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Influence of Photosynthetic Photon Flux Density (PPFD) and Air Temperature on Vegetative Growth and Side Shoot Formation in Greenhouse Grown Holly (*Ilex* x *meserveae* 'Blue Princess' S.Y. Hu)

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Summary

The influence of mean 24 hour air temperature and photosynthetic photon flux density (PPFD) on the vegetative growth of *Ilex* x *meserveae* 'Blue Princess' (S.Y. Hu) was investigated. Rooted cuttings were grown in greenhouse compartments at four mean air temperatures (18.3, 20.6, 23.9 and 25.8 °C) combined with four PPFD regimes (0.6, 2.1, 3.7 and 4.7 mol m^{-2} d^{-1}). The number of unfolded leaves was recorded continuously throughout the experiment. Shoot length, number of side shoots, number of leaves below the first side shoot and dry weight were measured at the end of the experiment. Periodic growth with two growth cycles could be observed in 3 treatments (2.1 mol m^{-2} d^{-1} and 25.8 °C; 2.1 mol m^{-2} d^{-1} and 23.9 °C; 0.6 mol m^{-2} d^{-1} and 25.8 °C) for the duration of the experiment. The number of side shoots reached an optimum at 20.6 °C and 3.7 mol m^{-2} d^{-1}. Side shoot formation only occurred after a dormant phase of the previous flush. The main shoot was significantly longer in the 25.8 °C treatment compared to all the other temperature treatments and also the number of leaves below the first side shoot was highest at the highest temperature. Dry weight increased linearly with increasing temperature and did not seem to reach an optimum within the tested temperature interval. For the production of a well-branched holly plant we would therefore recommend growing holly at 20.6 °C and 3.7 mol m^{-2} d^{-1}. Within all treatments individuals did not start growing simultaneously (non-synchronised), but we could find a trend towards better synchronization in the cooler treatments. The results indicate that further studies are needed on growth retardation and crop synchronization.

Zusammenfassung

Einfluss von Photonenflussdichte (PPFD) und Lufttemperatur auf das vegetative Wachstum und die Verzweigung von Stechpalmen (*Ilex* x *meserveae* 'Blue Princess' S.Y. Hu) unter Gewächshausbedingungen. Gegenstand dieser Untersuchung war der Einfluss der durchschnittlichen täglichen Lufttemperatur und der Photonenflussdichte (PPFD) auf das vegetative Wachstum von *Ilex* × *meserveae* 'Blue Princess' (S.Y. Hu). Bewurzelte Stecklinge wurden in Gewächshausabteilungen bei vier verschiedenen Durchschnittstemperaturen (18.3, 20.6, 23.9 and 25.8 °C) gezogen, die mit 4 verschiedenen Photonenflussdichten (0.6, 2.1, 3.7 and 4.7 mol m^{-2} d^{-1}) kombiniert wurden. Die Aufzeichnung der Anzahl der entwickelten Blätter wurde während des gesamten Experiments regelmäßig durchgeführt. Die Länge der Triebe, die Anzahl der Seitentriebe, die Anzahl der Blätter bis zur ersten Verzweigung und das Trockengewicht der Triebe wurden am Ende des Experimentes gemessen. Periodischer Zuwachs mit zwei vollständigen Zuwachszyklen während der Dauer des Experiments konnte in 3 Behandlungen (2.1 mol m^{-2} d^{-1} and 25.8 °C; 2.1 mol m^{-2} d^{-1} and 23.9 °C; 0.6 mol m^{-2} d^{-1} and 25.8 °C) beobachtet werden. Die Anzahl der Seitentriebe erreichte ein Optimum bei 20.6 °C and 3.7 mol m^{-2} d^{-1}. Eine Entwicklung von Seitentrieben erfolgte nur nach der Ruhephase des vorhergehenden Zuwachszyklus. Der Haupttrieb war signifikant länger in der 25.8 °C Behandlung als in den kälteren Behandlungen und auch die Anzahl von Blättern bis zur ersten Verzweigung war bei der höchsten Temperaturbehandlung am höchsten. Das Trockengewicht der Triebe stieg linear mit der Temperatur, ohne innerhalb des getesteten Intervalls ein Optimum zu erreichen. Für die Produktion von gut verzweigten Stechpalmen würden wir daher empfehlen, die Pflanzen bei 20.6 °C and 3.7 mol m^{-2} d^{-1} zu kultivieren. Innerhalb einer Behandlung starteten nicht alle Individuen gleichzeitig mit dem Zuwachs (unsynchronisiert), wobei jedoch ein Trend zu besserer Synchronisation in den kälteren Behandlungen vorhanden war. Unsere Ergebnisse zeigen weiterhin, dass weiterführende Studien zur Sprosslängensteuerung und zur Synchronisation der Individuen nötig sind.

