These results suggest that, in addition to temperature, there are other variables that influence the duration of each phenophase that have not been considered in this study. The second flush of sprouting was recorded for year one and two in November, with a duration of 19 days (27 %) and 22 days (18 %), approximately. In year three, the second sprouting, which stood out for both intensity and duration, began in September until the end of December, totaling 124 days. The TT5 required to initiate the second rhythm was 441.6 GD. The third occurrence of the phenophase was in March for year 1 with 66 days and February for years two and three, with 88 days and 102 days, respectively. The TT5 required to initiate the third sprouting rhythm was 317 GD.

Based on the evaluation and recording of the data, it could be considered that to trigger the phenophase initiation, and for the first rhythm, 951.3 GD are required; for the second rhythm 441 GD; and for the third rhythm 317 GD). When the three observed years are analyzed, positive correlations with precipitation are obtained (Table 1). For year one and two no correlations were detected (Tables 2 and 3). For year 3 (2017-2018) a positive correlation was recorded with the variable radiation and maximum temperature and a negative correlation with relative humidity (Table 4).

Table 1. Spearman correlations (rs) for vegetative phenophases of *Ilex paraguariensis* plants and the variables temperature, photoperiod, relative humidity, PAR radiation, precipitation and gravimetric water

Environmental variables	**V1**		**V2**		**V3**		**V4**		**V5**	
	rs	**p**	**rs**	**p**	**rs**	**p**	**rs**	**p**	**rs**	**p**
Mean Temperature	0,22	0,1876	0,21	0,2119	**0,61**	**0,0001**	**0,43**	**0,0087**	-0,31	0,0659
Minimum Temperature	0,28	0,1014	0,26	0,1286	**0,55**	**0,0005**	**0,48**	**0,0028**	**-0,34**	**0,0431**
Maximum Temperature	0,25	0,1489	0,14	0,4163	0,31	0,0669	0,24	0,1617	**-0,33**	**0,0471**
PAR Radiation	0,21	0,2225	0,31	0,0615	**0,42**	**0,0101**	0,14	0,4012	**-0,45**	**0,0061**
Precipitation	**0,37**	**0,0275**	0,14	0,4226	0,32	0,0598	**0,36**	**0,0292**	-0,2	0,2331
R.H. (%)	-0,1	0,5577	-0,09	0,5916	0,06	0,7442	0,11	0,5332	0,22	0,1938
Photoperiod	0,28	0,096	0,29	0,0872	**0,65**	**<0,0001**	**0,4**	**0,0149**	**-0,36**	**0,0325**
Gravimetric water (%)	0,08	0,6473	-0,08	0,637	-0,21	0,2191	0,12	0,4817	-0,02	0,8999

Evaluation 2015-2018. Significant values: p<0.05; p: significant values<0.05. rs: Spearman correlations

Average Temperature

Table 2. Spearman correlations (rs) for vegetative phenophases of *Ilex paraguariensis* plants and the variables temperature, photoperiod, relative humidity, PAR radiation, precipitation and gravimetric water

Environmental variables	**V1**		**V2**		**V3**		**V4**		**V5**	
	rs	**p**	**rs**	**p**	**rs**	**p**	**rs**	**p**	**rs**	**p**
year 1 (2015-2016)										
Mean Temperature	0,4	0,2011	0,18	0,578	**0,69**	**0,0123**	0,37	0,2384	-0,43	0,1653
Minimum Temperature	0,19	0,5573	0,05	0,87	**0,78**	**0,0027**	**0,65**	**0,0232**	-0,33	0,2888
Maximum Temperature	0,36	0,2487	0,28	0,373	0,19	0,5488	-0,09	0,7822	-0,51	0,0897
PAR Radiation	0,47	0,125	0,43	0,1617	**0,75**	**0,0046**	**0,5**	**0,0951**	**-0,86**	**0,0003**
Precipitation	0,51	0,093	0,35	0,272	0,41	0,19	0,13	0,6951	-0,2	0,5405
R.H. (%)	-0,27	0,3908	-0,29	0,356	-0,08	0,8021	0,03	0,9217	0,5	0,0992
Photoperiod	0,47	0,1258	0,29	0,365	**0,82**	**0,0011**	**0,52**	**0,0854**	**-0,59**	**0,0437**
Gravimetric water (%)	0,01	0,981	0,1	0,747	**-0,58**	**0,0474**	-0,37	0,2422	0,02	0,9416

