
EFFECT OF LIGHT AND TEMPERATURE ON PLANT CANOPY GROWTH

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INTRODUCTION

Environmental cues have a major impact on plant development. Only if a plant is well adapted to its environment it can reach theoretical potential and maximum yield. On the other hand when environment differs from the physiological range of a given species, it has to invest more resources to adapt. As the plant cannot move away from those suboptimal conditions, growth does not reach an optimal intensity which ultimately has an impact on biomass production and yield.

World arable area *per capita* is decreasing and the resources mankind can afford to invest in agriculture are more and more limited: we are running out of phosphorus (Cordell *et al.* 2009) and water supply is limiting for most agricultural systems. Arable land *per capita* declined by more than 50% from 0.43 ha in 1962 to 0.21 ha in 2007 (FAO 2010). Often, arable land is nutrient-depleted and degraded – an increase in salt concentration is amplifying this problem. Increases in world meat consumption are also driving the need for more cereal grain production. Biofuel production is now competing for the same arable land as crops, putting more pressure on agricultural productivity. With the world population and its demands rapidly increasing there is a priority to rethink our agricultural practices. This is why another green revolution is required if we are to feed the world population in the next decades. To achieve such a revolution, a better, more integrated understanding of crop growth and yield responses to environment is required.

Variations in growth dynamics in response to environmental conditions are not easy to measure. Both a detailed characterisation of the environmental conditions along with techniques able to grasp subtle changes in growth rate are required. Both spatial and temporal resolutions of these parameters are poor when studied with the naked eye, the technique commonly used by plant breeders. The greatest challenge here is to bridge the gap between the subtleties of the plant inner rhythm and our ability to monitor plant growth parameters and environmental variables.

Nutrient availability, water, light and temperature are key players driving plant development. It is critical to understand what effects they have on different time scales. As they are the most energy costly inputs in agricultural practices, we need to assess potential yield variation related to either supply or reduction of those inputs. The ability to predict the upcoming climate and to know how plants will react is at the base of a sustainable agriculture, optimising the yield and limiting inputs (energy and chemicals) (Dodd *et al.* 2005). We have to optimise the way plants are utilising available resources and then provide them only what is really needed to utilise their full potential.

Recent advances in non-invasive analysis techniques allow precise and continuous monitoring of changes in growth, identifying growth patterns that were otherwise concealed due to their low amplitude and timescale (Schmundt *et al.* 1998). Those non-invasive tools are powerful to improve our understanding of these dynamics and thus determine the ecophysiological rationale of those changes.

The knowledge of the way plants grow and make use of available resources is progressing. Nevertheless several questions remain unsolved, especially those related to dynamic processes involved in the adaptation of plant activities to fluctuating environmental conditions and the integration of the two major environmental components affecting plant growth: soil and atmosphere. Plant response towards changes in environment isn't only local. It is one aim of this study to focus on integrated aspects of plant responses (DeLucia *et al.* 1992). One case study of this response is observed after root cooling is applied and we analysed changes at whole plant scale for growth patterns, carbohydrate metabolism, transport of carbohydrates and water.

MOTIVATION

This work is meant to raise the awareness on the effect of temperature and light on potted plants. Many scientific studies are currently based on the assumption that we can mimic field conditions in a growth cabinet and thus directly use the knowledge gained in the lab to the field. The need to bridge the gap between lab-based experiments and field trials is great and phenomics strategies are based on this assumption but we should remain aware of the major differences between those two worlds. Current techniques allow monitoring plant growth rhythm when exposed to fluctuating climatic conditions. Acknowledging and measuring the physiological differences between lab and field are needed to know to which extent we can export the results gained from the lab.

This work aimed at describing the integrated response at different scales when climatic cues are altered.

- The effect of soil temperature at whole plant scale is investigated in the article #1.
- The effect of air temperature and light on various plant types is detailed in article #2.
- The article #3 investigates the effect of light distribution and low air temperature on chrysanthemum. It is also a case study on how one can improve energy use in horticulture.

SHOOT AND ROOT ARE LIVING IN DIFFERENT WORLDS

TEMPERATURE HETEROGENEITY

Temperature is the main variable to take into account to understand plant performance. Low temperature can have a large negative effect on most plant metabolic processes - in case of a freezing event it can induce formation of ice crystals and cell bursting. Several protection mechanisms are acting to prevent damages caused by frost because it is critical for plants to maintain structural integrity and compartmentalisation (Guy 2003). At the other extreme, high temperatures are usually associated with high Vapour Pressure Deficit (VPD) which ultimately leads to drought stress at the plant level. There is a fine regulation between stomata opening and amount of water that plants are ready to invest to maintain low leaf temperatures and the need to allow gas exchange to sustain high photosynthesis rates (Mott & Parkhurst 1991). Increasing temperature accelerates most enzymatic reactions (Breidenbach *et al.* 1974) to their maximum level but above a given threshold this activity declines (Sharkey & Zhang 2010) negating any potential gain due to protein instability and activity reduction. Temperature of the above ground environment is neither static in the short nor in the long term; air temperature is fluctuating markedly throughout a typical day even in temperate areas. Ultimately, those differences in soil and air temperature have an impact on plant growth and underlying metabolism (Walter *et al.* 2009). Some plants do require low temperature to be able to complete their development cycle. This phenomenon is known as vernalisation and can imply weeks at low temperature, typically between 5 and 10°C, for a plant to be able to flower (Dennis & Peacock 2009). Some plant reproductive organs need to maintain a temperature higher than surrounding air. Philodendron spadixes are known for their thermogenesis that can maintain up to 30 h after being cut off the stem (Grant 2010).

In the field, amplitude of day-night temperature course can easily reach 20°C in the air while Root Zone Temperature (RZT) is remaining fairly damped over 24 h. Moreover, there is a pronounced spatial temperature gradient in the soil, which fluctuates throughout 24 h (Füllner 2007). The deeper the roots in the soil, the lower the temperatures and the lower day-night amplitude they will experience (Hillel 1998). Experiments in a specific Australian soil show, for example, that at 25 cm depth, soil temperature varies by merely one degree over 24 h and at 100 cm depth the amplitude is less than a tenth of a degree. Data from Phenonet Leeton (NSW, Australia). Courtesy of High Resolution Plant Phenomics Centre.

In laboratory experiments carried out in growth chambers or greenhouses, this effect is of great importance as pot volume is very small and thermal equilibrium with the air is much greater than in the field (Passioura 2006). We observed that soil temperature in a pot left to equilibrate in controlled conditions can rise very quickly to temperatures higher than the air temperature cf. Fig. I-1*. The effect of temperature variation belowground is even further increased by a higher heat transfer capacity of soil compared to air.

* Nomenclature for citing figures from the three research articles compiled in this thesis is as following:
Fig. X-Y: where X refers to the paper I, II or III and Y is figure's original numbering.

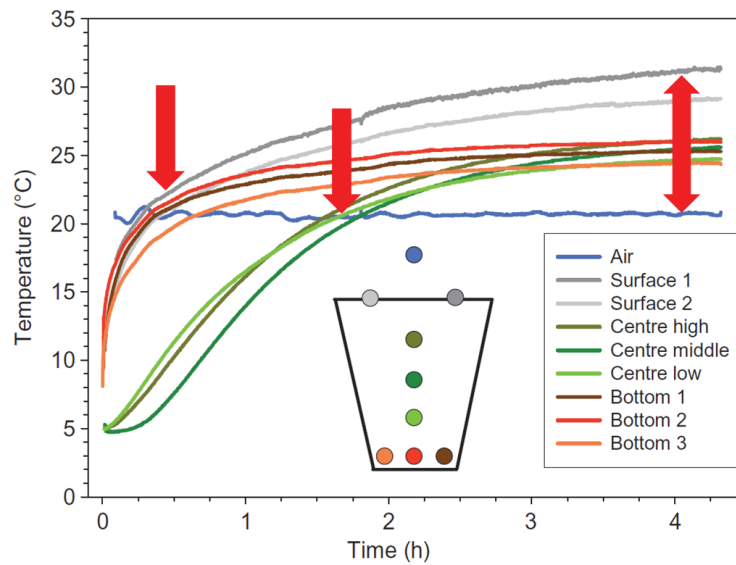


Fig. I-1: Temporal development of temperature at nine different locations of a soil-filled pot (1 L) transferred from 5°C to a growth chamber at 20°C. The pot was translocated from a cold room (5°C) to a climate chamber. Exact locations of temperature analysis are indicated in the scheme.

It takes only 30 to 90 minutes for the RZT to match the air temperature and after 4 hours the RZT can be up to 10 K higher than air temperature and increasing. This scenario is unlikely in the field but close to the average conditions for potted experiments. The effect of RZT at the whole plant scale isn't well documented but nevertheless a key issue we need to address to validate the knowledge we can gain from laboratory experiment and transpose them to the field. To control and assess for the effect of RZT we used a pot cooling system (Fig. A below) (Füllner 2007).

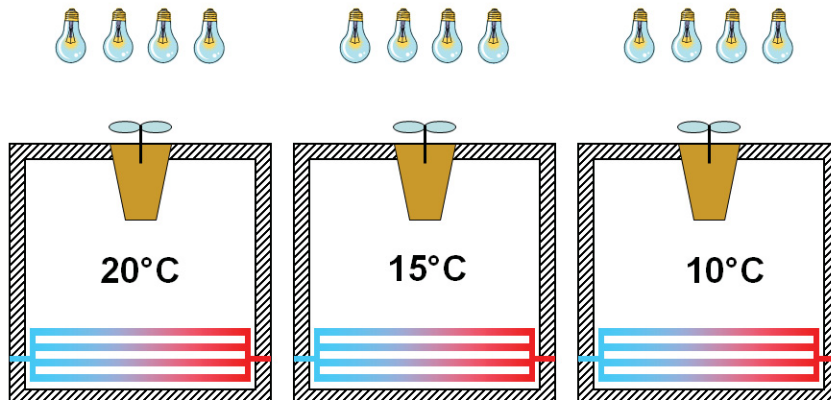


Fig. A: Schematic overview of the pot cooling system which consists of 3 insulated chassis containing a heat exchanger. Coolant is pumped through the heat exchanger and lowers the temperature inside the box compared to the outside. Several apertures are made in the upper side of this box, allowing to place the pots inside. The cooling pump allows controlling of coolant temperature independently in the 3 zones. It takes about 30 min to reach the desired temperature within the entire pot.

We investigated the effect of sustained root cooling during the first experiment by applying 3 constant root temperatures (10, 15 and 20°C) for 19 days. We observed that *Ricinus communis* total leaf area and Relative Growth Rates (RGR) were overall reduced by a factor 2 to 3 compared to the 20°C treatment (Fig. I-2a & I-2b). We observed the same growth pattern in all 3 treatments (Fig. I-2b). When RZT was permuted every 4 days in sequence, *Ricinus communis* was adjusting its leaf growth rate in less than 24 h after change (Fig. I-3). Using DISP to reach a higher temporal resolution we observed that nocturnal average growth rates scaled well with the temperature (Fig. I-4k). In turn, diurnal average growth rates are severely decreased to values close to zero by low RZT (Fig. I-4k) while nocturnal maximal growth rates are not affected by root-zone temperature (Fig. I-4a-i). This demonstrates that leaf growth reaction to root-zone or root temperature alteration cannot simply be scaled between long-term and short-term responses, but that diel and probably circadian variations of regulating mechanisms have to be taken into account. The effects of low RZT are at whole plant scale as we also observed that root elongation and ramification decreased rapidly after cooling but in this is reversible if the RZT is warmed up (Fig. I-5).

