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Original Article

Silviculture

Thermal Requirements and Photoperiod Influence in the Leaf Development of Two Forest Species

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ABSTRACT

The aim of this work was to evaluate the effect of thermal time methods and to identify the phyllochron and photoperiod response in seedlings of two forest species: *Corymbia citriodora* and *Eucalyptus urophylla*. A field experiment was installed under a completely randomized design, in a 2 × 11 factorial scheme, with two forest species and eleven sowing dates (E). Phyllochron is influenced by thermal time methods. The best thermal time method is the one that considers the base and optimum temperature and compares them with daily extreme temperatures. In addition, the influence of photoperiod and air temperature on phyllochron values was also verified. The lowest value occurred in E1 (*Corymbia citriodora*: 24.6 and *Eucalyptus urophylla*: 13.2 °C day leaf^{-1}) and the highest in E10 (*Corymbia citriodora*: 46.4 °C day leaf^{-1}) and E7 (*Eucalyptus urophylla*: 29.2 °C day leaf^{-1}), suggesting that the phyllochron is influenced by sowing dates.

Keywords: *Corymbia citriodora*, *Eucalyptus urophylla*, phyllochron, degree days, daylength.

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1. INTRODUCTION

Studies on the quantification and modeling of leaf development are important for scientific and practical reasons. They are a basis for the selection of species and cultivars more adapted to specific climatic conditions, evaluation of plant response in scenarios of climate changes (Streck et al., 2011; Ferreira et al., 2019) and may be useful to nurserymen in choosing strategies to optimize seedling production (Martins et al., 2007). In addition, by-products obtained in this study such as 3-PG and PnET are used in modeling of forest growth and yield (Xie et al., 2017).

Air temperature and photoperiod are the meteorological variables that most influence crop leaf development (LD) (Lisboa et al., 2012; Soltani & Sinclair, 2012). Air temperature is important due to its influence on physiological and metabolic processes, since it acts as a moderator of photosynthetic and respiratory rates, solute translocation, balance between water loss as steam to the atmosphere and CO_2 assimilation, which influence the number of leaves (NL) and leaf appearance rate (LAR) (Rosa et al., 2009; Freitas et al., 2017). Photoperiod is a parameter that improved the ability to capture CO_2, water fluxes and canopy development (Way & Montgomery, 2015). For this reason, it exerts strong influence on LAR, which directly affects solar radiation interception and, consequently, photosynthesis, phytomass production and productivity (Soltani & Sinclair, 2012).

The energy required for a plant to reach a certain stage of development is called thermal time (TT, °C day). TT is based on the daily accumulation of air temperature and its relation with cardinal temperatures (Freitas et al., 2017), and can be calculated by several methods. Different methods can result in different TT values, especially in periods when temperatures are below or above cardinal temperatures (Rosa et al., 2009; Ferreira et al., 2019), influencing LD quantification.

A way to relate TT to LD is through the phyllochron, defined as the time needed for the emission of consecutive leaves in the main stem (Martins et al., 2007), whose unit is °C day leaf^{-1}. Phyllochron varies mainly according to species, TT calculation method, sowing date and photoperiod (Rosa et al., 2009). It is necessary to consider the different TT methods, different sowing dates (E) and photoperiod to avoid bias in the analysis of phyllochron variation among species.

The effect of photoperiod is given by the day length available to plants. One of the ways to identify the influence of photoperiod is through field experiments with several sowing dates throughout the year (Rosa et al., 2009). Some studies have reported the effect of photoperiod on the flowering and germination of woody species (Rawal et al., 2014). However, there is no confirmation of the influence of photoperiod on LD in forest species, such as eucalyptus (Rawal et al., 2015). This information could help improving the quality of seedlings and increase the probability of success in the implantation and establishment of forests (Martins et al., 2007; Rawal et al., 2014; Ferreira et al., 2019).

The aim of this work was to evaluate the effect of thermal time methods and to identify the phyllochron and photoperiod response in seedlings of two forest species: *Corymbia citriodora* and *Eucalyptus urophylla*.