Interannual evaluation 2015-2016. Significant values: p<0.05; p: significant values<0.05. rs: Spearman correlations

Table 3. Spearman correlations (rs) for vegetative phenophases of *Ilex paraguariensis* plants with the variables temperature, photoperiod, relative humidity, PAR radiation, precipitation and gravimetric water

Environmental variables	V1		V2		V3		V4		V5	
	rs	p	rs	p	rs	p	rs	p	rs	p
year 2 (2016-2017)										
Mean Temperature	0,08	0,7987	0,24	0,4585	**0,58**	**0,0503**	0,41	0,1853	-0,47	0,1265
Minimum Temperature	0,29	0,3545	0,36	0,2459	0,46	0,13	0,49	0,1048	-0,43	0,1656
Maximum Temperature	0,2	0,5316	-0,03	0,9194	0,3	0,3356	0,41	0,1895	-0,35	0,258
PAR Radiation	-0,07	0,8177	0,3	0,348	0,39	0,2164	-0,19	0,556	**-0,58**	**0,0471**
Precipitation	0,24	0,4474	-0,05	0,8755	0,2	0,5264	0,46	0,1302	-0,25	0,4257
R.H. (%)	0,06	0,867	0,14	0,6766	0,34	0,3009	0,24	0,482	0,07	0,84
Photoperiod	0,14	0,6602	0,38	0,2174	**0,76**	**0,0042**	0,43	0,1583	**-0,68**	**0,0155**
Gravimetric water (%)	0,21	0,5145	0,11	0,7319	0,23	0,4639	0,19	0,5451	-0,02	0,9426

Interannual evaluation 2016-2017. Significant values: p<0.05; p: significant values<0.05. rs: Spearman correlations

Table 4. Spearman correlations (rs) for the vegetative phenophases of *Ilex paraguariensis* plants with the variables temperature, photoperiod, relative humidity, PAR radiation, precipitation and gravimetric water

Environmental variables	V1		V2		V3		V4		V5	
	r	p	r	p	r	p	r	P	r	p
year (2017-2018)										
Mean Temperature	0,39	0,2113	0,34	0,2763	0,52	0,0854	0,33	0,2879	-0,18	0,5772
Minimum Temperature	0,43	0,1598	0,32	0,3128	0,43	0,1664	0,34	0,2861	-0,17	0,5911
Maximum Temperature	**0,64**	**0,025**	0,38	0,2269	0,53	0,0789	0,47	0,1202	-0,41	0,188
PAR Radiation	**0,91**	**<0,0001**	0,55	0,0614	0,52	0,0861	0,36	0,2444	-0,33	0,2757
Precipitation	0,47	0,1269	0,24	0,4484	0,32	0,286	0,38	0,2207	-0,02	0,9445
R.H. (%)	**-0,7**	**0,0116**	-0,42	0,1792	-0,37	0,2338	-0,08	0,8154	0,4	0,1933
Photoperiod	0,5	0,0999	0,37	0,2338	**0,48**	**0,1095**	0,37	0,2395	-0,08	0,7808
Gravimetric water (%)	0,09	0,7787	-0,4	0,202	-0,39	0,2127	0,4	0,1919	-0,06	0,8518

Interannual evaluation 2017-2018. Significant values: p<0.05; p: significant values<0.05. rs: Spearman correlations

The absence of correlations in year two and with the other variables suggests that the phenophase, considered as bud swelling, was regulated by factors intrinsic to the species and by other factors not considered and their interaction.

Incipient leaves (V2)

This phenophase (Figure 5) showed a similar behavior to the bud swelling stage, showing three rhythms during the first two years and a fourth in the third year of evaluation. During year one, the first rhythm was recorded in September with approximately 13 chronological days (CD), another one started in October with a duration of 20 days and the last one started in March with 18 days. The second year was recorded in September, with 16 days, December, 20 days, and February, 41 days. The third year began in August with a duration of 34 days, October with 17 days, November 69 days and a fourth rhythm identified in February with 39 days.

