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Analysis of rhythmic growth in holly (*Ilex* × *meserveae*) grown in controlled conditions

Andrea Kosiba*, Margit Nothnagl, Beatrix W. Alsanius, Rolf U. Larsen

Department of Crop Science, Swedish University of Agricultural Sciences, P.O. Box 44, SE-23053 Alnarp, Sweden

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Abstract

The influence of 24 h mean air temperature (18.3, 20.6, 23.9 and 25.8 °C) and photosynthetic photon flux (PPF; 0.6, 2.1, 3.7 and 4.7 mol m^{-2} d^{-1}) on the growth cycles of vegetative growth in *Ilex* × *meserveae* ('Blue Princess' S.Y. Hu) was investigated. Plants propagated from top cuttings were grown in greenhouse compartments. The number of unfolded leaves was recorded continuously throughout the experiment. A modified sine function was fitted to collected data and the values for the amplitude and frequency of the growth curves were analysed. The sine function was tested as a method to evaluate the influence of climate on periodically flushing species. Both amplitude and frequency were significantly influenced by air temperature and PPF. The highest frequency of flushing was found at 23.9 °C and 3.7 mol m^{-2} d^{-1}. The function resulted generally in a good fit to collected data with R^2 values above 0.9. Growth curves of all individual plants were categorised with respect to their growth pattern as poor synchronisation within the treatments did not allow analysis of the mean values of the growth curves.

Keywords: Growth analysis; Blue holly; Rhythmic growth; Sine function

1. Introduction

Rhythmic growth patterns are a phenomenon found in many plant species. Oak (Borchert, 1975; Farmer, 1975), cacao (Greathouse et al., 1971) and *Ligustrum* (Kuehny

* Corresponding author. Tel.: +46 40 415375; fax: +46 40 460441.
E-mail address: andrea.kosiba@vv.slu.se (A. Kosiba).

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and Halbrooks, 1993) have been investigated with respect to their infradian growth rhythm (growth cycles from a few days to months). Millet and Bonnet (1990) review circadian, ultradian and infradian growth rhythms in herbaceous and woody plants and refer to applications in agriculture. Some species perform rhythmic growth patterns under controlled conditions, but not during natural conditions (Mertens and Wright, 1978; Viémont and Lambert, 1994; Kosiba et al., 2005). Studies on the flushing behaviour of *Gnetum* (Mialoundama and Paulet, 1986) and *Citrus* (El-Morsy and Millet, 1996) indicate that periodic growth rhythms originate from meristematic activity and Borchert (1973) based a model describing rhythmic growth of *Theobroma cacao* on internal feedback mechanisms in water balance. Moreover, hormonal balance (Favre and Juncker, 1989; Whalley and Loach, 1978) and assimilate distribution (Kuehny et al., 1997) have been discussed as possible mechanisms for periodic growth.

The mechanisms behind periodic growth are still not fully understood and might differ for different species. In greenhouse production, periodically growing crops can cause problems. For growers, timing of the crop is essential. The repeated change in growth rate over time at constant climate conditions makes it difficult to use traditional prediction.

Blue holly, as a small pot plant, may be a future potential greenhouse crop for summer production in Northern Europe. *Ilex* shrubs, grown in nurseries, normally perform one flush per growing season. When working with *Ilex* × *meserveae* we observed flushing behaviour under various greenhouse climate conditions. In preliminary studies, the amplitude and the frequency of the growth oscillations seemed to differ between different temperature regimes (Kosiba et al., 2005). When trying to determine the responses we found that the plants were not synchronised. A statistical analysis of the number of unfolded leaves produced after a specific time interval did not show significant differences due to the large standard deviations.