2. MATERIAL AND METHODS

A field experiment was carried out in the experimental area at 22° 30'S, 45° 27'W and 850 m of altitude and according to the Köppen-Geiger classification, the climate is Cwa–altitude tropical - with dry winters and hot and rainy summers (Abreu et al., 2015).

The experiment was conducted in a completely randomized design, with 2 × 11 factorial scheme and two species (*Corymbia citriodora* ((ex Hook) Hill & Johnson) and *Eucalyptus urophylla* ST Blake), eleven sowing dates, five replicates per treatment, totaling 110 experimental units (EU).

Each EU was composed of two plants cultivated in 8-L polyethylene pots filled with moderate A horizon of an Udic Dystrophic Oxisol. Soil belongs to a clayey textural class with high acidity in which pH in water was 5.0 and the amount of exchangeable calcium and magnesium was considered very low, according to the Soil Fertility Commission of Minas Gerais (CFSEMG, 1999). The contents of phosphorus, potassium, calcium, magnesium and micronutrients were considered low or very low and the soil was corrected according to the 5th Approximation (CFSEMG, 1999). Acidity and fertility correction was performed in each pot through the addition of 10.18 g simple superphosphate (18%), 0.31 g potassium chloride (60%), 0.20 g ammonium sulfate (20%) and liming with 12.32 g calcium carbonate according to the 5th approximation (CFSEMG, 1999). Daily watering was performed in the morning, and in the afternoon in days of vapor pressure deficit (VPD) ≥15 hPa (Abreu et al., 2015). On rainy days, watering was not performed since plants were under good water supplementation.

Sowing was performed at 30-day intervals, so plants were exposed to different meteorological conditions during their development (Lisboa et al., 2012) (Table 1).

LD was quantified through accumulated NL in the main stem during the seedling phase, which started when seedlings reached 50% emergence and finished when plants of each EU reached an average of 20 accumulated leaves (Martins et al., 2007; Abreu et al., 2015). NL count was performed once a week when each leaf presented limb ≥1.0 cm in length (Martins et al., 2007).

Daily average air temperature data were collected from the automatic weather station located 200 meters from the experimental area.

Six daily TT methods (methods 1 to 6) were used, which related air temperature and cardinal temperatures (Tb, Topt and TB) in different ways (Rosa et al., 2009) (Equations 1-6):

Method 1:

$$TT = T_{med} - T_b \cdot 1day \qquad T_{med} = \frac{T_{max} + T_{min}}{2} \tag{1}$$

when T_{med}<Tb, T_{med} = Tb.

Method 2:

Considered as a variation of method 1, with penalty in the minimum air temperature:

$$TTd = T_{med} - T_b \cdot 1day \tag{2}$$

when T_{min}≤Tb, T_{min} = Tb.

Method 3:

$$TTd = T_{med} - T_b \cdot 1day \tag{3}$$

when T_{med}< Tb, T_{med} = Tb; when T_{med}>Topt, T_{med} = Topt.

Method 4:

Considered as a variation of method 3, with penalty in the minimum and maximum air temperature:

$$TTd = T_{med} - T_b \cdot 1day \tag{4}$$

when T_{min}<Tb, T_{min} = Tb; when T_{max}>Topt, T_{max} = Topt.

Method 5:

$$\begin{aligned} &If\ \ Tb < T_{med} \le Topt: \mathrm{TTd} = \mathrm{T_{med}} - \mathrm{T_b} \cdot 1\mathrm{day} \\ &If\ \ Tot < T_{med} \le\ TB: \mathrm{TTd} = (\mathrm{Topt} - \mathrm{Tb}).\frac{(\mathrm{TB} - \mathrm{T_{med}})}{(\mathrm{TB} - \mathrm{Topt})} \end{aligned} \tag{5}$$

when T_{med}<Tb, T_{med} = Tb; whenT_{med}>TB, T_{med} = TB.