Low Night Temperature (LNT) of 12°C reduced chrysanthemum photosynthesis efficiency (F_v/F_m) from 0.77 to 0.72 (Fig. III-7) and it correlated well with a reduction of shoot growth (Fig. III-4). We did not expect the nocturnal temperature to possibly have an effect on diurnal metabolism but indicate that air temperature has a pleiotropic effect at whole plant scale.

LIGHT HETEROGENEITY

As for temperature, light conditions are highly variable over 24 h and plants have adaptive mechanisms to cope with those fluctuations. The amount of light that reaches the canopy depends largely on the latitude, the architecture of the canopy and agricultural management in case of crops. Canopy will tend to expand to intercept up to 100% of the incident light during most of the season. If Daily Light Integral (DLI) is low, plants can increase their photosynthetic efficiency by rearranging their photosynthetic apparatus and stacking several layers of thylakoids. We observed at high temporal resolution that a swift change of the light conditions (i.e. switching the lights on or off) of a growth chamber induce a growth artefact (transient increase of growth almost immediately followed by a transient growth depression; both effects lasting less than half an hour). This effect is only visible at very high temporal resolution (Fig. III-3), it has no effect on the average daily growth rate and can be overcome if needed by dimming the light source at dawn and dusk.

Many flowering plants use photoreceptors to sense seasonal changes in night length to trigger flowering. Some species need long days, some short days and others are not responding to day length to flower. Our experiments were carried out before flowering to avoid the metabolic changes that may interfere with the physiological parameters we were monitoring.

Growth chamber light conditions can be very heterogeneous; Photosynthetic Active Radiation (PAR) can fluctuate by 30% and more across few decimetres and have a large impact on plant development. Attention was kept in this study to grow plants only in growth chamber regions, where the light was very homogenous. This efficiency for light interception has been measured and successfully modelled for *Arabidopsis thaliana* and *Helianthus annuus*. The model even revealed that *Arabidopsis thaliana* increased its light interception efficiency when shaded (Chenu *et al.* 2008). Plants can take advantage of the night to increase their leaf area and minimise their self-shading by nyctinastic motions to explore areas with more favourable light distribution. Some species are also known for their heliotropic behaviour, they are able to track the sun during the day to optimise their light interception. We witnessed both phenomena in this study by using time lapse imaging techniques.

In case of high irradiance, plants have to find a way to cope with excessive light energy to prevent any damage. There are several light avoidance mechanisms at work depending on the duration of the high irradiance episode. If the high irradiance episode is swift, the plant can reduce the stiffness of the stem, petiole and leaf blade (Vandiver & Goriely 2008) to display less leaf area and to reduce overall light interception. If the high irradiance episode is longer other photoprotective mechanisms are engaged such as the down regulation of efficiency of the Light Harvesting Complex (LHC) (Fig. III-6) to limit production of Reactive Oxygen Species (ROS). ROS management is very important for long term light avoidance strategy as the plant cannot afford to accumulate ROS because of their high toxicity (Bowler *et al.* 1992).

Light effects on plant performance can also be caused by light quality as the plant can sense the differences in the intercepted spectrum. Phytochromes and cryptochromes are sensitive to red and blue light, respectively and they provide specific information to the plants concerning their neighbourhood (Franklin & Whitelam 2005). Red light or more specifically a low Red/Far Red (R/FR) Ratio indicates the proximity of other plants and competition for the available light resource. Similar to the reaction at low light conditions, plants increase their growth upward at low R/FR Ratio to remain above their competitors until the R/FR Ratio goes back to higher values. (Devlin *et al.* 1999) Photoperiod is for most temperate species a trigger of flowering along with R/FR Ratio. Flowering time is a major determinant of crop performance and thus it is of a great value to understand the mechanisms that are triggering flowering to design more efficient plants.

UV light is also a major component of the light spectrum quality. UV light has mutagenic effect on DNA and triggers DNA repair mechanisms of the plants (Dempel & Harrison 1994). The increase in lyase activity competes with other primary metabolic activities and reduces the overall growth. The growth reduction induced by UV light has been studied in other experiments of our group at the same time as this thesis was performed. This was done both in controlled conditions with exogenous UV sources (UV-A and UV-B) and under greenhouse conditions by comparing roofing materials differing with respect to their UV-B transparencies (i.e. Phytex greenhouse at ICG3 - Jülich - Germany) (Tittmann *et al.* 2010).

NUTRIENT HETEROGENEITY

Nutrient availability in soils is highly variable and heterogeneous. It is essential for the plant to make the best use of available resources (Jobbágy & Jackson 2001). There are different strategies to increase root soil exploration and availability of the main nutrients (Nitrogen, Phosphorus and Potassium) to provide ample supply to sustain both root and shoot growth. Common strategies are involving preferential branching, alteration of root growth rates and production of fine roots. Phosphorus shortage will be a major limitation to agricultural production in the near future and a major research focus must be toward improving Phosphorus Use Efficiency (PUE) (Cordell *et al.* 2009). It will be a new challenge to use less fertilisers and more degraded soils. However, as this topic is not a primary focus of the current study, pre-fertilised soil (ED73, Balster Einheitserdewerk, Fröndenberg, Germany) was used throughout all experiments to ensure that all nutrient needs of our plants were met. We acknowledge that this potting mix represents an artificial homogeneity in the soil properties but it reflects best the average laboratory and greenhouse conditions compared to more artificial media such as hydroponic, aeroponic or agarose gel systems. It is possible to use soil cores taken from the field as alternatives to the potting mix. Collecting and using such a core is possible but remains complex as the core should be large enough to minimise border effects and as it requires a lot of care to retain the spatial heterogeneity during the sampling. The sampling process required state of the art excavation tools and a crane. Those soil cores are integrated in a controlled environment to simulate a complete ecosystem (<http://www.ecotron.cnrs.fr>).

TEMPERATURE SENSING

TEMPERATURE AS SIGNAL - CIRCADIAN CLOCK

No simple temperature sensor has been identified *in vivo* but some proteins are affected by temperature and may act as temperature sensor in addition to their primary functions (Franklin 2009). Not only the absolute ambient temperature but also the dynamics of temperature change can trigger plant response. Temperature sensing mechanisms have been reported for *Arabidopsis thaliana* (Kumar & Wigge 2010); there, histone H2A.Z and DNA folding seem to be key elements in the transduction of temperature changes as low as 1 K.

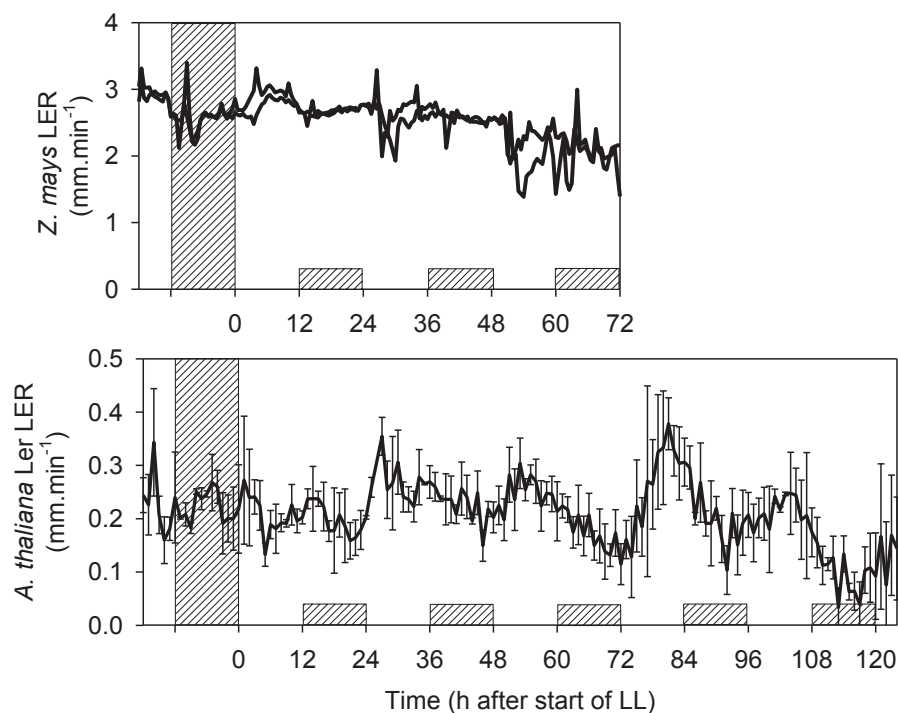


Fig. II-6: Leaf growth of *Z. mays* and *A. thaliana* transferred from 12/12 h day/night to a continuous light regime. Hatched bars represent subjective night in the continuous light (LL) treatment. For *Z. mays*, replicate plants are shown with different lines; for *A. thaliana*, mean value and SD (n=4) are shown.

While plants are able to sense changes in air temperature over a 24 h period, their rhythmicity is assured by a circadian clock which is sensitive to temperature but can retain its rhythm for many days under constant temperature (Fig. II-6 *A. thaliana*). Many processes throughout in plants are controlled by the circadian clock, the degree to which the circadian clock affects other processes differs between tissues, organs, and species (McClung 2006). There is an evolutionary advantage for plants to be adapted to a 24 h day length compared to plants that are not adapted (Dodd *et al.* 2005) In the dicot model species *Arabidopsis thaliana*, leaf growth, as well as hypocotyl growth, seems to be controlled by the circadian clock. Plant motions are mostly concealed at our time scale but time lapse imaging allows perceiving them. They can be either diurnal such as the heliotropism of sunflower or nocturnal such as nyctinastic motions. Both heliotropism and nyctinastism are reported to be controlled by the circadian clock (McClung 2006). From an evolutionary point of view, a correct phasing between

plant metabolism and forthcoming environmental conditions represents an advantage over organisms with a coarser regulation. Monitoring plants with combined thermal and chlorophyll fluorescence imaging can provide information applicable to the improvement of crop adaptability and yield performance. Combining those techniques allows continuous circadian plant monitoring of plant response at individual leaf and whole plant scale (Chaerle *et al.* 2007). The circadian clock is in control of both stomatal conductance and photosynthesis (Dodd *et al.* 2005). Therefore, it is necessary to monitor both at a high temporal resolution to understand those regulatory mechanisms. A basic yet fully functional living oscillator based on cyanobacterial KaiC protein has been reconstructed *in vitro* demonstrating the stability of the period regardless of the incubation temperature (Nakajima *et al.* 2005). Circadian clock is temperature compensated and does not tick faster as temperature increases (period remains 24 h). Temperature compensation is an essential feature of the circadian clock, the periodicity and amplitude of rhythmic processes that are controlled by the circadian clock are affected only to a minor extent by temperature variations Harmer (2009). We witnessed for dicotyledon species a decay in growth rate toward the end of the night phase when the plant is “expecting” the light phase (Fig. I-4a).

The effect of endogenous rhythms on leaf growth was analysed under continuous light in *Arabidopsis thaliana*, *Ricinus communis*, *Zea mays*, and *Oryza sativa*. No rhythmic growth was observed under continuous light in the two monocotyledons, while growth rhythmicity persisted in the two dicotyledons (Fig. II-6). We concluded that while monocotyledons respond to external temperature by adjusting their growth rate, they do not display any rhythmicity. The rhythmicity observed for the dicotyledons (Fig. II-2) was merely modulated, but not dominated by temperature confirming that in dicotyledons, the circadian clock provides an important element of control and synchronisation between leaf growth and underlying processes.

TEMPERATURE AS ENERGY SOURCE - THERMAL TIME

The concept of thermal time arose from agronomy where it was required to be able to compare plant performance in the field across multi-plots and multi-year trials. As field conditions cannot be controlled breeders needed a way to normalise the yield (Granier & Tardieu 1998).

To do so agronomists and plant breeders express their results using a developmental scale which integrates not only time but also temperature. Temperature is a major controller of development and the relationship between growth rate and temperature is not linear (Granier *et al.* 2002); it follows a threshold function with respect to temperature and below a critical temperature there is no growth. This threshold is the so called “base temperature”.