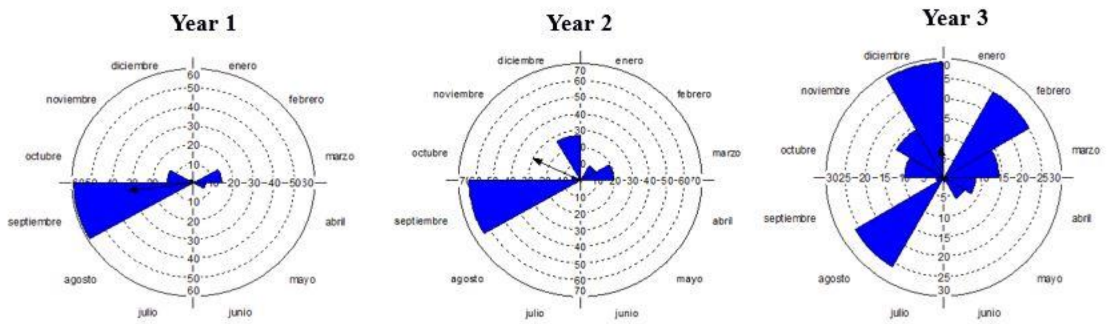

Figure 5. Circular histograms of monthly frequency for vegetative phenophase V2 (incipient leaves) of *Ilex paraguariensis* for years one (2015-2016), two (2016-2017) and three (2017-2018)

The calculated thermal time for the first, second and third paces was 297, 416 and 540 GD. The fourth rate exceptionally recorded for the third year was 766 GD. The incipient leaf phenophase showed no correlations either within or between years with any of the environmental variables evaluated (Tables 1, 2, 3, 4). This suggests that, in particular, this phenophase would be strongly regulated by intrinsic factors of the plant, or by factors not considered in this work.

Leaves attached at the apex (V3)

This phenophase represented marked differences in behavior during the period evaluated. For year one, the phenophase beginning occurred in September and ended in November, with a duration of approximately 37 days, and a second rhythm, from December to May, with a duration of 174 days, with the highest intensities in January. During year 2 the first rhythm was recorded in September ending in mid-November, 53 days, and a second rhythm that starts at the end of November and ends in March totaling 134 days, with the highest intensities in December (Figure 6).

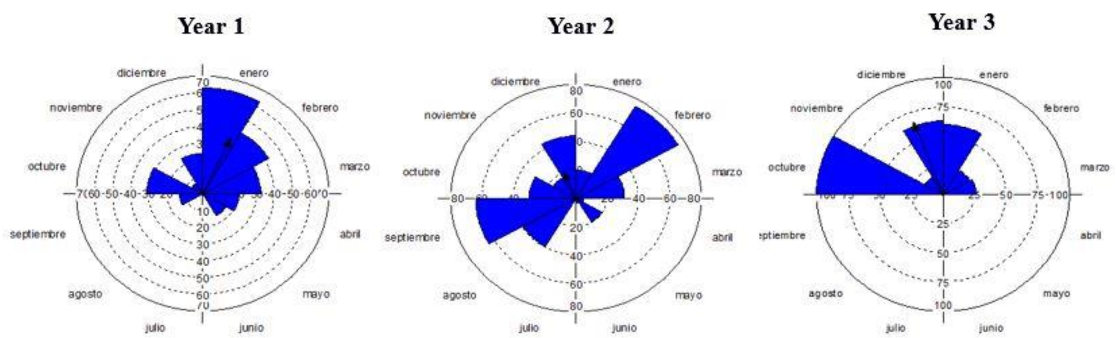

Figure 6. Circular histograms of monthly frequency for vegetative phenophase V3 (leaves attached at the apex) of *Ilex paraguariensis* for years one (2015-2016), two (2016-2017) and three (2017-2018)