Method 6:

Considered as a variation of method 5, with penalty in the minimum and maximum air temperature:

$$\begin{aligned} &If\ \ Tb < T_{med} \le\ Tot: \mathrm{TTd} = \mathrm{T_{med}} - \mathrm{T_b} \cdot 1\mathrm{day} \\ &If\ \ Tot < T_{med} \le\ TB: \mathrm{TTd} = (\mathrm{Topt} - \mathrm{Tb}).\frac{(\mathrm{TB} - \mathrm{T_{med}})}{(\mathrm{TB} - \mathrm{Topt})} \end{aligned} \tag{6}$$

Table 1. Air temperature and photoperiod characterization during eleven sowing dates of the field experiment for *Corymbia citriodora* and *Eucalyptus urophylla* seedlings. Itajubá, MG, 2014/2015.

Sowing Dates (month/day/year)		*Corymbia citriodora*						*Eucalyptus urophylla*					
		Temperature* (°C)			Photoperiod (h)			Temperature* (°C)			Photoperiod (h)		
		Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min
E1	04/04/2014	17.1	20.2	13.9	11.7	12.2	11.6	17.1	20.2	13.9	11.7	12.2	11.6
E2	05/05/2014	18.5	26.5	13.9	12.3	13.7	11.6	18.4	26.5	13.9	12.2	13.6	11.6
E3	06/02/2014	18.8	26.5	14.0	12.4	13.6	11.6	18.8	26.5	14.0	12.4	13.6	11.6
E4	07/02/2014	20.2	26.5	14.0	12.8	13.9	11.9	20.4	26.5	14.0	12.9	14.0	11.9
E5	08/19/2014	22.3	26.5	15.6	13.8	14.4	12.6	22.2	26.5	15.6	13.7	14.4	12.6
E6	09/16/2014	22.7	26.5	15.6	14.0	14.4	13.1	22.7	26.5	15.6	14.0	14.4	13.2
E7	10/15/2014	23.4	27.3	19.1	14.1	14.4	13.7	23.4	27.3	19.4	14.1	14.4	13.7
E8	11/18/2014	23.6	27.3	19.1	14.1	14.4	13.5	23.6	27.3	19.1	14.1	14.4	13.5
E9	12/18/2014	23.2	27.3	19.1	13.7	14.3	12.9	23.2	27.3	19.1	13.7	14.3	12.8
E10	01/21/2015	20.9	26.1	15.2	12.6	13.8	11.6	--	--	--	--	--	--
E11	02/20/2015	20.0	24.7	15.3	12.3	13.4	11.6	20.3	24.7	15.3	12.4	13.4	11.6

*Values obtained by arithmetic means of the average temperature (Tmed), maximum and minimum air temperature, adapted from Freitas et al. (2017). - when there was loss of all EU.

when T_{min}< Tb, T_{min} = Tb, and when T_{max}> TB, T_{max} = TB; where: TTd= daily thermal time (°C.day); T_{med}= daily medium air temperature, obtained by the arithmetic average of the maximum (T_{max}) andminimum air temperatures (T_{min}) obtained from automatic weather station (°C); Tb = base temperature (°C); Topt = optimum temperature (°C); and TB = maximum temperature (°C) of leaf development, whose values were Tb = 8.7 °C and TB = 41.3 °C for *C.citriodora* and Tb = 11.5 °C and TB = 40.5 °C for *E.urophylla*. Topt was 17.1 °C for both species (Freitas et al., 2017).

TTd was accumulated from the emergence date (*i*) until the end of the seedling phase (*n*) and resulted in the accumulated thermal time (TTa, °C day), obtained by Equation 7:

$$TTa = \sum_{i=1}^{n} TTd \tag{7}$$

For each TTd method (method 1 to 6) and for each EU, phyllochron was determined by the inverse angular coefficient (a) of the linear regression between NL and TTa (Lisboa et al., 2012) (Equation 8):

$$NL = a \cdot TTa + b \tag{8}$$

For each TTd method, the standard deviation (SD) of the phyllochron variable was obtained among the 11 sowing dates for each species. SD was used to select the best TT method, as proposed by Rosa et al. (2009). After choosing the best TT method, phyllochron means were submitted to the Shapiro-Wilk's test (α = 0.05) to evaluate data normality. Ln (x) transformation was used in data that violated the normality assumption (Freitas et al., 2017). Subsequently, phyllochron values were submitted to analysis of variance (ANOVA) to evaluate the effect of sources of variation (species and sowing dates), followed by the comparison of means by the Scott-Knott's test (α = 0.05), through SISVAR software.