Base temperature can be determined experimentally plotting the plant growth rates at various steady state meristem temperatures. The intersection of the fit line with the temperature axis indicates the base temperature. Knowing the base temperature of a given species allows the calculation of its thermal time for development or physiological age. This is done by subtracting this base temperature from the daily average meristem temperature.

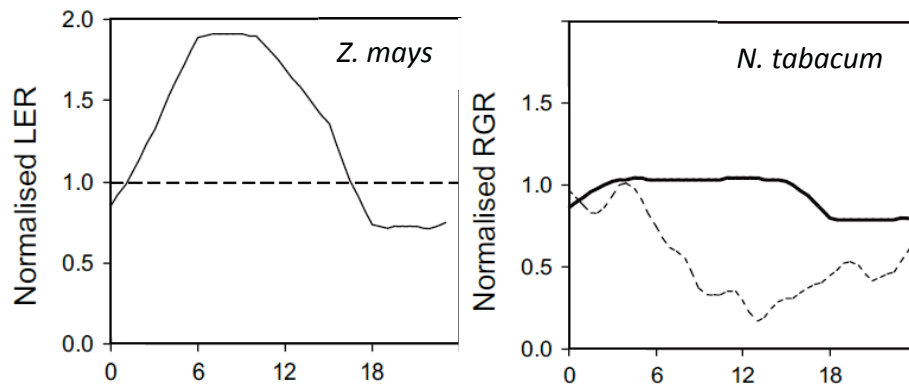


Fig. II-7. Addition of the effects of temperature and endogenous rhythms in *Z. mays* (left) and *N. tabacum* (right). Effect of temperature (solid line) and of endogenous rhythms (dotted line) simulation from the superposition of the two effects.

Thermal time, then, represents the physiological age of a plant. Taking into account all processes that are accelerated by higher temperatures and thus expressing growth against thermal time allows normalising the results of different experiments carried out under uncontrolled conditions. It is crucial for this normalisation to keep record of the temperature experienced by the shoot meristem of the plant with a data logger in the field.

It is possible to establish a temperature compensation curve that takes into account the effect of temperature only on leaf growth (Fig. II-7). The effect of temperature on plant growth depends largely on whether it is a monocotyledon or a dicotyledon. In monocotyledons we were able to relate most of the leaf growth variation to the temperature while dicotyledons remain largely unaffected in their rhythm. There is a duality between the concepts of thermal time and circadian clock but our observations and modelling attempts managed to demonstrate that both hold true, depending on the time scale considered. Thermal time is best suited for representing ecophysiological effects on a day to day basis while the circadian clocks rules the plant physiology at a sub 24 h scale.

MONOCOTYLEDONS - DICOTYLEDONS

Not all plant species are experiencing time and temperature in the same way and while thermal time provides a good estimate of the daily amount of growth a plant can achieve (Ben-Haj-Salah & Tardieu 1995) it is not the most suited measure to explain temperature effects on a shorter time scale. The largest discriminant of interspecific difference in response toward temperature may lay in their shoot meristem physiology. The meristem is the most important tissue related to plant growth. There, cells divide and expand. We can distinguish two types of plants, monocotyledons and dicotyledons, differing in the organisation of their leaf meristems and differing in their strategy to achieve maximum growth (Fig. B).

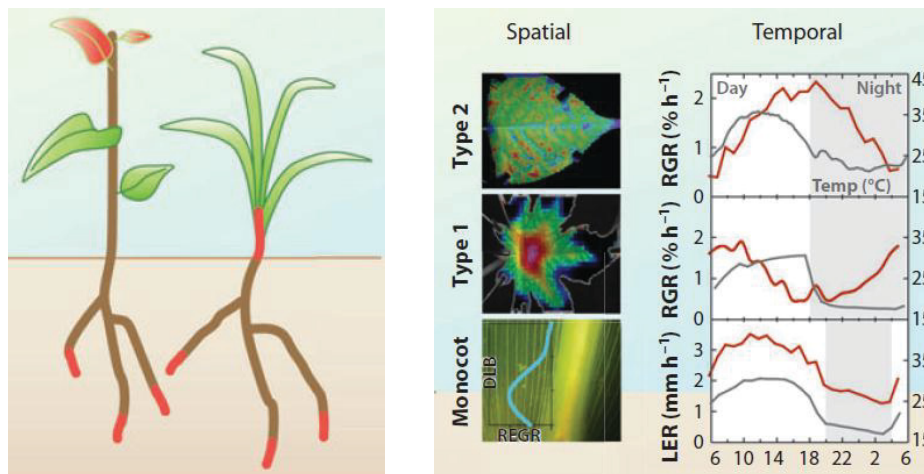


Fig. B: Schematic overview of position of shoot and root meristems in dicots and monocots (left panel), growth zone positioning and spatiotemporal leaf growth patterns (right panel). From Walter *et al.* 2009.

Dicotyledonous species have their shoot meristems on the leaf blade, exposed to the sun and the surrounding conditions (Fig. B). This means that growing tissues are vulnerable to the environment and this location imposes a trade-off between growth, temperature regulation and photosynthesis when the growth conditions are suboptimal (high VPD) (Sharkey & Zhang 2010). Some dicotyledons got around this constraint by shifting their leaf growth toward the night (Fig. II-3). This night growth allows a better use of the available resource by decoupling photosynthesis during the day and leaf growth at night.

Monocotyledon plants have their meristems buried deep into the stem and thereby protecting it against most environmental pressure. The apparent part of their leaves consists of non-growing tissues which in essence are less sensitive to stressful environment. The positions of meristems in plant types may also be an important determinant of their sensitivity toward temperature change.

We observed that monocotyledonous leaf growth rate would mainly follow the air temperature regardless of the light supply (Fig. II-6c & Fig. II-6d) while dicotyledonous species seems to obey an internal growth pattern that is merely modulated by temperature (Fig. II-2 & Fig. II-3). Dicotyledonous species will retain this rhythmicity for days

and weeks even under constant temperature and continuous light (Fig. II-6a & Fig. II-6b). This rhythm seems to be directly related to the circadian clock which is extremely robust against external cues (Nakajima *et al.* 2005). Monocotyledons on the other hand seem to respond only to air temperature - they do not seem to possess an endogenous rhythm and they don't withhold their growth potential if conditions are favourable (Fig. II-7).

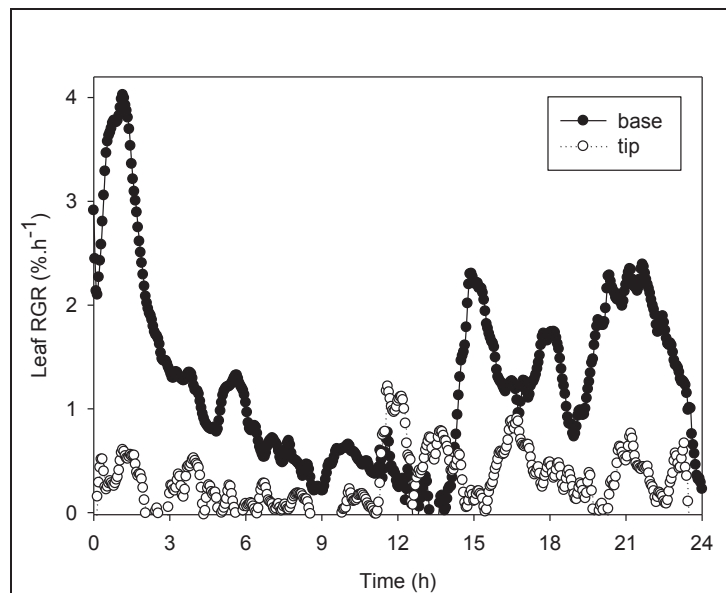


Fig. C: relative growth rate of *Flaveria bidentis* leaf measured at base and tip.

Soybean, a dicot was reported to be lacking a base-tip growth gradient (Ainsworth *et al.* 2005) and our study was able to identify that *Flaveria bidentis* has a very steep base-tip growth gradient which reminds more of a monocotyledons growth pattern. The growth is on a daily average 5 times greater at the base than at the tip of the leaf (Fig. C). This makes the fixation for Digital Image Sequence Processing (DISP) measurement more complex to follow growth rates over several days.

The simplest leaves are flat, providing the highest light interception per unit area when the leaf blade is facing the light source. As the sun's position fluctuates during the day some leaves have beneficially evolved complex architectures thereby minimising self-shading and establishing complex 3D canopy structures (Lecoeur *et al.* 2010). Differential growth rates of the layers that compose a leaf or flower induce a growth shift that is no longer in the plane but a more complex 3D shape.

In literature, the shift from 2D to 3D was studied at the biophysical level to find out where and when this singularity occurs leading to a complex 3D leaf (Prusinkiewicz & Barbier de Reuille 2010). It is shown there, that one can fully explain most complex leaf architecture using only differential growth rates among cell layers and physical properties. The complexity of leaf structure can make the estimation of leaf area more difficult. While it is relatively easy to estimate the area of a flat leaf, uneven 3D elements are harder to measure accurately as the error increases when calculating the leaf growth rate. While it is relatively simple to measure linear extension of a monocot leaf using a ruler, a Rotation Resistance Transducer (RRT) or a Linear Variable Differential Transformer (LVDT) the measurement of complex leaf require either leaf fixation (leaf DISP) (Walter *et al.* 2002a) or 3D imaging (Biskup *et al.* 2007).

PHOTOSYNTHESIS TYPE

Light is at the base of the energy chain in the plant. Photosynthesis transforms light energy to chemically bound energy. As photosynthesis provide the carbohydrates, the elementary structural brick of the plant, its efficiency is critical for the whole-plant growth performance.

We can distinguish at least three main types of carbon fixation as well as intermediate types. C₃ plants possess a photosynthesis pathway that uses RuBisCO and a three carbons compound as the first intermediate in the carbon fixation as in the following reaction: $\text{CO}_2 + \text{RuBP} \rightarrow (2) \text{ 3-phosphoglycerate}$. C₃ plants perform well under low VPD conditions but the RuBisCO enzyme tend to be wasteful under high VPD when water is limiting, temperature high or low CO₂ levels. C₃ photosynthesis has been modelled (Farquhar *et al.* 1980). C₃ plants are transpiring up to 97% of the water they are collecting through with their root system making them very resource expensive in arid countries. C₄ plants photosynthesis is not relying on RuBisCO for first step of the fixation of CO₂. C₄ plants are fixing CO₂ as a four carbons compound (oxaloacetate or malate). This makes C₄ plants more efficient in high VPD scenario as they do not bear the drawback of photorespiration and excessive water loss. CAM (Crassulacean Acid Metabolism) plants are mostly succulent plants and are well adapted to arid conditions. They make the best use of limited resources and unlike C₃ plants are much more efficient under high VPD while consuming little water.

Some plants are able to switch from C₃ to CAM metabolism depending on the climatic conditions (Walter *et al.* 2008). This makes C₃-CAM plants a great model to study specific features of those photosynthesis mechanisms. *Flaveria bidentis* as C₄ dicot (*Flaveria* has also members in C₃ and C₃-C₄ types) is of great interest to study the evolution and the shift from C₃ to C₄ photosynthesis.

We hypothesised that photosynthesis type may have a role in the relationship between carbon availability, temperature and diel growth pattern. This was investigated on various plant species, either C₄ monocot or C₃ dicot.

We observed larges fluctuations in the pool of carbohydrates of both C₃ and C₄ members, monocot and dicot types (Fig. II-5). While all investigated species had variations in starch levels over 24 h, this trend was less clear for fructose and sucrose. This can indicate a different metabolism or the usage of another carbohydrate as storage and transport form of carbon.