Key words. Blue Holly – branching pattern – greenhouse climate – rhythmic growth – periodic growth – episodic growth

Introduction

In Scandinavia, greenhouse production of potted plants meets a weak market during summer and autumn. The production and sale of almost all existing ornamental crops is unprofitable during this season even if the climatic conditions are most favourable for a cheap production. This discrepancy in potential supply and actual demand causes economical problems for greenhouse nurseries especially if they wish to keep qualified labourers. This problem could be solved by producing woody hardy ornamentals as potted plants during the summer. The finished product could be stored outdoors until the sales period for Christmas. Due to its traditional use, holly (e.g. *Ilex* × *meserveae* 'Blue Princess' S.Y. Hu) is a potential potted plant for Christmas marketing. In contrast to traditional nursery production, the greenhouse industry is interested in producing small plants with red berries for indoor decoration. The production period should not exceed six months to fit into the non-profitable part of the year and avoid interference with the profitable production of bedding plants during spring and early summer. To achieve this, information is needed of how to manipulate the greenhouse climate in order to control growth and timing of the crop. Very little is known about the influence of climate on growth and development of *Ilex* species. *Ilex* spp. shrubs normally perform one annual flush of shoot growth (GALLE 1997). However, when grown in the greenhouse, with adequate moisture and nutrition, several flushes during one season can be observed (MERTENS and WRIGHT 1978; GILLIAM and WRIGHT 1978). Periodic growth phenomena have been described for oak (BORCHERT 1975; REICH et al. 1980). FARMER Jr. (1975) reported that temperatures above 23 °C promoted rapid and frequent flushing in juvenile Northern red oak. SALE (1968) showed that the periodic flushing behaviour of cocoa (*Theobroma cacao*) was influenced by the climate. At higher temperatures plants also lost their apical dominance. Shading in greenhouses is a common method to keep temperatures down during the summer. The process of branching is often influenced by the light intensity and might be affected by shading treatments. In *Draceana* (DONSELMAN and BROSCHAT 1982) and young apple trees (LINDHAGEN 1996), high light intensities have been reported to promote side shoot formation.

We consider an ideal pot plant for the Scandinavian market to be compact, not larger than 20 cm from the pot edge to the shoot tip, with as many side shoots as possible. Indicative for an acceptable plant would be a low number of leaves below the lowest positioned side shoot. Hand shearing and the use of growth retardants are common methods to shape *Ilex* × *meserveae* (BORDEN and CAMPELL 1984). However, suitable greenhouse climate management might be a valuable tool for manipulating plant shape. The objective of this study was to investigate the possibilities to grow holly plants in small pots during commonly encountered summer greenhouse conditions at Northern latitudes. Moreover we wanted to evaluate the use of shading screens in the greenhouses to influence the branching patterns of *Ilex* and if periods of dormancy would occur when *Ilex* is grown under greenhouse conditions.