In order to verify the influence of photoperiod on leaf development of both forest species, linear regressions between mean phyllochron (arithmetic average of 5 EU) and mean photoperiod (Pmed) were adjusted as proposed by Rosa et al. (2009). Pmed was obtained by the arithmetic average of the daily photoperiod (P) from emergence (i) until the end of the seedling phase (n) by the method of Keisling (1982) (Equation 9):

$$\begin{aligned}
&P = \frac{2}{15} \cdot \arccos\left[\cos(\alpha) \cdot \sec(\varphi) \cdot \sec(\delta) - \tan(\varphi) \cdot \tan(\delta)\right] \\
&\alpha = 90 + B \\
&\delta = \arcsin(0.39779) \cdot \sin(\lambda) \\
&\lambda = M + 1.916 - \mathrm{sen}(M) + 0.020 \cdot \sin(2M) + 282.565 \\
&M = 0.985600 \cdot DOY - 3.251
\end{aligned} \tag{9}$$

where P = daily photoperiod (hours); α = zenital angle (degrees); Φ = latitude (degrees); δ = solar declination (degrees); 0.39779 = 23.45° sine (23°27'); M = mean solar anomaly (degrees); DOY = day of the year; B = angle below the horizon (6°).

Positive and significant values (α = 0.05) of the linear regression coefficient indicate typical photoperiodic response of short-day plants. Negative and significant values (α = 0.05) indicate typical photoperiodic response of long-day plants (Streck et al., 2007; Rawal et al., 2015). Long-day plants develop faster, advancing their cycle, when photoperiod is above the critical photoperiod and short-day plants develop faster when photoperiod is below the critical photoperiod (Soltani & Sinclair, 2012).

3. RESULTS

During the experiment, plants were submitted to temperature and photoperiod variations (Table 1). Temperature ranged from 3.8 °C (minimum absolute) at E2, E3 and E4 to 35.7 °C (maximum absolute) recorded at E3 to E9, while photoperiod values ranged from 11.6h to 14.4h. The difference among weather conditions also influenced the duration of the seedling phase (Table 2). There was a tendency to decrease the seedling duration as the medium air temperature increased, except for E1 and E7.

No temperatures ≥TB were observed for both species. Daily temperature values close to Topt and Tb were recorded, mainly in E1 and E6, which had a slightly shorter duration compared to the other sowing dates. In addition, it was verified that there was no change in the duration of the seedling phase between species, with 87 days for *C. citriodora* and 80 days for *E. urophylla*, both between E2 and E8.

Both species, in the six thermal time methods (1 to 6) and in the eleven sowing dates (E1 to E11) presented linear regressions between NL and TTa with high determination coefficient (R^2) values above 0.95, indicating that phyllochron estimation through these linear regressions is appropriate and can be applied to both forest species.The same situation was observed in perennial crops such as olive trees (Lisboa et al., 2012; Martins et al., 2012) and other eucalyptus species (Martins et al., 2007).

There was variation in phyllochron values in relation to the six thermal time methods, with values between 35.70 and 67.16 °C day leaf^{-1} for *C. citriodora* and between 21.44 and 48.65 °C day leaf^{-1} for *E. urophylla* (Table 3), both for methods 5 and 2, respectively. Methods 1 and 2 presented similar phyllochron values due to the consideration in the TTd calculation, in which there is only Tb penalization, increasing linearly above this value.In addition, for most of the days, Tmed was not lower than Tb in both species and Tmin was lower than Tb in 24 days for *C. citriodora* and in 111 days for *E. urophylla*, from April to October 2014.