Flaveria bidentis as a C₄ dicot helped disentangling whether growth pattern is inherited from the meristem position or the photosynthesis type. *Flaveria bidentis* leaf growth response to temperature was of a dicot type and we couldn't identify any property that could be related to the photosynthesis type. The position of the meristem seems to play a much more important role in the phasing of the shoot growth than the photosynthesis type (Fig. II-3). The existence of intermediate and cross type species (e.g. *Flaveria bidentis* and *Clusia minor*) allows testing those hypotheses.

We investigated the effect of time of day application of light in chrysanthemum plants (Kjaer *et al.* 2010). The light supply was designed to provide the same quantity of light but distributed over different photoperiods. We could measure that if the DLI is constant then the daily growth will remain the same. We could also identify a relationship between the quantity of light that a chrysanthemum plant can assimilate and the night temperature. For a given DLI we measured dramatic photosynthetic efficiency decreases when plants were exposed to LNT of 12°C compared to higher night temperature (Fig. III-6 & Fig. III-7). If temperature is comparable the effect of the distribution of light is negligible. This opens new opportunities in term of light supply in agricultural practices. Chrysanthemums are able to make use of an extended photoperiod. It is thinkable to provide additional light, either by extending the photoperiod or even by adding a light phase in the middle of the night if economically rentable. The dependencies between the photosynthesis efficiency and the circadian clock have been established

(Dodd *et al.* 2005) nevertheless photosynthesis remains mainly driven by the available light energy. This variation in carbohydrate supply translates into whole plant effects which determine the plant performance (Sulpice *et al.* 2009).

We observed that when *Ricinus communis* is exposed to low RZT it induced an accumulation glucose and fructose content of growing leaves, with values increased by a factor of four, whereas sucrose and starch concentrations were increased by a factor of two to three (Fig I-6). Roots contained negligible amounts of starch either during or after root cooling; for sucrose, fructose and glucose, lower concentrations compared with leaf samples were found, with much higher concentrations in treated plants.

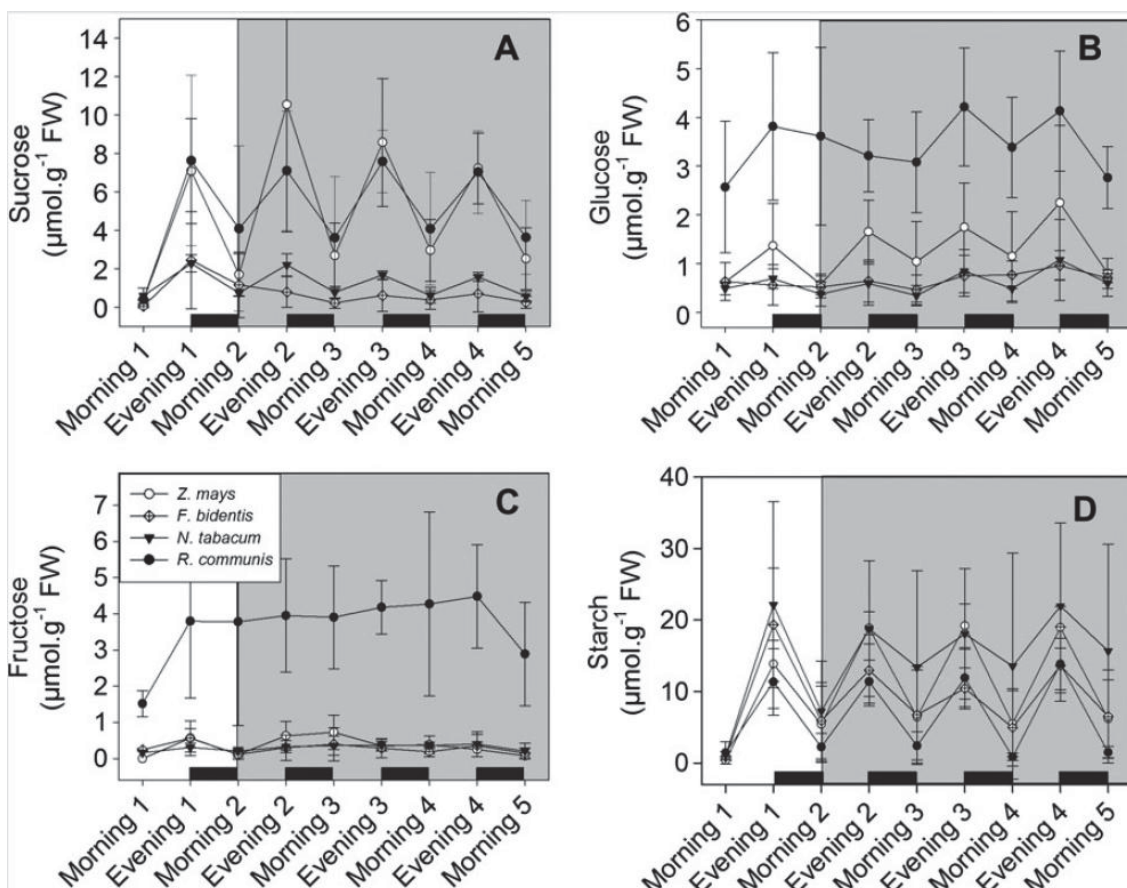


Fig. II-5: Carbohydrate concentrations of growing leaves. (A) Sucrose, (B) glucose, (C) fructose, and (D) starch. During day 1, plants were exposed to constant temperature, thereafter (grey background) temperature fluctuated as shown in Fig. 2. Mean value and SD (n=4).

We observed for all species we investigated large fluctuations in the carbohydrate pool over the day in response to the light supply and temperature. Photosynthesis has been identified as the main limitation to further yield increase on modern cultivars (Zhu *et al.* 2010). This opens the way to new approaches aiming toward the engineering of new plants with more efficient photosynthesis. The greatest challenge at the moment is to implement a C_4 photosynthesis apparatus into a C_3 organism like rice (Sage 2004). Such initiative will insure food safety in future.

TRANSPORT

CARBOHYDRATES

Carbohydrates are the elementary bricks of the plant structural framework. In order to optimise plant growth it is necessary to understand metabolic processes underlying carbon fixation and remobilisation. This knowledge is required to explain why a species or genotype is more efficient than another under given climatic conditions. Current technology allows accurate monitoring of leaf photosynthesis rate *in situ* (Li-6400; Li-Cor Biosciences GmbH, Bad Homburg, Germany) (Long & Bernacchi 2003) but it is still not easy to determine how the photosynthesis products are allocated within the plant. The tissue growth is related to the available carbohydrates so the understanding of the transport dynamics is needed if we want to engineer plants with better performances under degraded environmental conditions.

By destructive sampling we observed that lower RZT induced shoot growth reduction associated with an accumulation of carbohydrates (starch and soluble sugars as Fructose, Glucose and Sucrose) in both shoot and root (Fig. I-6).

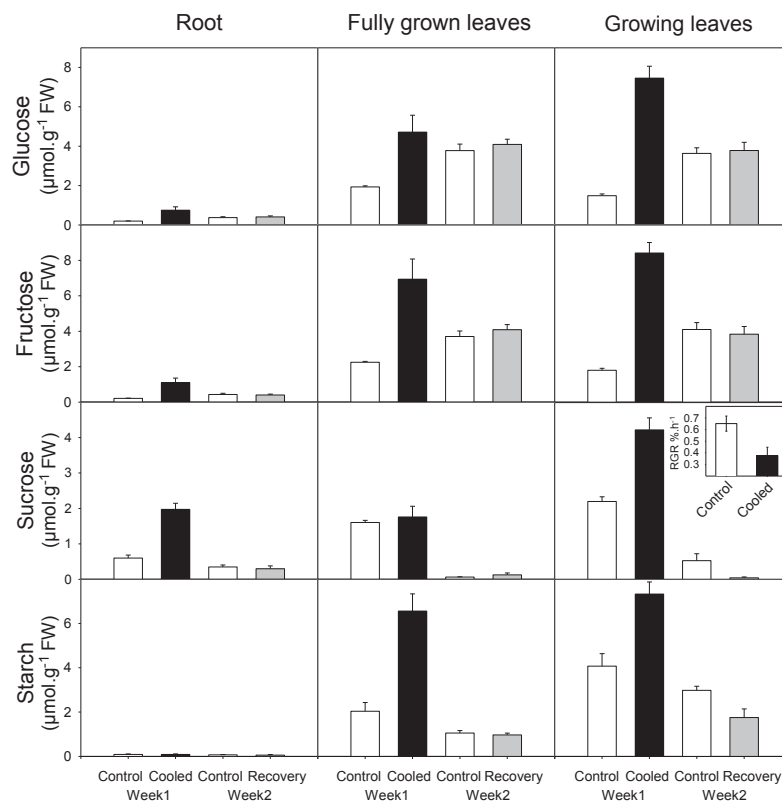
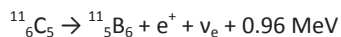


Fig. I-6: Starch and soluble sugar content of tissues from two populations of *Ricinus communis* plants exposed either to constant root-zone temperature throughout the experiment (control, T = 20°C) or to 5 d of cold root-zone temperature (cooled, T = 10°C) followed by a return to control temperature (recovery, T = 20°C). *n* = 6-9, mean value and standard error. The inset shows relative growth rate (RGR) of the total leaf area of the two populations during cold root-zone temperature (*n* = 10, mean value and SE).

By sampling plant tissues at specific time points we can observe in all the investigated species a regular pattern of the carbohydrate concentrations, essentially starch, over a 24 h period (Fig. II-5 & Fig. III-5). The carbohydrate concentrations of a plant directly reflect the metabolic status of plant responses to climatic cues. Carbohydrates are accumulated over the day and processed at night to sustain various metabolic outputs including tissue growth. Altered carbohydrate metabolism, in turn, leads to instantaneous and often very specific growth reactions in leaves (Sulpice *et al.* 2009, Smith & Stitt 2007).

The ability of a plant to grow vegetatively, intercept light and perform photosynthesis is only the first step of the strategy to reach the maximum potential. In agronomy, Harvest Index (HI) is not about total biomass but related to the fraction of carbohydrate which is invested into the seeds. HI results from the biological efficiency of a plant in both fixation and translocation of carbon, thus transport mechanisms are to a large part explaining the variability observed between different genotypes. Short lived isotopes can be used to monitor the carbohydrate flow non-invasively (Minchin & Thorpe 2003). They are especially well suited to measure the dynamics of transport mechanisms under fluctuating cues. The carbon isotope ^{11}C requires a complex setup and a cyclotron but allows monitoring the allocation of freshly assimilated photosynthesis products into the phloem stream. ^{11}C is decaying as follows:



The half-life of ^{11}C is 20.38 min allowing around 2 hours of continuous monitoring before the activity level becomes too low to be measured accurately. ^{11}C experiments performed with castor bean show a preferential carbon allocation to the shoot when the roots are cooled down to 10°C (Poiré *et al.* 2010). The carbon flow can be reverted quickly if soil temperature is set back to higher temperature (20°C) (Fig. I-8). We hypothesise that the transport reduction is caused by combination of differential sap loading and changes in the water fluxes along the stem.