Year 3 behaved notably different, with the greatest inequalities occurring both in the beginning and in the duration of the phenophase. The first rhythm was recorded in August and ended in the first half of September with a duration of 34 days, a second rhythm at the end of September until mid-November with 41 days, a third rhythm starting at the end of November and ending in May, 154 days, a third rhythm starting at the end of November and ending in May, 154 days, and a fourth rhythm starting in May and ending in June, 30 days. The lowest values were recorded in November. This could indicate that during the month of November and for the three years evaluated, the lowest values of intensity or the absence of the phenophase (year 1 and 2) of the state of leaves attached at the apex were recorded. With respect to degree days, 980, 197.67, 241 and 273 GD were accumulated for the first, second, third and fourth rates observed, respectively. When correlation values obtained within each year were observed, in year one a positive correlation was verified with minimum temperatures; mean, radiation, photoperiod and a negative correlation for gravimetric water. For year two, positive correlations were obtained for mean temperature and photoperiod (Table 1). In year three the results showed a positive correlation for the photoperiod variable (Table 3). Spearman correlations for the three years of study showed a positive correlation for minimum temperatures; mean temperature, radiation, and photoperiod (Table 4). In temperate climates, the regulation and rhythmicity of trees is mainly regulated by temperature and photoperiod. Some authors observed that yerba mate meristems remained in a resting state when winter temperatures were below 10 °C and photoperiod was short (about 10 h) [(18)]. During months evaluated, minimum temperatures of 7° and 1 °C (August, September 2015), between 9 and 5 °C (April, May, June and July 2016), 7 and 5 °C, were recorded during June and July 2017 and 2018. These months coincide with the highest presence values of the mature leaf phenophase, with a marked decrease of the apex-attached leaf phenophase.

Unfolded leaves (V4)

For the three years evaluated, differences are shown for both the beginning and the end of the phenophase. For year one, the greatest episodes of unfolded leaves, considered full budbreak, were recorded in October, November and February, with the absence or end of the phenophase from July to August (Figure 7) (year 1). In year 2, the months of October, the second half, November, January, April and May stood out for their frequency. In year three, it was recorded in September, with the highest intensities in October, January and March, the phenophase was absent from May to July. For year one, results showed a positive correlation with the variables minimum temperature, radiation and photoperiod. Year 2 and 3 showed no correlation with any of the variables evaluated. When analyzing the three years evaluated, the Spearman correlations were positive for the variables minimum temperature, mean temperature, photoperiod and precipitation.

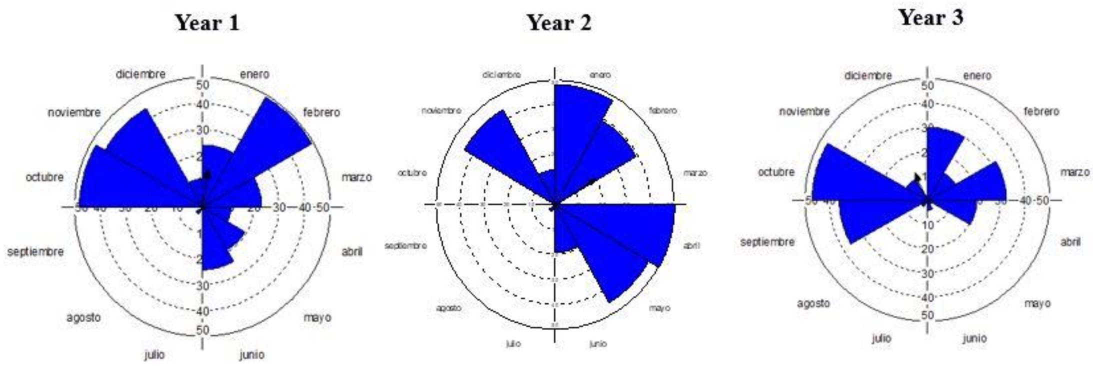

Figure 7. Circular histograms of monthly frequency for vegetative phenophase V4 (unfolded leaves) of *Ilex paraguariensis* for years one (2015-2016), two (2016-2017) and three (2017-2018)

The correlations found show that the species is mainly conditioned by photoperiod and minimum temperatures (Tables 1, 2). It is important to highlight how absicic acid is involved in the drought response of *I. paraguariensis* [19]. These and other mechanisms typical of subtropical perennial species could explain the correlations found in some years with the variable precipitation. It is worth mentioning that the response of the plant may be a function of the duration of this water stress [20].

The evaluation of the phenophase in the observed period had a duration of 41 to 127 chronological days. The onset of the phenophase occurred after accumulation of 1073, 409 and 197 GD for the first, second and third rates, respectively.