Phyllochron SD varied among TT methods, from 5.47 °C day leaf^{-1} day in method 4 to 15.88 °C day leaf^{-1} day in method 2. Thus, method 4 was considered the best method to be used for phyllochron estimation due to its lower SD value, being a variation of method 3, which considers the penalization of extreme temperatures during the day (maximum and minimum air temperatures), Tb and Topt. These results are different from those found by Rosa et al. (2009) and Streck et al. (2007), who considered method 6 as the best for phyllochron estimation for wheat and soybean.

With the best thermal time method, phyllochron was estimated for both species in the eleven sowing dates (E1 to E11) and their replicates. Phyllochron values did not follow normality by the Shapiro-Wilk's test (α=0.05) and were transformed by the naperian logarithm [Ln (x)]. The analysis of variance for the phyllochron variable was significant (p≤0.05) for the following sources of variation: species and sowing date, but there was no significant interaction between sources of variation (p=0.3485). Thus, the comparison of means through the Scott-Knott test was performed separately for species and sowing dates (Table 4).

Phyllochron differed among sowing dates, with the lowest phyllochron value at E1 (24.65 and 13.17 °C day leaf^{-1},

Table 2. Sowing dates, emergence and duration of eleven sowing dates for *Corymbia citriodora* and *Eucalyptus urophylla* seedlings. Itajubá, MG, 2014/2015.

Sowing Dates Month/day/year		*Corymbia citriodora*		*Eucalyptus urophylla*	
		Seedling emergence Month/day/year	Duration of the seedling phase (days)*	Seedling emergence Month/day/year	Duration of the seedling phase (days)*
E1	04/04/2014	04/22/2014	97	04/22/2014	97
E2	05/05/2014	05/15/2014	166	05/15/2014	159
E3	06/02/2014	06/26/2014	117	06/26/2014	117
E4	07/02/2014	07/30/2014	97	07/30/2014	104
E5	08/19/2014	09/07/2014	122	09/07/2014	124
E6	16/09/2014	09/30/2014	99	10/02/2014	99
E7	10/15/2014	10/29/2014	105	10/29/2014	114
E8	11/18/2014	12/03/2014	79	11/30/2014	79
E9	12/18/2014	12/28/2014	89	12/28/2014	89
E10	21/01/2015	02/05/2015	116	--	--
E11	02/20/2015	02/27/2015	105	02/27/2015	94

*Period from emergence- when 50% of seedlings were visible above the ground until the seedling phase- when each EU reached, on average, 20 leaves accumulated in the main stem - when there was loss of all EU. Adapted from Freitas et al. (2017).

Table 3. Mean ± standard deviation of the phyllochron (° C day leaf^{-1}) for *Corymbia citriodora* and *Eucalyptus urophylla* in eleven sowing dates and six thermal time methods. Itajubá, MG, 2014/2015.

Species	Thermal time methods					
	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6
C. citriodora	66.69 ± 15.76	67.16 ± 15.88	43.67 ± 12.28	38.73 ± 9.01	35.70 ± 12.64	35.85 ± 12.86
E. urophylla	47.38 ± 11.15	48.65 ± 11.33	25.22 ± 7.49	23.39 ± 5.47	21.44 ± 7.93	23.08 ± 10.45
Average	57.04 ± 13.46	57.91 ± 13.61	34.45 ± 9.89	31.06 ± 7.24	28.57 ± 10.29	29.47 ± 11.66