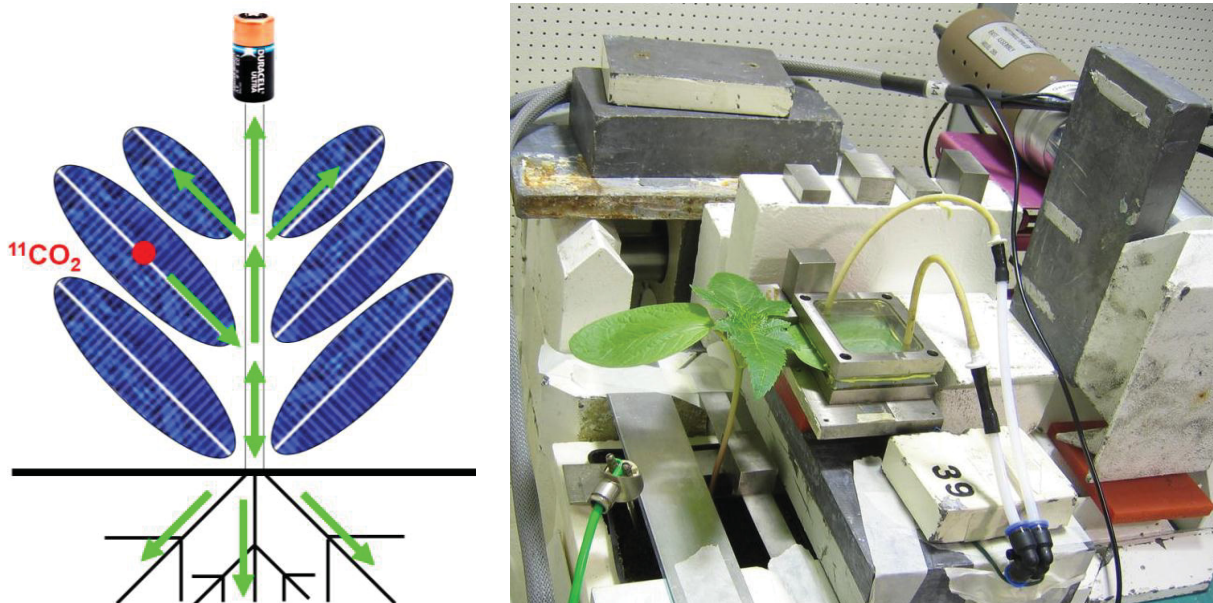


Fig. D: Schematic overview of the ^{11}C experiment principle with flow of photosynthesis products (left) and photograph of the experimental setup (right).

WATER

Water availability remains an issue in many countries and current breeding efforts focus on the increase of the Water Use Efficiency (WUE) (Sinclair *et al.* 1984). In future we will have to sustain higher crop production level and the global warming we are experiencing means that plants will be exposed to dryer conditions than it is currently the case. Plants are usually responding to soil drought by closing their stomata and limiting further water losses (Holbrook *et al.* 2002). Drought has dramatic effects on plant performance (Davies & Zhang 1991). When water is limited plants cannot regulate leaf temperature causing the overall photosynthesis efficiency to decrease (Sharkey & Zhang 2010). This effect is transient if the stress is moderate and short but it can induce ROS production if the stress is sustained. It is crucial to measure how water limitation is perceived by the plant to understand what mechanisms have to be improved to breed next generation crops.

Different methods are available to measure the water status of a plant but when it comes to the analysis of the dynamics of the plant reaction to a short stress event then the pressure probe is a powerful tool (Zimmermann *et al.* 2004). Water pressure is an important driver of plant growth and the pressure probe can establish a connection between the reduction of water availability and growth. Water deficit experiments have to be carried out carefully as a proper and controlled drought environment is complex to establish and characterise.

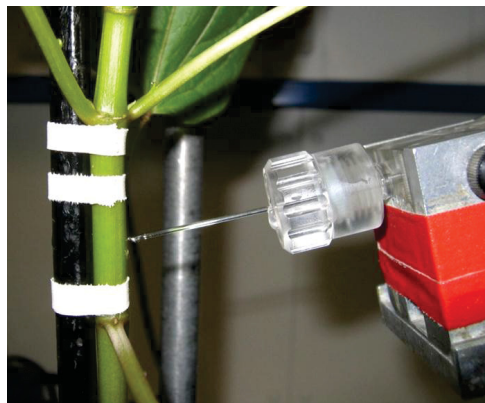


Fig. E: close-up view of the entry point of the pressure probe on a *Ricinus communis* stem.

Pressure probes allow a minimally invasive procedure capable of monitoring the hydrostatic pressure in the xylem by establishing a continuum between the xylem vessels, a glass capillary filled with degassed water and a pressure gauge (Fig. E). This technique allows real time monitoring of hydrostatic pressure profile when the plant is exposed to variation in the water availability or climatic conditions.

We measured no reduction in transpiration rate when plants were exposed to low RZT (Fig. I-7a) but with the pressure probe we were able to measure a fast decrease in the hydrostatic tension induced by the reduction of soil temperature from 20 to 10°C (Fig. I-7b). The reduction of the hydrostatic tension was in this case associated with a

$$\frac{dV}{dtV} = \phi(P - Y)$$

V: cell volume
Φ: extensibility
P: turgor pressure
Y: Yield threshold

reduction of the growth level in both shoot and roots supporting the theory that turgor pressure is the main driver of plant growth (Lockhart 1965). One advantage of this technique is that it allows measuring a change in pressure long before it would be possible to measure a decrease in leaf growth rate. This ability to measure the effect of

short term events can be crucial to understand the dynamic changes of transport processes when a plant is exposed to various climatic conditions.

In an attempt to increase our analysis throughput, we explored the use of an open microwave resonator to estimate biomass and water content of chrysanthemum tissues. The technique is relatively new and in future it has the potential to batch process large number of biological samples. Magnetic sensors are being developed to indirectly estimate the turgor pressure non-invasively. Those Leaf Patch Clamp pressure Probes (LPCP) are less invasive than the regular pressure probe, thereby theoretically allowing high throughput analyses (Zimmermann *et al.* 2008).

Plant structural stiffness is realised by its inner turgor pressure acting against a network of rigidly connected fibres. The composite nature of plant tissues is largely determined by the organisation of these fibrous networks, which are composed of an inner, soft and extensible part and an outer layer which is flexible but non-elastic. Taken individually those two materials cannot sustain the weight of a plant and would simply collapse but coupled together and adding only turgor pressure, a composite material is obtained that offers amazing structural properties (Vandiver & Goriely 2008).

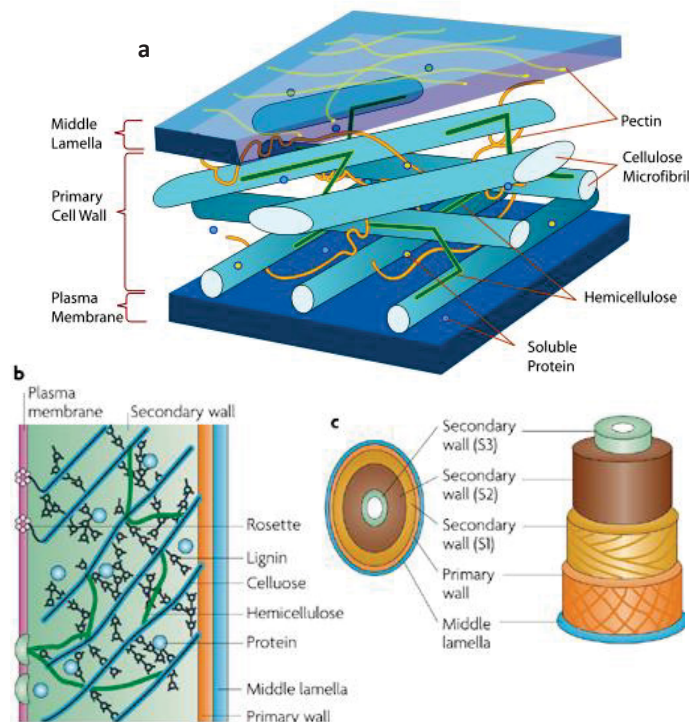


Fig. F: Plant plasma membrane and cell-wall structure. **a** | Cell wall containing cellulose microfibrils, hemicellulose, pectin, lignin and soluble proteins. **b** | Cellulose synthase enzymes are in the form of rosette complexes, which float in the plasma membrane. **c** | Lignification occurs in the S1, S2 and S3 layers of the cell wall (Sticklen 2008).

Hydraulic forces allow maintaining plant structure and are the main driver of plant growth. Thus it is essential for the plant to maintain an inner pressure that is able to sustain photosynthesis, structural stiffness and the hydraulic component of leaf growth. This also explains the drivers of the hydraulic theory of plant growth. The pressure needs to overcome the resistance of cell walls to achieve growth (Lockhart 1965). According to the Lockhart equation, cell growth can be improved either by acting on the turgor pressure (aquaporin) or by altering the composition of cell walls to improve extensibility and yield threshold.

PLANT GROWTH

NON-INVASIVE METHODS

Monocotyledonous leaf growth proceeds mainly in length making measurement relatively straightforward. A ruler can provide a good estimate of the leaf area analysed on a day to day basis. One can increase the temporal resolution by using Rotational Resistance Transducers (RRT) and strings attached to the tip of the leaf. This technique can be used with multiple devices side by side to provide high throughput analysis of large populations of monocots (Sadok *et al.* 2007). This technique was used successfully also in this thesis on maize and rice (Fig. II-6c and Fig. II-6d).

Dicotyledon plant leaves are not only growing in length but also in width. Therefore, imaging techniques are required to monitor their growth processes. For this purpose we used an industrial camera mounted above the plant canopy. The camera was able to record time lapses movies of one growing leaf of over several days. This technique has few limitations inherent to its nature. It is required that the leaf grows in the focal plane of the camera and remains within it at all time during the acquisition. The leaf was fixed using six nylon threads attached to the edge of the leaf and spun over a ring encasing the leaf. The threads were tensioned by weights and provided the elastic fixation needed for the image acquisition. The choice of the weights was very important to be able to maintain the leaf in place without disturbing the growth rhythm. The appropriate weight has been determined for each species we monitored with leaf DISP.



Fig. G: Photograph of a *Ricinus communis* leaf fixed on a ring to allow leaf DISP image acquisition.

DISP imaging requires that the camera remains unaffected by the visible light provided to the plant in order to monitor growth during night and days. This is achieved by using Near Infrared (NIR) interference filter in front of the camera and using NIR Light Emitting Diodes (LED) matched with the wavelength of the filters as illumination source. The combined use of LED and filter provides a consistent brightness level to the camera regardless of the PAR supplied to the plant.

DISP technique is very versatile and can monitor growth rate fluctuations for a broad range of species. DISP was the main technique used to investigate growth in dicot species of this study (Fig. I-4, II-3 & III-3).

The image acquisition frame rate is dependent of multiple factors such as the focal length of the lens, the distance to the object but also the timescale of the event to monitor. In this study, pictures were acquired at a rate of one every 180 s as it focused on growth dynamics changes occurring during minutes and hours after the application of a climatic event. The technique allows using frame rates as low as one image per second to measure growth rate changes induced by swift events such as light pulses or osmotic stress.

The DISP processing algorithm is based on the Brightness Constancy Constrain Equation (BCCE) (Schmundt *et al.* 1998) and assumes that the grey value of a pixel of an image remains the same over the whole sequence. Processing a stack of images provides a displacement vector for each pixel of the image and allows calculating RGR of an area of interest. DISP allows grasping the smallest variations of growth rates of either shoot (Walter *et al.* 2002a) or root (Walter *et al.* 2002b; Nagel *et al.* 2009) by processing time lapse movies of the growing tissues.

Leaf growth can also be measured using simpler experimental design for the image acquisition. The alternative method requires a digital Single Lens Reflex (SLR) camera placed above the canopy. This method is suited for the estimation of projected leaf area of rosette plants on a day to day basis (unpublished data from drought experiment performed in the course of this thesis in Montpellier in 2006) and allows to identify growth rate reductions from tobacco plants grown with five different soil water contents (18, 21, 25, 30, 40%). The SLR method loses some temporal resolution compared to the leaf DISP but canopy does not need to be constrained anymore. The use of multiples cameras or multiple views allows 3D reconstruction of canopy (Biskup *et al.* 2007). Compared to manual measurement this greatly increases the throughput at which plants can be measured. The development of custom-made software allows fast thresholding of green pixels in the image and estimating the projected leaf area of each plant. This setup brings high resolution imaging techniques directly in the field and greenhouse to measure differences in canopy development.