The problems mentioned above underlined the need to make a more detailed growth analysis on *Ilex* based on the previously collected data. In this study, we tested a modified sine function (Larsen and Kosiba, 2002) as a tool to describe periodic growth phenomena. Traditionally, sine functions are used to describe yearly variations in light or diurnal variations in temperature (Charles-Edwards, 1986) and they are not based on underlying biological explanations. The use of such a function would, in theory, enable the determination of the frequency and amplitude of the growth rhythms when fitted to data from individual plants. The objectives of the study were to see if the sine function could be used as a tool for rhythmic growth analysis and if different climate treatments resulted in any significant difference in estimated amplitude, frequency and synchronisation of the growth rhythms.

2. Materials and methods

The study, previously described by Kosiba et al. (2005), was arranged as a two-factor experiment in a block design with two blocks and 20 replicates per treatment. Due to the problem with synchronisation, a single plant had to be considered as one experimental unit. The combination of four PPF regimes and four air temperature regimes resulted in 16 treatments with a total of 320 plants. By fitting an adapted sine curve to the growth pattern,

the amplitude and frequency of the leaf-unfolding rate could be determined. These parameters were further analysed for differences between the treatments.

2.1. The sine function

The accumulated number of unfolded leaves, L, was calculated from the following adjusted sine function (Larsen and Kosiba, 2002):

$$L = \frac{A}{B}(-\sin Bt) + At \tag{1}$$

where the parameter A (leaves) is the amplitude, B (days) the frequency of the oscillations and t denotes time (days) (Fig. 1). In its derivative form the function has the following expression:

$$\frac{dL}{dt} = -A\cos Bt + A. \tag{2}$$

To be able to adjust the starting point of Eq. (1) in relation to collected data, two more parameters, C (days) and D (leaves), had to be introduced. The parameters C adjusts the time phase on the x-axis and D denotes the number of unfolded leaves when $t = 0$. The final form of the equation fitted to the data was:

$$L = \left(\frac{A}{B}(-\sin(B(t+C))) + A(t+C)\right) + D \tag{3}$$

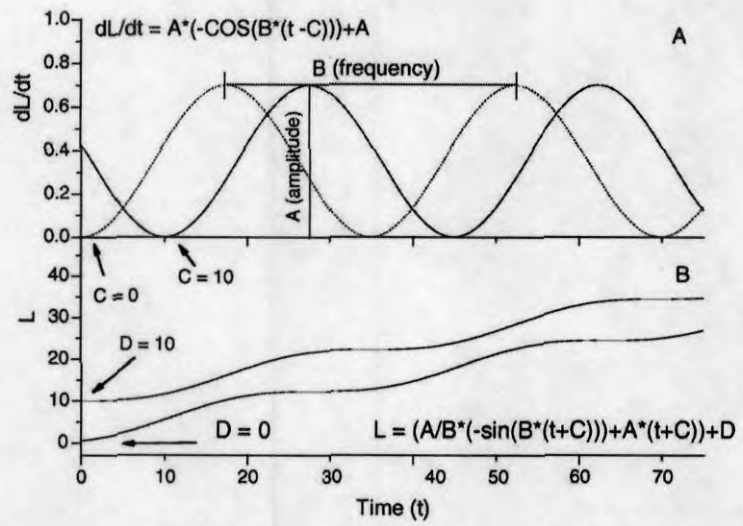

Fig. 1. Illustration of Eq. (2) (growth rate) (A) and Eq. (3) (accumulated number of leaves) (B). (A = amplitude; B = frequency; C = correcting factor on the x-axis; D = correcting factor on the y-axis; L = number of unfolded leaves; t = time).