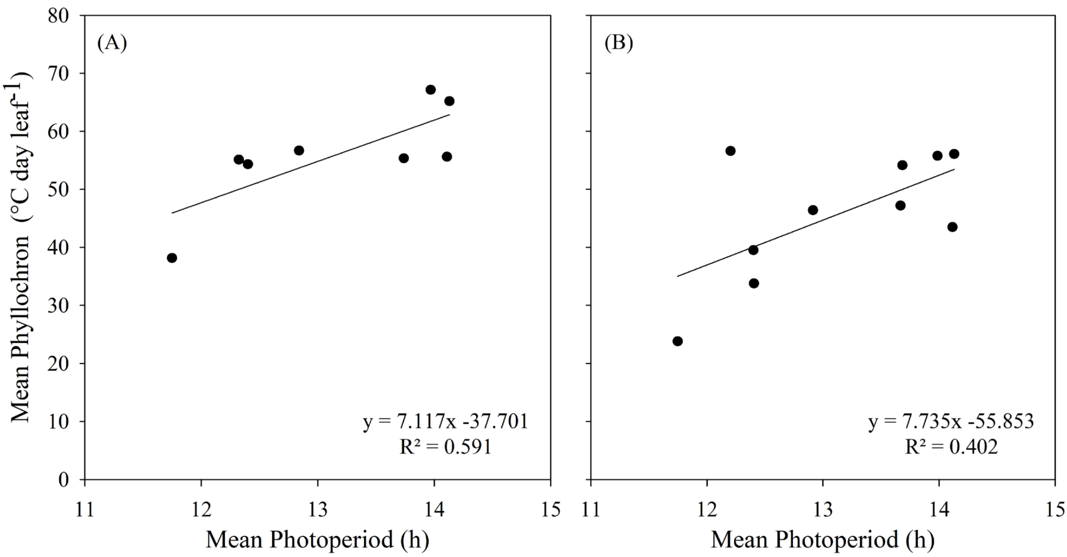

Figure 1. Relationship between mean phyllochronvalue and mean photoperiod in *Corymbia citriodora* (A) and *Eucalyptus urophylla* (B) seedlings. Itajubá, MG, 2014/2015.

Table 4. Mean phyllochron values (C° day leaf^{-1}) calculated by thermal time method 4 for *Corymbia citriodora* and *Eucalyptus urophylla* in eleven sowing dates. Itajubá, MG, 2014/2015.

Sowing Dates	*Corymbia citriodora**	*Eucalyptus urophylla**
E1	24.65a	13.17a
E2	46.96c	28.46d
E3	31.47b	26.61d
E4	31.65b	21.22c
E5	55.44c	26.99d
E6	41.40c	29.02d
E7	39.80c	29.20d
E8	33.27b	21.99c
E9	34.31b	25.18d
E10	46.39d	-
E11	35.90b	18.69b
Average	38.17	24.16

*Means followed by equal letters do not differ by the Scott-Knott's test at 5% probability; - all experimental units were lost in sowing date E10.

respectively) differing from the other sowing dates. At E1, plants accumulated smaller amount of energy to emit a leaf on the main stem, while at E2, E5, E6, E7 and E10 (*C. citriodora*) and E2, E3, E5-E7 and E9 (*E. urophylla*), there was greater amount of accumulated energy. Particularly, E10 was a sowing date extremely hot with precipitation below the climatological average. For this, all EU of *E. urophylla* were lost, while *C. citriodora* showed higher phyllochron and less development (Table 4), as observed by Freitas et al. (2017).

Both species presented different duration and phyllochron values during sowing dates ($p<0.05$), which indicates the influence of photoperiod (Rosa et al., 2009; Lisboa et al., 2012; Ferreira et al., 2019). The angular coefficients (a) of linear regressions between mean phyllochronand mean photoperiod were significant for both species, proving that there is influence of photoperiod on the phyllochron of *C. citriodora* and *E urophylla*. In addition, 'a' values are positive (Figure 1), indicating that both forest species present a typical photoperiodic response of short-day plants (Rawal et al., 2015). This explains the lower phyllochron value obtained in E1, which concomitantly presented milder temperature and shorter day length.

4. DISCUSSION

Different environmental conditions, such as those occurring during the 11 sowing dates (Table 1), affected the leaf appearance rate and, consequently, the leaf development rate and are important in plant development studies (Ferreira et al. 2019). The presence of an inverse relationship between air temperature and duration of the seedling phase indicated that lower air temperature extends the seedling phase in both species. The same trend is observed for other perennial crops, such as olive tree MGS ASC 315 (Lisboa et al., 2012), *Bixa orellana* and *Citharexylum myrianthum* (Ferreira et al., 2019).