ECONOMICAL IMPACT - OUTLOOK

HORTICULTURE

In horticulture there is a need to optimise plant growth conditions while maintaining the production costs as low as possible. Ultimately product quality is determined not only by the raw biomass but also the fitness and aesthetic value of the plant. Fertilisers were a main component of the green revolution but their extensive uses makes gain potential very limited now. The increasing need for sustainability should lead to reduce the use of fertilisers and pesticides by breeding plant lines that make a better use of the available resources. The energy needed to grow horticultural plants is a major production cost and we need to find ways to minimise them. Cost reduction can be achieved in different ways, for instance by making use of power plant by-products like CO₂ and the heat generated. This allows improving plant growth conditions at low cost. Renewable energies are explored to minimise the energetic footprint of greenhouses.

In Denmark where about 75% of the overall electricity production is provided by windmills the prices per kW are set according to weather forecasts and broadcasted 24 h in advance. Breeders can then decide to make the best use of this cheap energy to supplement their plants with more heat or light (Aaslyng *et al.* 2003). This has the effect of speeding up all plant metabolic reactions associated with biomass production and goes toward the ultimate goal of achieving a more sustainable production, faster while remaining cheap. This can mean for the plant an extra light phase in place of the putative night. Beyond the obvious effect on the internal rhythm of the plant we have to make sure that the health of the plant is overall maintained and also that the final horticultural product remains aesthetic and with a consistent and predictable flowering time. This also means that on the days where energy is costly they may have to reduce the use of it by reducing the temperature in their greenhouses. The experiments performed in the course of this thesis (Kjaer *et al.* 2010) have helped to improve future horticultural management practices by exploring the connections between growth of a horticultural crop, temperature and light intensity.

LINKING LAB-BASED PHENOTYPING TO FIELD-BASED BREEDING

Phenomics approaches and the use of High Throughput (HT) screens are a novel way to study plant performance under diverse climate conditions (Furbank 2009). Those phenotyping techniques, essentially based on non-invasive tools are able to handle large population of plants and provide physiologically relevant parameters. Those non-invasive techniques can include high resolution imaging at various wavelengths but are not limited to it. The power of those tools is coming mainly from the large number of plants they can process in a reasonable time opening the way to new scientific approaches and selection screens. New HT screens need validation from higher resolution methods to assess for the physiological relevance of the parameters measured. We need to improve the characterisation and standardisation of climate parameters to compare and reproduce studies carried out in different laboratories (Massonnet *et al.* 2010). Lab-based phenotyping must be done carefully to insure that the growth conditions are not too biased compared to the field conditions (Passioura 2006).

The recent advances in the fast determination of plant area as proxy for biomass allowing batch processing of a large amount of plants in a short time. This allows some new possibilities of HT screening of plants based on pictures taken from different angles in Red, Green and Blue (RGB) colour space (GROWSCREEN: Forschungszentrum Jülich ICG-3; Scanalyzer 3D: Lemnatec; PlantScan: High Resolution Plant Phenomics Centre). Those imaging techniques can then be extended to other wavelengths and thus providing insights not only on the

area but also on the physiological status of the plant. The UV spectrum is rich of information related to the photosynthesis state of a plant. (FLUORO GROW SCREEN: Forschungszentrum Jülich ICG-3; TrayScan: High Resolution Plant Phenomics Centre). Ultimately any physiological advantage will result into an increase in biomass and harvest index and this can also be assessed non-invasively using emerging HT methods like microwave resonators (Menzel *et al.* 2009). Plant 3D architecture is still a limitation for high throughput approaches. There is a need to improve real 3D acquisition platforms to account for the complex architecture of plants with the help of multiple views reconstruction, X-Ray Computed Tomography (XRCT) or Light Detection And Ranging (LIDAR). More phenomics platforms are becoming available and it is necessary to implement them in breeding program to improve future crops in term of PUE and WUE (Richards *et al.* 2010).

Canopy architecture can be complex to characterise when plants are growing at high density but attempts have been made to characterise it for modelling purposes. L-system is a “parallel rewriting system” suited to reproduce patterns found in nature from spiral, Fibonacci sequences to the golden ratio (Prusinkiewicz & Barbier de Reuille 2010). Joint efforts on both phenotyping and biophysical modelling (unlike statistical models) can account for non-linear interactions between plant and environment. Modelling approaches together with good parameterisation can reveal complex developmental strategies adapted to specific environment (Lecoeur *et al.* 2010). Modelling simulations have the potential to bridge the gap between lab based observations to the field (Ma *et al.* 2007, Ma *et al.* 2008).

There is a need to improve our understanding of plant physiology at high resolution to establish high throughput screens that will contribute solving the agricultural challenges mankind will be facing in the future.

RESEARCH ARTICLE #1

Richard Poiré, Heike Schneider, Michael R. Thorpe, Arnd J. Kuhn, Ulrich Schurr & Achim Walter (2010) Root cooling strongly affects diel leaf growth dynamics, water and carbohydrate relations in *Ricinus communis*. *Plant, Cell & Environment* **33**: 408-417

Root cooling strongly affects diel leaf growth dynamics, water and carbohydrate relations in *Ricinus communis*

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ABSTRACT

In laboratory and greenhouse experiments with potted plants, shoots and roots are exposed to temperature regimes throughout a 24 h (diel) cycle that can differ strongly from the regime under which these plants have evolved. In the field, roots are often exposed to lower temperatures than shoots. When the root-zone temperature in *Ricinus communis* was decreased below a threshold value, leaf growth occurred preferentially at night and was strongly inhibited during the day. Overall, leaf expansion, shoot biomass growth, root elongation and ramification decreased rapidly, carbon fluxes from shoot to root were diminished and carbohydrate contents of both root and shoot increased. Further, transpiration rate was not affected, yet hydrostatic tensions in shoot xylem increased. When root temperature was increased again, xylem tension reduced, leaf growth recovered rapidly, carbon fluxes from shoot to root increased, and carbohydrate pools were depleted. We hypothesize that the decreased uptake of water in cool roots diminishes the growth potential of the entire plant – especially diurnally, when the growing leaf loses water via transpiration. As a consequence, leaf growth and metabolite concentrations can vary enormously, depending on root-zone temperature and its heterogeneity inside pots.

Key-words: ^{14}C ; biomass; image analysis; root growth; starch; sucrose; transpiration.

INTRODUCTION

The immediate environment of growing roots and leaves differs strongly in temperature, light, humidity and other environmental factors to which the growing organs are exposed. For example, soil temperature, like air temperature, has sinusoidal oscillations on a diel (24 h) scale (Hillel 1998). However, depending on soil depth and moisture, changes in soil temperature are delayed and lower in amplitude than the atmospheric temperature variation. In a typical diel pattern (shown in Walter, Silk & Schurr 2009), atmospheric temperature varies by 16 K and reaches a

maximum around 1400 h, whereas soil temperature at 10 cm depth varies by merely 3 K and reaches a maximum around 1600 h. Soil temperature variation is even more damped at greater depth and the diel average temperature to which shoot and root are exposed can differ strongly, depending on weather and season. The importance for the plant of different temperatures at root and shoot has been analysed in relatively few studies throughout the last decade. Yet, in studies that did expose root and shoot to different temperatures, a clear influence of root temperature on leaf growth and on partitioning of biomass between roots and leaves has been reported (Watts 1972; Davies & Van Volkenburgh 1983; DeLucia, Heckathorn & Day 1992; Engels 1994).

Most greenhouse and growth chamber experiments use plants cultivated in relatively small, black containers although a small rooting volume can strongly hamper shoot growth (Thomas & Strain 1991). Moreover, it has to be expected that roots experience uncommon temperature regimes there and that this may affect shoot growth. In a study with soybean it has been shown for example that watering the plants with cold water (15 °C instead of 25 °C for control plants) leads to a 10% growth decrease (Passioura 2006). Yet, more often, root temperature in pot experiments will be higher than in the field because of radiative heating of the black pots. Increased root temperature can also decelerate leaf growth because of strongly increased root respiration and other factors, which was demonstrated in a study on the C_4 -grass *Andropogon gerardii* (DeLucia *et al.* 1992) that had optimal leaf growth at a root temperature of 25 °C. At low root temperature, it is well known that root water uptake is reduced because of decreased transmembrane transport (Markhart *et al.* 1979), which can lead to wilting (Bloom *et al.* 2004) or other symptoms resembling salt stress that are induced by the decreased shoot water availability (Davies & Van Volkenburgh 1983).

From experiments where the leaf growth zone temperature has been controlled along with root temperature, it seems that the close proximity of monocot leaf meristems to the soil leads to a strong influence of soil temperature on monocot leaf processes (DeLucia *et al.* 1992; Engels 1994). In fact, for maize (Watts 1972), leaf extension rate was proportional to the root temperature when the meristem temperature was not controlled, but when meristem

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temperature was actively kept constant, leaf extension rate was constant too, regardless of the soil temperature. In dicot plants, it is much less clear, how root temperature affects the dynamics of leaf growth, which are governed to a much higher extent by intrinsic temporal patterns probably regulated by the circadian clock (Walter *et al.* 2009). Experiments exposing the root system of *Phaseolus* to a constantly low temperature throughout 24 h indicate that leaf growth is inhibited during the day but not at night (Davies & Van Volkenburgh 1983). Such a daytime leaf growth inhibition seemed to prevail even when root temperature was increased for 8 h within a 12 h day (Ainsworth, Walter & Schurr 2005).

As carbohydrates regulate leaf growth in a diel pattern (Smith & Stitt 2007; Wiese *et al.* 2007; Sulpice *et al.* 2009), it is important to learn how carbohydrate concentrations react to altered root temperature. Presumably, shoot photosynthesis will not be directly affected by cooling the root. Yet, shoot carbohydrate would be likely to increase if the root's demand was reduced, leading to end-product inhibition. As the diel leaf growth cycle in dicot plants is strongly affected by the interaction of water relations and carbohydrate metabolism (Walter *et al.* 2009), it is important to perform physiological experiments taking all of these aspects into account.

Hence, it was the aim of this study to investigate how cooling the root system throughout the diel cycle affects leaf growth dynamics in the dicot plant *Ricinus communis*. Leaf growth analyses were complemented by investigations of water relations, carbon translocation and carbohydrate concentrations that are a vital part of the physiological framework facilitating the diel leaf growth cycle. *Ricinus communis* was selected as an experimental system as, in contrast to rosette plants, leaves and roots are separated widely enough to independently control their temperature, and as leaf growth dynamics, water relations and carbohydrate metabolism have been analysed intensively in this plant species.

MATERIALS AND METHODS

Plant material

Ricinus communis L. (ecotype Carmencita rot) plants were germinated and grown in a greenhouse for 10 d. The plants were then translocated to the growth chamber some days before the experiment started (usually 14 d after germination). Plants were grown in 40 pots (1 L) filled with a pre-fertilized soil (ED73, Balster Einheitserdewerk, Fröndenber, Germany). During germination and for the duration of the experiment soil water content was kept at optimal level.