2.2. Cultivation practise

On December 7, 2000, un-branched top-cuttings with eight leaves were taken from stock plant material (Splendor Plant, Sweden). The cuttings were wounded on one side and dipped into a 0.6% solution of IBA (indole–butyric-acid, Sigma, Sweden) to stimulate root formation and stuck in commercial peat based growing medium (Hasselfors–K, Sweden) mixed with 30% of perlite. After the rooting in a mist chamber at 22 °C for 6 weeks the cuttings were transplanted in pots (∅ 11 cm) in the growing medium with 50% of perlite (v/v). The plants were placed in four greenhouse chambers with 16-h additional lighting with high-pressure sodium lamps (400 W, Phillips) with a PPF of 40 μmol m^{-2} s^{-1} and a daily mean air temperature of 18.3, 20.6, 23.9 and 25.8 °C. Additional lighting continued until the natural photoperiod reached 16 h (April 17, 2001). Three weeks after transplanting, controlled-release fertilizer (3 kg m^{-3}, 18N–6P–12K, 8 months) was added as a top dress. After two more weeks the uppermost new-grown shoot over the first leaf was pinched to start the experiment. All other sprouting vegetative shoots were removed to avoid competition. In each temperature chamber the shading was achieved as described in Kosiba et al. (in press), resulting in a daily mean PPF of 0.6, 2.1, 3.7 and 4.7 mol m^{-2} d^{-1}.

2.3. Climate recordings and measurements

Air temperature was recorded with two thermocouples (type T, copper and constantan) in each temperature chamber. The photosynthetic photon flux (PPF) was recorded at plant level using Skye SKP 215 quantum sensors (Skye Instruments Ltd., Llandringdod Wells, UK). The climate data were collected continuously at 5-s intervals using a datalogger (Intab, Sweden). From April 12th until May 28th the number of unfolded leaves was counted every second day. A leaf was determined as unfolded when the leaf base had loosened from the shoot tip.

2.4. Statistical procedure

The number of unfolded leaves was plotted against time and Eq. (2) was fitted to the data set for every single plant (Fig. 2) and for the mean values of each treatment. All model fitting was done in Origin 5.0 (Origin Lab Corporation, Northhamton, USA) with a non-linear regression procedure. For every plant, a visual evaluation on the accuracy of the sine curve's representation of the data was done and the percentage of plants with good fit within every treatment was calculated. The parameters C and D, which were introduced to be able to adjust the function along the x- and y-axis, were also used to describe the level of synchronisation. For every treatment the mean values estimated for C were plotted against D together with their standard deviations. Estimated values for A and B were tested for interaction using the ANOVA test with a GLM procedure and then analysed with a one-way ANOVA for every climate treatment (Minitab 12.0, Minitab Inc., USA). Data points in every treatment were connected with a spline function curve in Origin 5.0.

To describe all the occurring growth patterns found in the 16 treatments, we created seven categories. In the category 1F (Fig. 3A) one flush was performed during the experimental period of 93 days. In the category 1FL (Fig. 3B), one flush was performed but a long resting period of at least 30 days followed the active growth phase. Category 2F

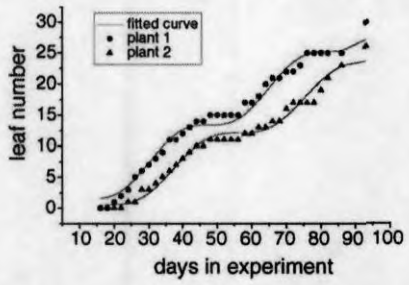

Fig. 2. Example of two poorly synchronised growth curves from one treatment fitted to the same function.

(Fig. 3C) described two full flushes with a resting period of 10–30 days. When two full flushes with a resting period longer than 30 days were performed, the category was called 2FL (Fig. 3D). Plant growth patterns with a trend towards two flushes, but with a resting period shorter than 10 days, were categorized with 2FT (Fig. 3E). Plant growth patterns with a tendency for three flushes (resting periods shorter than 10 days) were named 3FT (Fig. 3G) and three flushes with resting period between 10 and 30 days was called 3F (Fig. 3F).