Phyllochron estimation through linear regressions between NL and TTa was considered appropriate for annual crops such as wheat (Rosa et al., 2009) and rice (Streck et al., 2011), and perennial crops such as olive (Lisboa et al., 2012) and other eucalyptus species (Martins et al., 2007). Phyllochron values were higher in methods 1 and 2, intermediate in methods 3 and 4 and lower in methods 5 and 6 (Table 3), similar to values observed for rice (Streck et al., 2007) and wheat (Rosa et al., 2009). This decrease in phyllochron values in methods 1 and 2 to methods 5 and 6 occurred due to the decrease in TTd values by including Topt (methods 3 and 4) and Topt and TB (methods 5 and 6) in TTd calculations. This occurs mainly on hotter days or in the hottest periods of the year, when minimum, maximum and medium daily air temperatures were equal to or above cardinal temperatures, mainly above Topt (17.1 °C for both species, Freitas et al., 2017).

The phyllochron value obtained in method 1 (66.69 and 47.38 °C day leaf^{-1}for *C. citriodora* and *E. urophylla*, respectively) is similar to that found by Freitas et al. (2017) for *C. citriodora* (62.95 °C day leaf^{-1}) and *E. urophylla* (46.03 °C day leaf^{-1}). Small variation may have occurred due to the different waymean air temperature was obtained (Streck et al., 2011; Ferreira et al., 2019). Freitas et al. (2017) calculated the daily mean air temperature based on the average hourly values recorded in the automatic weather station. This study used the average between minimum and maximum daily air temperatures (average of extreme values).

Differently from results observed by Rosa et al. (2009) and Streck et al. (2009) who found lower SD values for methods 5 and 6 in the phyllochron estimation of wheat and soybean, the lowest SD values were found for methods 3 and 4. These methods include Tb and Topt and exclude TB. This difference probably occurred because there were no records of air temperatures equal to or greater than TB for both forest species throughout the experiment. Therefore, there were no penalty of TB conditions in methods 5 and 6.

Another important point is that methods that compare cardinal temperatures with Tmin and Tmax have presented better performances than methods that consider only Tmed. They indicate that the appearance of *C.citriodora* and *E. urophylla* leaves should be described in mathematical models in response to extreme daily temperatures and not according to the average air temperature.

E. urophylla presented lower phyllochron value (24.16 °C day leaf^{-1}) when compared to *C. citriodora* (38.17 °C day leaf^{-1}), indicating that *E. urophylla* requires less accumulated energy to emit leaves on the main stem and, consequently, develops faster than *C. citriodora*.This result is consistent with results observed in field, in which *E.urophylla* leaves appeared faster than *C. citriodora* leaves. This difference between the development of the two species is not noticed when the number of days to complete the seedling phase is considered (Table 2), demonstrating that phyllochron represents the development better than the civil calendar, which is the measurement commonly used to detect the duration of seedling phase in nurseries (Ferreira et al., 2019).

Values similar to *E. urophylla* phyllochron (24.16 ± 6.33 °C day leaf^{-1}) were found in perennial plants such as: Arbequina olive cultivar (21.7 °C day leaf^{-1}) (Martins et al., 2012), *E. saligna* (30.7 °C day leaf^{-1}), *E. grandis* (32.0 °C day leaf^{-1}) (Martins et al., 2007) and annual plants such as watermelon (23.4 °C day leaf^{-1}) (Lucas et al., 2012). On the other hand, *C. citriodora* presented phyllochron value (38.17 ± 10.09 °C day leaf^{-1}) higher than the crops above and similar to olive cultivar MGS ASC315 (41.6 °C day leaf^{-1}) (Martins et al., 2012). One way to interpret the practical results of phyllochron for both species is considering that the transplanting height of eucalyptus seedlings is 20 to 30 cm, similar to that observed in this study. At this time, plants present approximately 20 leaves (Martins et al., 2007), *C. citriodora* reaches transplanting height at 763.4 °C day after emergence, whereas *E. urophylla* reaches this point at 483.2 °C. These values are lower when compared to those found by Martins et al. (2007) for *E. grandis* (640 °C day) and *E. saligna* (614 °C day). This difference may be caused by the thermal time method and development differences between species.