When these pots were exposed to a temperature change in a preliminary experiment, temperature inside the pot acclimated to a new steady-state value within 1 to 4 h, depending on the exact location within the pot (Fig. 1). In this experiment, a soil-filled pot was cooled down overnight to 5 °C and then placed in a climate chamber

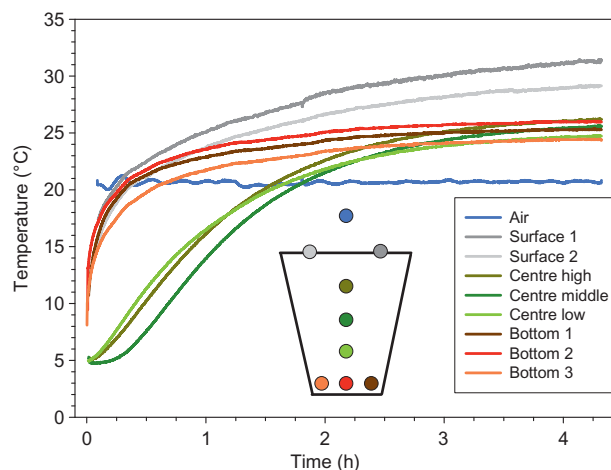


Figure 1. Temperature at nine different locations of a soil-filled pot. The pot was translocated from a cold room (5 °C) to a climate chamber. Exact locations of temperature analysis are indicated in the scheme.

(air temperature = 20 °C, relative humidity = 60%, light intensity = 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation). At each position within the pot, a temperature higher than air temperature was reached within 15 to 100 min. At the bottom of the pot, steady-state temperature was reached fastest, exceeding air temperature by 3 to 5 °C. At the top, temperature still increased markedly after 4 h of incubation in the new temperature regime and it exceeded air temperature already by 10 K at that time.

Experimental schedule and environmental parameters

The entire study consists of a suite of four subsequent experiments with different root temperature treatments. In each of these experiments, different parameters were monitored. In each of these experiments, air temperature was kept at 22 °C in the growth chamber and the light intensity measured with a quantum light sensor (LI-190SB, Li-Cor, Lincoln, NE, USA) was 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 12 h a day. Photosynthetic active radiation, ultraviolet (UV)-A and UV-B were measured using an optometer (X1₂, Gigahertz-Optik GmbH, Puchheim, Germany). The light was provided by an array of fluorescent lamps. The root cooling system consisted of an insulated chassis containing a heat exchanger. Coolant was pumped through the heat exchanger and thus lowered temperature inside the box compared with the outside. Several apertures were made in the upper side of this box, allowing to place the pots inside. The cooling pump allowed controlling of coolant temperature and it took about 30 min to reach the desired temperature within the entire soil volume of the pot (Füllner 2007). Up to four cooling boxes (each containing maximally 10 plants) were used for these experiments.

In the first experiment, root temperature was kept constant for 19 d with three different treatments (10, 15 and

20 °C). Leaf growth and shoot biomass was monitored. In experiment two, root temperature was modified in each cooling box every 4 d. In box A, roots were first exposed to 20, then to 15, then to 10 °C for 4 d, respectively. In box B, roots were first exposed to 15, then to 10 and then to 20 °C. In box C, roots were first exposed to 10, then to 20 and then to 15 °C. In this experiment, leaf growth and diel leaf growth cycles were analysed. In experiment three and four, only the two extreme root temperature treatments were applied, as experiments one and two showed intermediate results for the intermediate temperature treatment. In experiment three, roots were exposed to 20 °C for 7 d, then to 10 °C from day 8 to day 12 and again to 20 °C thereafter. Here, leaf growth, root growth, carbohydrate concentrations, transpiration rate and xylem pressure were analysed. In experiment four, roots were exposed to 20 °C except for a 24 h period, during which they were exposed to 10 °C. Here, carbon partitioning was analysed.

Ten thermocouples (type T, cooper/constantan and 0.5 mm in diameter) were used (three on the meristems, six in the soil, and one measuring air temperature at canopy level) to record temperature. All temperature data were recorded using a laptop PC and signal interfaces (cFP-1804; cFP-TC-120; cFP-CB-3, National Instrument, Austin, TX, USA). Data were pooled every second and an averaged value recorded every 10 s using a Labview-based (National Instrument) program. Air temperature and air relative humidity were also recorded every 10 min using an independent data logger (Testo 175-H2, Testo AG, Lenzkirch, Germany).

Leaf growth

The length and width of initiated leaves were manually measured every other day during experiment one. Rank specific leaf form factors (0.78 for cotyledon, 0.72 for primary leaves and 0.79 for the following leaves) were used to calculate leaf area from leaf dimensions (length × width × form factor = area). For diel leaf growth assessment, primary leaves were mechanically fixed in a stationary position (compare Walter, Feil & Schurr 2002a; Fig. 1 therein) to keep growing leaves in the focal plane of the camera. Each investigated leaf was fixed at the petiole using a stripe of Parafilm (Parafilm M®, Pechiney Plastic Packaging Company, Chicago, IL, USA) and kept flat with five nylon threads, clamped to the edge of the leaf using shortened hair clips and fabric tape to protect the leaf. Each of the threads was pulled with a weight of 12 g and spun over a metal ring surrounding the leaf, a procedure that does not affect temporal or spatial growth patterns (Walter *et al.* 2002a). Leaf images were acquired with high resolution, monochrome CCD cameras (Scorpion SCOR-20SO, Point Grey Research, Vancouver, BC, Canada), positioned above the plants and equipped with a standard objective lens (25 mm; Cosmocar/Pentax; The Imaging Source, Bremen, Germany) and an infrared interference filter (880 nm; Edmund Optics, Karlsruhe, Germany). Constant illumination throughout day and night was provided by six

infrared diode clusters (880 nm; Conrad Electronics, Hirschau, Germany). Grey value images were taken every 180 s and were saved in a multi-tiff format. Image sequences were evaluated with algorithms based on a structure-tensor approach (optical flow via the brightness constancy constraint equation (BCCE) (Bigün & Granlund 1987; Schmudt *et al.* 1998) that calculates velocities from all moving visible structures at the leaf surface within the image sequence of a growing leaf. Area relative growth rates (RGRs) were calculated as the divergence of the estimated velocity field by selecting an area of interest (AOI) within the image and tracking the structure within this AOI with time. For more details, see Schmudt *et al.* (1998), Walter *et al.* (2002a) and Matsubara *et al.* (2006).

Root growth and water losses

During experiment three, 10 plants were grown with the root system and the surrounding soil (1 L) in a transparent plastic bag sealed at the base of the stem. This allowed carefully taking the bag out of the pot every second day for direct access to the roots growing at the surface of the bag. The position of each root tip was marked every other day. From this data, the increase in root length and root number was calculated. Furthermore, the pots were weighed at the beginning and end of each day and the lost water was replaced to give a direct estimate of transpired water as the increase in plant biomass was negligible (less than 1% of the weight difference caused by water loss via transpiration).

Carbohydrates

In experiment three, tissue sections of roots and leaves were sampled for carbohydrate analysis in the afternoon. Two discs per leaf (diameter: 8 mm) were punched out of full-grown cotyledon and growing primary leaves, were weighed, pooled, frozen in liquid nitrogen and stored at -80 °C for further extraction. Two pieces of root tissue were sampled from the main root (length: 1 cm; diameter: 1–2 mm), were rinsed with water, gently dried with tissue, pooled, frozen and stored at -80 °C. Soluble carbohydrates were extracted from frozen leaf material and glucose, fructose, sucrose and starch concentrations were analysed in a coupled enzyme assay (Jones, Outlaw & Lowry 1977) using a multiplate spectrophotometer (ht II; Anthos Mikrosysteme GmbH, Krefeld, Germany) as described in Walter *et al.* (2002a) and Wiese *et al.* (2007).

Carbon partitioning

The short-lived carbon isotope ¹¹C decays with a half-life of 20.4 min, resulting in the emission of photons that can be detected non-invasively, because of their high energy. For plants in experiment four, ¹¹CO₂ was applied to one cotyledon, and we monitored all of the radiolabelled photoassimilate exported from the leaf, using scintillation detectors to measure radiotracer in both shoot and root, with radiation shielding arranged to limit the regions of each plant 'seen'

by the detectors. Pairs of plants were set up in standard conditions, and after 24 h of acclimation to the shielding and detection setup, with root and shoot at 20 °C, pulses of ^{14}C were applied three times on two sequential days at 0900, 1130 and 1400 h. Soil temperature of one plant of the pair remained at 20 °C, and in the other plant it was reduced to 10 °C at 1600 h on day 1, and returned to 20 °C at 1000 h on day 2. From the time-series of radiotracer in shoot and root we used transfer-function analysis with allowance for tracer decay (Minchin & Thorpe 2003), to calculate the fraction of exported tracer being transported to root and shoot, both before and after changing the soil temperature. To show the treatment effects relative to controls, the shoot/root partitioning ratio of the treated plant calculated for each tracer pulse was divided by the partitioning ratio for the control plant measured at the same time, to allow for strong developmental effects in the young plants. The shoot/root ratio for a cotyledon's photoassimilate changes with plant development in favour of the roots because the true leaves become carbon sources for shoot growth.

Xylem pressure

The xylem pressure probe consisted of a water-wettable pressure transducer mounted in a 50 μL Plexiglas chamber and sealed to a glass microcapillary that was inserted into the xylem vessel as described in more detail in Zimmermann *et al.* (2004). The plant stem was attached to a fixed metal rod, with tape above and below the insertion zone, and the microcapillary was inserted slowly into the stem using a micromanipulator. When the tension dropped suddenly, indicating that the tip had reached a xylem vessel, the microcapillary was held at that position. Pressure equilibrium between the vessel and the probe was established within a few seconds, and was usually stable for several hours, allowing a minimally invasive direct reading of the pressure of the xylem sap.

Statistical analysis

Comparisons between treatments were performed using two-tailed Student's *t*-tests (software: SPSS Sigmatat).

RESULTS

Shoot growth

In experiment one, low root temperatures strongly reduced leaf development (Fig. 2a). At day 34, plants with 20 °C root-zone temperature reached 881 cm² of total leaf area, whereas plants with root temperatures of 15 and 10 °C gave leaf areas of only 471 and 288 cm², respectively. Lower root-zone temperatures led to decreased leaf growth rates throughout the entire experiment and not only during an initial phase (Fig. 2b). As leaf area and biomass might differ in their response, we also monitored shoot biomass (Fig. 2c). The biomass difference between populations with a root-zone temperature of 10 and 20 °C is in the same

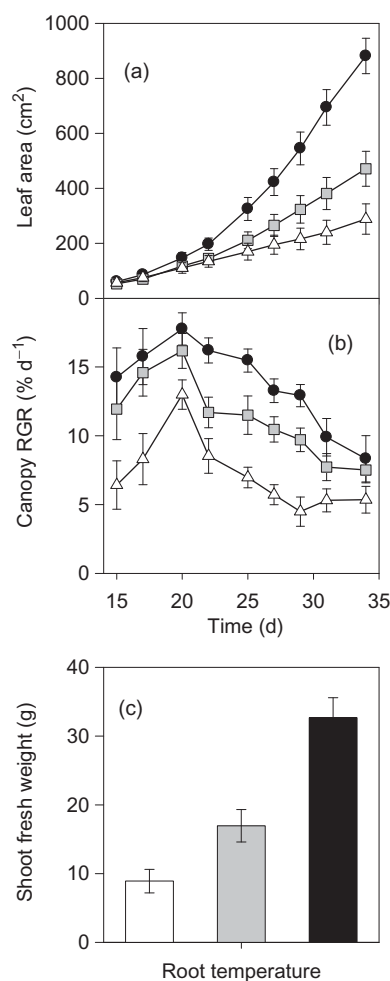


Figure 2. (a) Total leaf area of three populations of *Ricinus communis* plants exposed to three constant root-zone temperatures (10 °C white symbols, 15 °C grey symbols and 20 °C black symbols; $n = 10$; mean value and standard error). (b) Leaf relative growth rate calculated from the increase in total leaf area depicted in (a). (c) Shoot fresh weight determined at the end of the experiment.

range as the difference in leaf area: The final fresh weight of plant shoots grown at 10 °C root-zone temperature was a factor of three lower compared with the fresh weight of plants grown at 20 °C. Plants grown at a root-zone temperature of 15 °C showed intermediate fresh weights.