Materials and Methods

Unbranched top-cuttings of *Ilex* x *meserveae* 'Blue Princess' (S.Y. Hu), with eight leaves, were taken from stock plant material (Splendor Plant, Sweden) on December 7th 2000. The five lowest leaves were removed and the stem was wounded on one side and dipped into a 0.6 % solution of indole-butyric-acid (IBA, SIGMA, Sweden) to stimulate root formation. The cuttings were stuck in a commercial peat based growing medium (Hasselfors K, Sweden) mixed with additional 30 % perlite and rooted in a mist chamber at 22 °C for 6 weeks. The rooted cuttings were transplanted into pots (Ø 11 cm) in the growing medium with additional 50 % (v/v) of perlite and pinched below the uppermost leave. The plants were divided in four groups and placed in greenhouse compartments at the temperature set points: 15, 18, 21 and 24 °C which resulted in actual daily mean temperatures of 18.3, 20.6, 23.9 and 25.8 °C (Table 1). Day extension was achieved by 16-hours supplemental lighting with high-pressure sodium lamps (400 W, Philips; 2.3 mol m^{-2} d^{-1}). Three weeks after transplanting, the plants were fertilized with a controlled-release fertilizer (Multicote, 3 kg m^{-3}, 18N-2.6P 9.9K, 8 months). On February 25th 2001 the experiment was started by distributing the plants to the shading treatments (10 plants per treatment) and by pinching them at the uppermost new-grown shoots over the first leaf. Only one shoot emerging from the cutting was allowed to develop and all other possible sprouting vegetative shoots on the cutting were removed throughout the experiment to avoid competition with the observed shoot. The shading treatment was achieved by positioning the plants under a metal frame (130×100×50 cm) covered with layers of white shading material of different thickness (Vattex, LS 56 and Lutrasil 18 (3 layers)). Light quality was not affected by the shading cloth, which was tested with the Licor Portable Spectroradiomenter LI-1800. The shadings resulted in photosynthetic photon fluxes (PPFD) of 0.6, 2.1 and 3.7 mol m^{-2} d^{-1}. The 4.7 mol m^{-2} d^{-1} treatment was unshaded (Table 1). In every greenhouse section we placed two blocks of all four shading treatments to guarantee exactly the same temperature treatment of both blocks. Day extension continued until the natural photoperiod reached 16 hours (April 17th 2001). The plants were watered two to three times a week, when the substrate surface started to dry up.

Table 1. Air temperature and PPFD treatments for *Ilex* × *meserveae* 'Blue Princess'.

Air temperature Measured daily mean (°C)	PPFD Measured daily mean (mol m^{-2} d^{-1})
18.3 ± 1.7	0.6 ± 0.3
20.6 ± 1.4	2.1 ± 1.0
23.9 ± 1.3	3.7 ± 1.8
25.8 ± 0.5	4.7 ± 2.2

Climate recordings and measurements

Air temperature was recorded with two thermocouples (type T, copper and constantan) per greenhouse section which were placed within the canopy in the unshaded and the 0.6 mol m^{-2} d^{-1} treatment in every temperature section. The PPFD (400–700 nm) was recorded using Skye SKP 215 quantum sensors (Skye Instruments Ltd, Llandringdod Wells, UK), which were placed at plant height and adjusted weekly. Both PPFD and air temperature were recorded at 15-second intervals and the mean value was stored every 15 minutes in a datalogger (Intab, Sweden) and mean values of day air temperature and daily light integrals for the entire experimental period (Table 1) were computed from the collected data. 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. At the end of the experiment, on June 18th 2001, the plants were harvested and the following growth variables were recorded: dry weight, length of main shoot, number of side shoots per plant and the number of leaves below the first side shoot. The length of the shoot was measured from the lowest point where the shoot was emerging from the leaf axis, to the shoot tip. Dry weight was determined for the observed new-grown shoot, which was dried in paper bags at 80 °C for 48 hours.

Statistical procedure

The treatments were arranged in a two-factor block design with 2 blocks. The average of 10 plants under one shading frame was considered as the experimental unit. The combination of four PPFD and four temperatures resulted in 16 treatments. Due to the layout of the experiment with the shading treatments it was impossible to do a randomisation. Both PPFD and temperature treatments were fixed factors. Data were analysed by ANOVA using the GLM procedure of SAS/PC. Interactions between temperature and PPFD effects were tested. The significance of temperature and PPFD on plant characteristics was determined using a Tukey test at 5 % level. Linear and exponential curve fittings were done in Origin 5.0 (Microcal Inc., Northampton, USA). Both regression lines where selected according to the light saturation curve and the temperature optimum curve (with the linear response in the ascending arm of the curve) presented in TAIZ and ZEIGER (1998). To control, if the rise of dry weight with temperature is significant, the GLM procedure was used with temperature as the covariate.

Results

For all variables tested, except number of leaves below the first side shoot, the ANOVA analysis showed no significant interaction between the two factors air temperature and PPFD and no significant block effects (Table 2). The results are presented for each climate factor separately.