Both forest species presented lower phyllochron value during the period with milder temperatures (E1), which is a different pattern from that found by Rosa et al. (2009) for wheat crop, but similar to that observed by Ferreira et al. (2019) for native forest species and Lisboa et al., (2012) for olive cultivar MSG ASC315. Normally, the opposite is expected to occur, which is lower phyllochron value for warmer periods and higher phyllochron value for milder periods. This proves that the development response of plants is strongly influenced by temperature and its interaction with photoperiod (Rawal et al., 2015). When photoperiod changes, it affects TTd and TTa, but it remains unclear whether photoperiod may impose constraints on the physiology of fully developed leaves (and LAR) during relatively favorable temperature conditions (Way & Montgomery, 2015). There is evidence that the photoperiod can also regulate the physiological activity of leaves (Way & Montgomery, 2015), as observed in temperate deciduous trees, where seasonal variation in photosynthetic capacity is more closely correlated with photoperiod than with air temperature (Basler & Körner, 2012).

In this study, photoperiod plays an important role in this response, affecting LAR (Lisboa et al., 2012). Both species presented typical behavior of short-day plants (Figure 1). There was higher leaf appearance rate and consequently higher development when the air temperature was cooler and the daywas shorter (E1, seeded on 04/04/2014).

The effect of the photoperiod on the development of perennial plants, especially of the genus eucalyptus, are incipient, controversial and inconclusive (Basler & Körner, 2012; Rawal et al. 2015; Way & Montgomery, 2015). Moreover, Rawal et al. (2014) found that photoperiod and air temperature associated with air and soil moisture have a significant influence on growth in diameter and height of *E. polyanthemos*, *E. obliqua* and *E. radiata*. However, the three species present typical long-day plant behavior (Rawal et al., 2014), suggesting that the development is greater in longer days, differing from the pattern observed in this study. Rawal et al. (2015) verified that *E. tricarpa* is an obligate short-day plant and increasesin temperature associated with shorter photoperiods benefited flowering in *E. microcarpa*, similar to this study considering the seedling phase.

Phyllochron and photoperiod have been vegetative development variables widely used in ecophysiological studies and simulation models of annual crops development such as rice (Streck et al., 2011), wheat (Rosa et al., 2009) and perennial crops such as eucalyptus (Freitas et al., 2017) and olive trees (Martins et al., 2014). However, the results found in this study are important to demonstrate the effect of photoperiod on leaf development, represented by the seedling phase of both species.

This kind of information is useful to know the development pattern of forest species that have economic interest, mainly because it provides subsidies for the selection of species most adapted to the local climate, the choice of the best sowing or transplanting date, avoiding stress at the transplanting time and ensuring greater success in the establishment and uniformity of seedlings in the field.

5. CONCLUSIONS

1. Phyllochron of *Eucalyptus urophylla* and *Corymbia citriodora* is influenced by the thermal time method. The method that includes the base and optimum temperature and compares them with the daily extremes temperature (Method 4) was the best method;
2. Phyllochron varied between species and the eleven sowing dates. *Eucalyptus urophylla* presented lower phyllochron (24.2 °C day leaf^{-1}) and *Corymbia citriodora* presented higher phyllochron (38.2 °C day leaf^{-1}) for leaf development;
3. Phyllochron varied among sowing dates in response to photoperiod and air temperature. For both species, the lowest phyllochron occurred in E1, and the highest in E10 (*Corymbia citriodora*) and E2, E3, E5, E6, E7, E9 (*Eucalyptus urophylla*);
4. Our results confirm the photoperiod sensitivity in the seedlings of both species.

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