V5 mature leaves

The record of the V5 phenophase or mature leaves, although irregular, showed a continuity of the event in the period between October-July (year one), November-August (year two) and October-August (year three). This phenophase (Figure 8) is characterized by an increase in leaf size, change in color and texture, showing a leathery appearance and dark green color. If the correlations within each year are analyzed, similar results were obtained for year 1, with negative correlations for photoperiod and radiation, as well as for year 2, radiation and photoperiod (Table 1). For year 3, no correlations were found (Table 3). The analysis of the phenophase between the years evaluated shows a negative correlation of the phenophase with minimum temperature, maximum temperature, radiation and photoperiod (Table 4). This reflects the importance of light, in terms of radiation, photoperiod and minimum and maximum temperature in plant development.

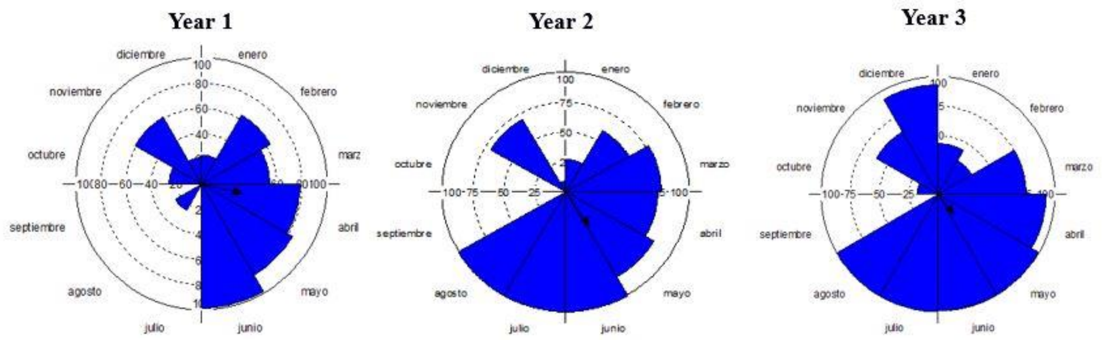

Figura 8. Circular histograms of monthly frequency for vegetative phenophase V5 (mature leaves) of *Ilex paraguariensis* for years 1 (2015-2016), 2 (2016-2017) and 3 (2017-2018)

A study carried out to verify the influence of luminosity on macronutrient and tannin contents in yerba mate leaves, formulated the hypothesis that in shadier environments, yerba mate has more constant metabolic activity, presenting smaller variations in N contents between year seasons (21).

The greater efficiency of PAR radiation use in yerba mate, under intercropping with pine, reveals the shade importance for the crop (22). This could explain, in part, the correlations found with radiation for the V5 or mature leaf phenophase, since for high density conditions, leaves depend more on actual photosynthesis than on nutrient remobilization, thus maintaining a more constant metabolic activity throughout the seasons. It is also known that the photomorphogenic responses of young yerba mate plants show that they have a shade-avoiding response, a strategy of increasing individual leaf area (23).

The thermal time required for the onset of phenophase was 938.31, 641.49 and 842 GD for the first, second and third rates, respectively.

CONCLUSIONS

- The analyses carried out allow us to conclude that the methodology of phytophenological observation in the yerba mate crop allows us to know the behavior of the plant throughout the year.
- Yerba mate has five vegetative phenophases: bud swelling (V1), incipient leaves (V2), leaves attached at the apex (V3), unfolded leaves (V4) and mature leaves (V5).
- Three sprouting rhythms were identified for the vegetative phenophases.
- When rainfall is abundant, four rhythms are verified for the phenophase incipient leaves (V2) and leaves attached at the apex (V3).
- Temperatures, photoperiod, precipitation and PAR radiation condition the presence and intensity of each phenophase throughout the crop cycle.

- The calculated DG allow knowing the thermal requirements for each rhythm and phenophase identified.

RECOMMENDATIONS

Long-term phenological observations should be made in order to improve the interpretations proposed in this work on a preliminary basis. These first contributions to the study of phenology are an initial step to understand the dynamics of the species and, in the long term, will contribute to generate models that will allow us to simulate the behavior of the crop.

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