Leaf unfolding

At the highest temperature treatments (25.8 °C and 23.9 °C), leaf unfolding started approximately 15 days after the plants were pinched, while at the lowest temperature (18.3 °C), plants started growing after 25–30 days. The pattern of leaf unfolding differed between the treatments. In some treatments (e.g. 4.7 mol m^{-2} d^{-1} and 25.8 °C) the plants grew nearly continuously while in others, one evident flush occurred and then no more leaves where produced (0.6 mol m^{-2} d^{-1} and 18.3 °C). In three treatments (2.1 mol m^{-2} d^{-1} and 25.8 °C; 2.1 mol m^{-2} d^{-1} and 23.9 °C; 0.6 mol m^{-2} d^{-1} and 25.8 °C) two flushes were produced (Fig. 1). A detailed analysis of these repeated measurements is presented in Kosiba et al. (submitted). After 93 days the plants in the warmest treatment (25.8 °C) had produced most leaves on the main shoot (data not shown), while, due to the high number of side shoots, the total number of leaves was highest at 20.6 °C (Fig. 2/A). Within this temperature treatment a PPFD of 2.1 and 3.7 mol m^{-2} d^{-1} resulted in the highest number of leaves and differed significantly from the lowest and the highest PPFD level. The lowest number of leaves was produced in the 0.6 mol m^{-2} d^{-1} and 18.3 °C treatment (data not shown). The growth and development of one flush could be divided into different stages (Fig. 3). The flush was initiated when the dormant terminal bud (stage A) started swelling (stage B). Then the first leaves started unfolding and the stem started to elongate (stage C). Leaf unfolding and stem elongation slowed down and the last leaves were unfolded (stage

Table 2. ANOVA of effects of block, PPFD and temperature on canopy dry weight, number of leaves below first side shoot, length of main shoot and number of side shoots.

	df	Significances of F (%)				
		No. Leave[v]	Shoots[w]	Leaves[x]	Weight[y]	Length[z]
Main effects						
block	1	0.4275	0.1504	0.6222	0.2704	0.4933
temperature	3	0.0042	<.0001	<.0001	0.0034	0.0007
PPFD	3	<.0001	0.0002	<.0001	<.0001	<.0001
Interactions						
temperature × PPFD	9	0.0942	0.086	0.0009	0.5232	0.28

[v] Final number of leaves; [w] Side shoots; [x] Leaves below first side shoot; [y] Canopy dry weight; [z] Length of main shoot.

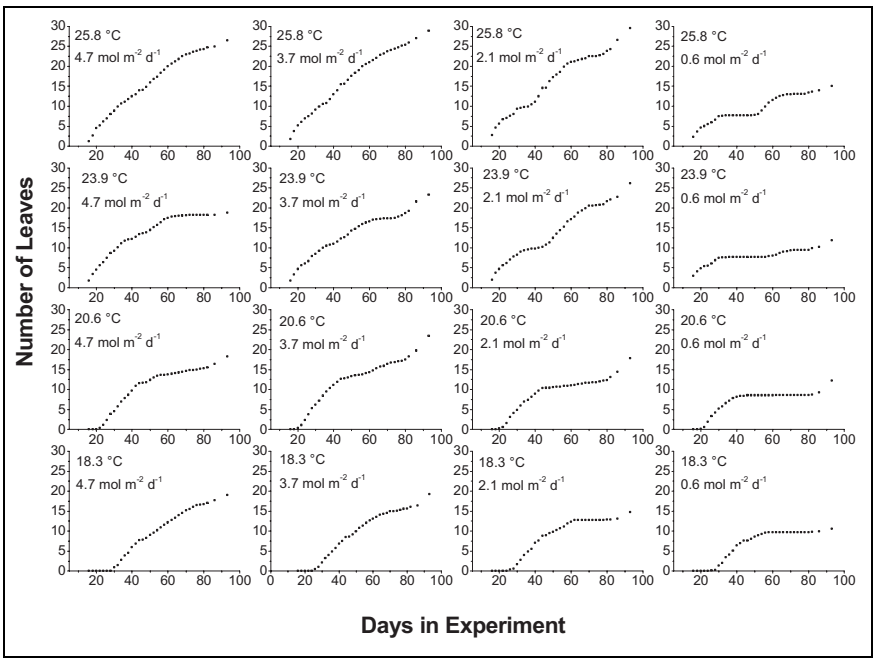


Fig. 1. Number of leaves unfolded in *Ilex* x *meserveae* under 16 combinations of 25.8, 23.9, 20.6 or 18.3 °C and 4.7, 3.7, 2.1 or 0.6 mol m^{-2} d^{-1}. Each measurement point represents the mean of 10 plants. Only the number of leaves on the main shoot is shown.