3. Results

The percentage of plants where the sine function could reflect the periodic growth behaviour was highest (95%) in the combined treatment including 23.9 °C and

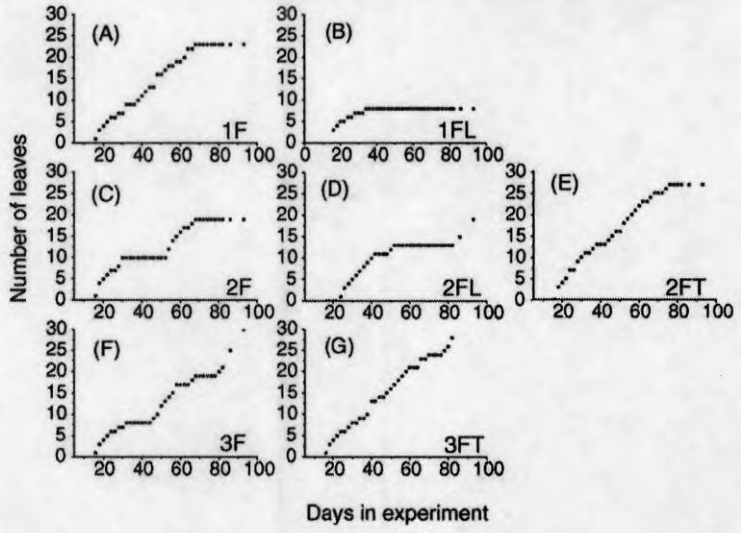

Fig. 3. Different growth patterns in greenhouse grown *Ilex*. 1F = one flush; 1FL = one flush with a resting period > 30 days; 2FT = a trend towards two flushes is visible, but the resting period is shorter than 10 days; 2F = two visible flushes with a resting period between 10 and 30 days; 2FL = two flushes with a resting period longer than 30; 3FT = a trend towards three flushes is visible, but the resting periods are shorter than 10 days; 3F = three visible flushes with a resting period between 10 and 30 days.

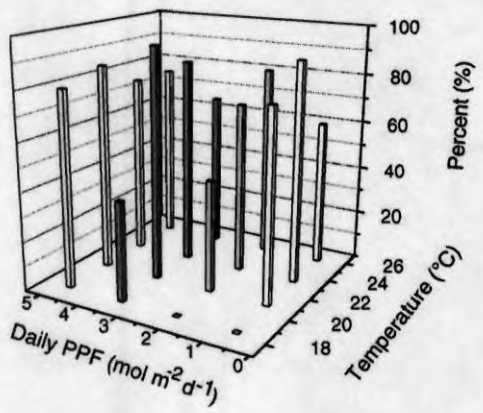

Fig. 4. Percentage of plants in every treatment with good fit of the modified sine curve to the collected data. The fit of the curve oscillation to the data was evaluated visually ($n = 20$).

2.1 mol m^{-2} d^{-1} treatment. In the 18.3 and 20.6 °C treatment at 0.6 mol m^{-2} d^{-1}, all plants showed long resting periods (category 1FL and 2FL) and the function could not be adapted to reflect those growth curves (Fig. 4).

When comparing the growth curves based on the mean values for every treatment, only four of the treatments resulted in visible periodic growth patterns (Fig. 5). High PPF and high temperature lead to nearly continuous growth while the cooler treatments with low PPF conditions only produced a single flush during the 93 days of experiment. After categorizing every single plant we found in almost every treatment individuals with oscillating growth behaviour, but the proportions varied substantially (Fig. 6). In the 23.9 °C and 2.1 mol m^{-2} d^{-1} treatment 95% of all plants showed clearly visible flushes (category 2F and 3F). In the lowest PPF treatments apart from the highest temperature level, around 50% of the plants performed two flushes with a long resting period (category 2FL), the remaining plants only one flush (category 1FL). Also the amount of categories occurring in the different treatments varied. In four treatments (18.3 °C; 20.6 °C and 23.9 °C at 2.1 mol m^{-2} d^{-1} and 25.8 °C and 4.7 mol m^{-2} d^{-1}) six of the seven categories could be found within one treatment while only two categories (1FL and 2FL) occurred in the two coldest treatments with the lowest PPF level (18.3 °C and 20.6 °C at 0.6 mol m^{-2} d^{-1}). Plants with long resting periods (category 1FL and/or 2FL) dominated the treatments with the lowest PPF level except for the warmest treatment, the two coldest treatments within the 2.1 mol m^{-2} d^{-1} treatment and the 23.9 °C 4.7 mol m^{-2} d^{-1} treatment. In treatments with a dominating amount of plants with clear oscillations (category 2F and 3F; 23.9 °C and 2.1 mol m^{-2} d^{-1}, 25.8 °C and 2.1 mol m^{-2} d^{-1}, 25.8 °C and 0.6 mol m^{-2} d^{-1}) no plants with long resting periods could be found.

The amount of plants that only showed a trend towards periodic growth (category 2FT and 3FT) dominated the two treatments with the highest temperature and the two highest PPF levels (3.7 and 4.7 mol m^{-2} d^{-1} at 25.8 °C). In the lowest PPF level no plants were found within those categories.

Due to poor synchronisation, the growth pattern of the average curve could differ considerably from the growth curves of individuals. Large deviations in estimated values of

Fig. 5. Comparison of the effect of 16 climate treatments (combination of 18.3, 20.6, 23.9 and 25.8 °C and 0.6, 2.1, 3.7 and 4.7 mol m^{-2} d^{-1}) on the periodic vegetative growth of *Ilex* × *meserveae*. Each data point represents the mean of 10 plants and the non-linear curve fitting was done with the function presented in Eq. (2).

Air temperature and PPF altered the amplitude and the frequency of the growth cycles of *Ilex* significantly. These results confirm our second hypothesis and also show that the estimation of amplitude and frequency is a suitable tool for growth analysis of plant species with periodic growth. Furthermore, our findings are supported by investigations on cacao (Sale, 1968), where the growth pattern was altered by climate and the number of flushes increased with raising temperatures.

The basic reason for the more detailed growth analysis in the present study was the poor synchronisation of the plants within the treatments. Farmer (1975) faced a similar problem when working with red oak seedlings, where he found a better synchronisation in colder treatments than at higher temperatures. Unfortunately, the synchronisation of plants in treatments with pronounced oscillations was not very good, making it difficult to use the sine function for applied deterministic prediction modelling. For prediction purposes a stochastic approach would be needed including the variations of parameters C and D in the model.

Micropropagation could be a possibility to get a more homogenous plant material in *Ilex* × *meserveae*. Only *Ilex aquifolium* has been micropropagated earlier (Morte and Olmos, 1991) but no further growth analysis has been performed. El-Morsy and Millet (1996) showed for *Citrus* that shoots derived from in vitro culture displayed the same infradian rhythm as stock plants. Dimmock et al. (1985) described in their work that the growth cycles of caribbean pine in Southern Queensland were unsynchronised both at plant and stand level. The authors concluded from their results that the branch tips were receptive for climatic growth stimuli only at a specific stage of bud development. This appears to be unlikely for *Ilex*, since six out of seven possible growth patterns could be observed at specific climate conditions although the plants were exposed to the same climate. A number of factors are considered to affect internal growth rhythms (Greathouse et al., 1971), such as water balance (Borchert, 1975), interspecific competition (Collet and Frochot, 1996), hormonal control (Abo-Hamed et al., 1981), internal C and N concentrations (Kuehny et al., 1997) or assimilate distribution (Dickson et al., 2000a,b). In our case the last option seems the most likely. Pronounced oscillations occurred in high temperature/low PPF and low temperature/high PPF conditions. This could be a sign, that the assimilate production might not be sufficient to support continuous root and shoot growth simultaneously, thereby forcing the plant into a periodic growth pattern with alternating root and shoot growth. This conclusion is supported by investigations on *cacao* (Sleigh et al., 1984), where root and shoot growth were studied and assimilate distribution was traced using radioactive markers. To strengthen the case for *Ilex*, further investigations on root and shoot growth are planned.

The modified sine function used in this investigation gave satisfying results to describe and analyse periodic growth behaviour where the underlying mechanisms are not known yet. Together with the fact that greenhouse climate influenced the growth cycles future work should focus on the development of a prediction model for the vegetative growth of holly.

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