D). Stem elongation terminated, a terminal bud was formed and leaves expanded to full size (stage E). Finally the leaves became dark green and leathery, the lignification of the stem started and stage A is reached again.

Branching and dry weight

The number of side shoots was highest at 20.6 °C and differed significantly from all the other temperature treatments (Fig. 2/B). In the 2.1 and 3.7 mol m^{-2} d^{-1}

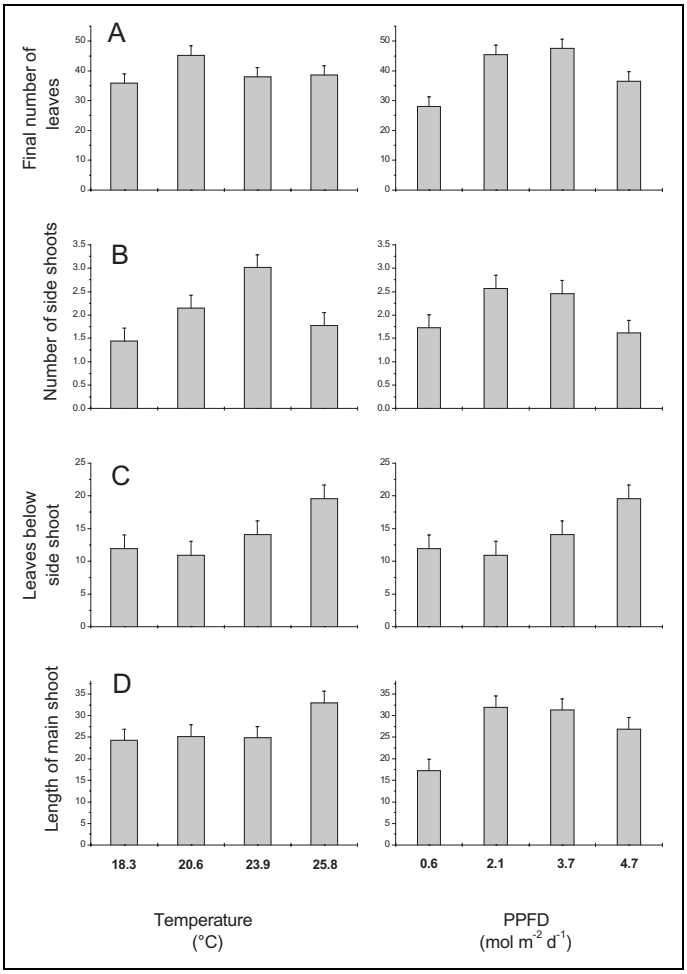


Fig. 2. Effect of air temperature and PPFD on A) Length of main shoot, B) Number of leaves below the first side shoot, C) Number of side shoots and D) Final number of leaves (leaves on main and side shoots). Bars showing the temperature response are mean values of all PPFD data for one air temperature treatment and *vice versa* for the different PPFD treatments (n = 80). The error bar indicates the minimum significant difference of the Tukey grouping ($p<0.05$).

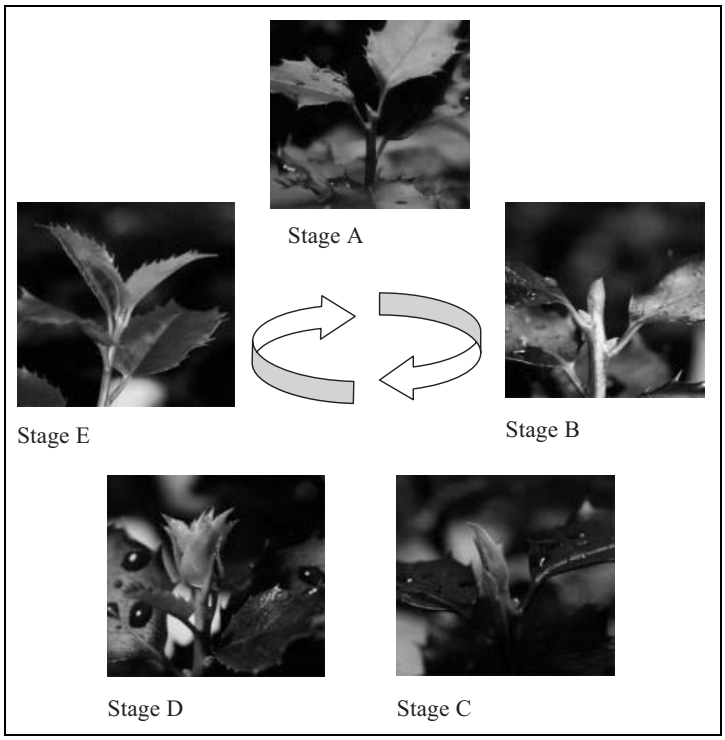


Fig. 3. Developmental stages of the vegetative growth of Blue Holly (*Ilex* x *meserveae* 'Blue Princess'). Stage A) Dormant terminal bud. B) Terminal bud starts swelling. C) The first leaves start unfolding and the stem starts elongating. D) Leaf unfolding and stem elongation slow down. E) Stem elongation is terminated, the terminal bud is formed and the leaves expand to full size.

treatments more than two side shoots emerged from the main shoot and this differed significantly from both the 0.6 and 4.7 m^{-2} d^{-1} treatment. Side shoots only developed after the dormant phase of the shoot (data not shown). Side shoots emerged from the base of the new grown shoot, i.e. the oldest nodes of the flush. In treatments, which resulted in a high number of side shoots, side shoot even emerged from the youngest nodes of the previous flush. A comparison between Fig. 1 and Fig. 2/B indicate that the most side shoots were initiated at climate conditions that caused pronounced periodic growth. Climate conditions inducing only one flush during the experimental period, resulted in poor side shoot formation. The number of unfolded leaves below the first side shoot was the highest in the 25.8 °C treatment and differed significantly from the 18.3, 20.6 and 23.9 °C treatments. At the lowest light level (0.6 mol m^{-2} d^{-1}) the plants produced significantly less leaves under the first side shoot compared to all the other PPFD treatments (Fig. 2C). In the 25.8 °C treatment the main shoot was significantly higher compared to all the other temperature treatments (Fig. 2D). The length of the main shoot was significantly shorter in the 0.59 mol m^{-2} d^{-1} treatment

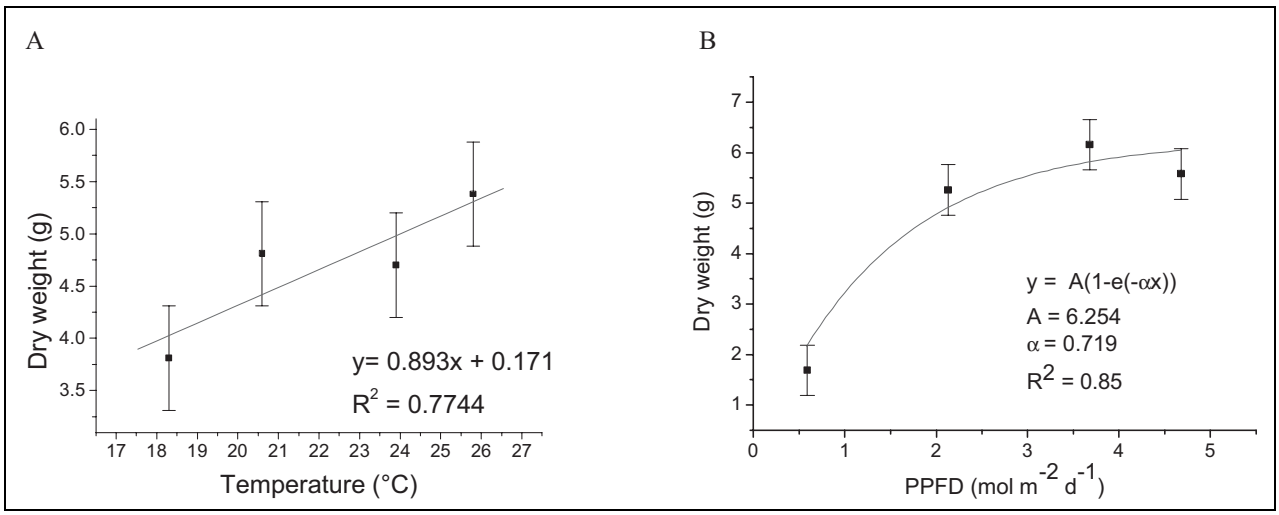


Fig. 4. Canopy dry matter production of *Ilex* x *meserveae* as a function of temperature (A) and PPFD (B) after 93 days in experiment. Each data point represents the mean of 8 plants and the error bars the minimum significant difference of the Tukey grouping ($p<0.05$).

compared to all the other PPFD treatments. The highest amount of dry weight was produced in the 25.8 °C temperature treatment and differed significantly from the lowest temperature treatment (Fig. 4A). The slope of the linear response curve differed significantly from zero. There was a significant difference in dry weight production between the lowest light level and the other three light intensities, which gave a good fit to an exponential saturation curve (Fig. 4B).

Discussion

From the growers´ point of view, an ideal situation would be if all individuals of a crop grow simultaneously (synchronised), leaves are unfolded regularly, and a maximum number of side shoots is developed. Our results indicate that this is impossible in Blue Holly since climate conditions, that promote side shoot formation the most, do not give the most compact plants. Favourable climate conditions for side shoot formation lead to a high number of leaves below the first side shoot and the individuals did not start growing simultaneously after the rooting phase. Plants with a high number of side shoots also show a pronounced dormant phase (LANG et al. 1987). Hence, this break in leaf unfolding seems to be crucial for side shoot formation. However, the connection between side shoot formation and periodic growth was masked as the oscillations in leaf unfolding rate could be extinguished when the mean values for all 10 plants in a treatment were calculated. In treatments with higher temperatures, displaying poorer synchronisation, this problem is more evident than in the cooler ones. In general, the influence of the climate on the leaf unfolding rate could not be quantified, because the beginning and end of the dormant period was not possible to identify in an accurate way. This required a new procedure for data analysis. To avoid the problems related to poor synchronisation a *sine* function has been adjusted (LARSEN and KOSIBA 2002) and used for a vegetative-growth analysis. This detailed analysis would have exceeded the present paper and is presented in Kosiba et al. (submitted). Contrary to the expectations that the high PPFD would be needed to stimulate side shoot formation (DONSELMAN and BROSCHAT 1982; LINDHAGEN 1996) our results indicated that a moderate PPFD close to 4 mol m^{-2} d^{-1} elicited most side shoots and highest dry matter production. This low value might appear surprising. However, it matches well with recommendations from traditional gardening where *Ilex* is considered a shade plant (RUGE 1966; DRAKE 1993).

Previous studies on other plants suggested that complex endogenous mechanisms are involved in controlling periodic shoot and root growth. Researchers have correlated periodic growth in oak and cocoa to climate and nutrients (SALE 1968; PHARES 1971; FARMER Jr. 1975), changes in water status (BORCHERT 1975), hormonal status (ABO-HAMED et al. 1981; FAVRE and JUNCKER 1989) and carbon translocation (HANSSON et al. 1988; DICKSON et al. 2000) In the present study on Blue Holly we have only been concerned with the growth response of the shoot. Since PPFD and temperature seemed to have a marked effect on the presence or absence of periodic growth it is tempting to speculate that cyclic behaviour of the carbon assimilation or carbon distribution in the plant might be a crucial factor. There may be a cyclic distribution pattern where limited amounts of available assimilates are alternately being partitioned mainly to the shoot or the root. However, at this point we do not know how root growth is related to periodic shoot growth. From a commercial point of view side shoot formation should have first priority. Therefore we conclude that climate conditions close to 20 °C and 4 mol m^{-2} d^{-1} should be recommended for the industry. The suggested climate conditions can easily be achieved in greenhouses at Northern latitudes during summer. However, a deeper understanding of how periodic growth can be controlled by PPFD and temperature would give us the possibility to grow small bushy holly plants without any retardants or chemical treatment.

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