When plants were exposed to controlled root-zone temperatures in experiment two for 12 d and root-zone temperature was altered two times during this period, leaf growth acclimated reversibly within some days to altered temperature regimes (Fig. 3). In this experiment, the effect of root-zone temperature on leaf RGR is most clearly shown in the population exposed to 15 °C in the beginning of the experiment (Fig. 3b). Leaf RGR was initially low (7% d⁻¹) but increased to values around 17% d⁻¹, which were comparable with the values in experiment one at that developmental stage (Fig. 2b). Transfer to 10 °C root-zone temperature resulted in a rapid decrease of leaf RGR to

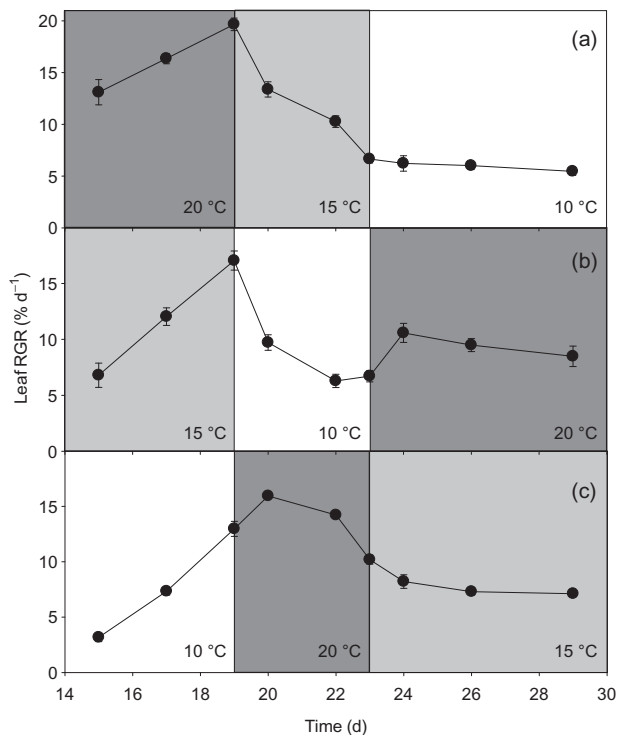


Figure 3. Relative growth rate (RGR) of the total leaf area of three populations of *Ricinus communis* plants exposed to three root-zone temperature sequences: a = 20, 15 and 10 °C; b = 15, 10 and 20 °C; c = 10, 20 and 15 °C. $n = 10$, mean value and standard error.

values around 6% d⁻¹, which were comparable with growth rates reached by the 10 °C population in experiment one. When root-zone temperature was finally set to 20 °C, leaf RGR increased again to a similar level as that of the respective population at this developmental stage in experiment one (around 11% d⁻¹, Fig. 3b). Populations exposed to other permutations of root-zone temperature (Fig. 3a,c) showed similar effects, clearly demonstrating that the inhibition of leaf growth induced by lowered root-zone temperature is reversible and can be relieved within some days. The reversibility of this effect is demonstrated by the fact that the final area of the three populations shown in Fig. 3 were almost the same at the end of the experiment (a: 276 ± 31 cm²; b: 275 ± 37 cm²; c: 248 ± 26 cm²), regardless of the root-zone temperature sequence they experienced.

Diel leaf growth variation

Because leaf growth varies strongly throughout the diel cycle, the growth of single leaves was investigated at higher temporal resolution. The general decrease of leaf growth with decreasing root-zone temperature was also obvious here (Fig. 4a–i). Compared with the diel leaf growth cycle without any control of root-zone temperature (Fig. 4k), three main effects of low root-zone temperature on diel leaf growth activity can be pointed out: firstly, diurnal (daytime)

growth activity almost vanished, independent of the exact temperature setting (Fig. 4j,k). Secondly, average nocturnal growth intensity was correlated with the selected root-zone temperature (Fig. 4k). Nocturnal average RGR decreased in the first population shown in Fig. 4a–c from the first investigated stage shown in a (root-zone temperature at 20 °C) to b (15 °C) to c (10 °C). In the second population, it decreased from d (15 °C) to e (10 °C) and increased again at the latest investigated stage to f (20 °C). Finally, in the third population, average nocturnal RGR increased from g (10 °C) to h (20 °C) and decreased to i (15 °C). Also, when average nocturnal RGR was compared between populations with developmental stage fixed, growth rates correlated with root-zone temperature: a > d > g; h > b > e, f > i > c. These results correspond very well to the results for the growth of the total leaf canopy (Fig. 2b). Thirdly, timing, amplitude and shape of the nocturnal RGR peak were not strongly affected by root-zone temperature. A nocturnal maximum RGR of approximately 1.5% h⁻¹ was reached a few hours before the onset of light, whether or not root temperatures were regulated.

Root growth

In experiment three, decreasing root-zone temperature from 20 to 10 °C showed that both the velocity of root tips and the induction of new lateral roots was strongly inhibited (Fig. 5). With 3 d cold, velocity of roots was a factor of five lower than in control plants: the main root grew by only 5 mm whereas control roots grew by 25 mm. The reduction in root growth was much stronger than that in leaf growth. Nevertheless, velocities of root tips and lateral root initiation rate never decreased to zero and they resumed when the root-zone temperature was reset to 20 °C.

Carbohydrates and water relations

In experiments three and four, several physiological parameters defining the framework of leaf growth control were monitored. Carbohydrates in roots, cotyledons (full grown) and primary (growing) leaves (Fig. 6) of plants with low root-zone temperature were all increased compared with the control plants. Strongest differences between cooled and control plants were observed for the glucose and fructose content of growing leaves, with values increased by a factor of four, whereas sucrose and starch concentrations were increased by a factor of two to three. Roots contained negligible amounts of starch either during or after root cooling; for sucrose, fructose and glucose, lower concentrations compared with leaf samples were found, with much higher concentrations in treated plants.

When cooled plants were allowed to recover in the following week, carbohydrate concentrations were comparable in control plants and formerly cooled plants in all tissues – with the exception of sucrose and starch values in

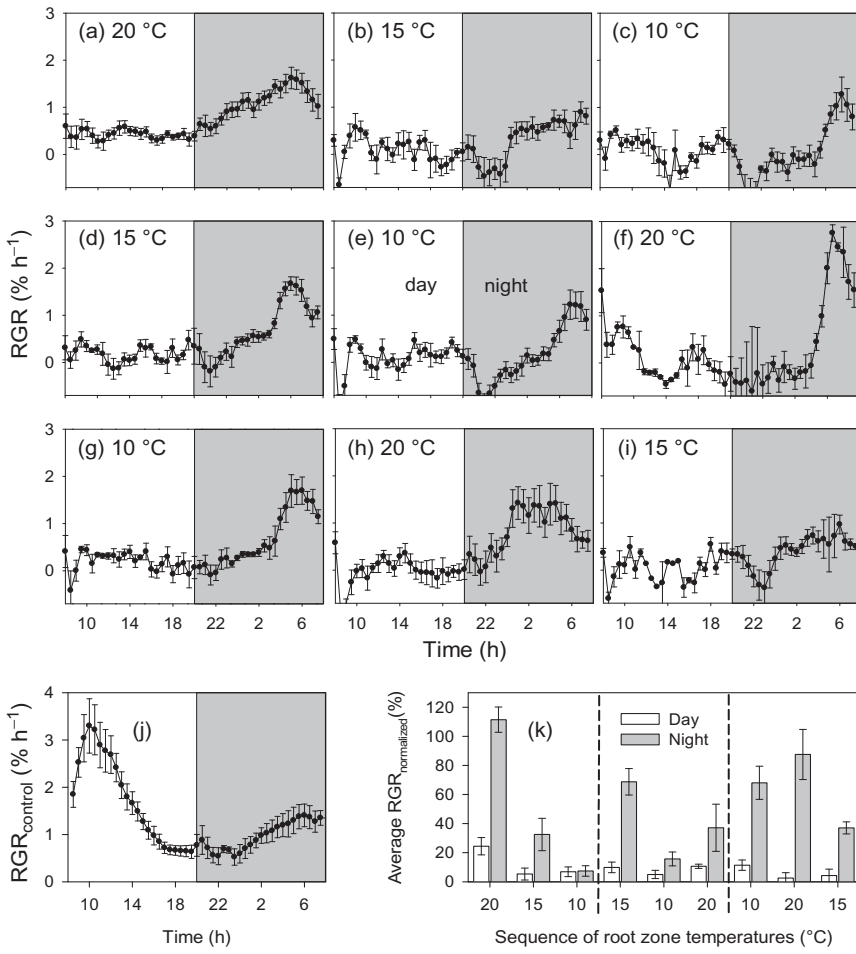


Figure 4. (a–i) Average diel cycle of leaf relative growth rate (RGR) of three populations of *Ricinus communis* plants exposed to different sequences of root-zone temperature. Population one (a–c) was exposed to 20 °C for 4 d, then to 15 °C for 4 d and then to 10 °C for another 4 d (populations two and three: root-zone temperatures as specified in panels d–i, respectively). (j) Average diel cycle of leaf RGR with unregulated root temperature ('control' plants of this experiment). (k) Normalized average diurnal and nocturnal leaf RGR from a–i. Average diurnal (or nocturnal) RGR was related to average diurnal (or nocturnal) RGR of control plants, respectively. $n = 4–12$, mean value and standard error.

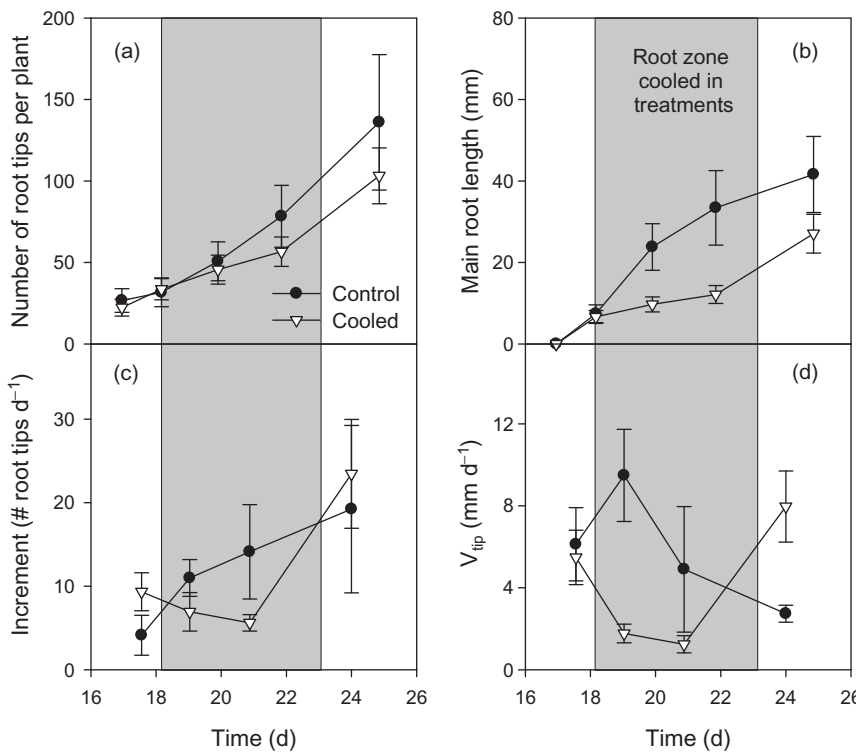


Figure 5. Root growth of *Ricinus communis* plants exposed to different root-zone temperatures. (a) Number of root tips counted in control plants ($T = 20$ °C) and cooled plants ($T = 10$ °C between day 18 and 23 and $T = 20$ °C else). (b) Length of the main root relative to initial value at day 17. (c) Increase in number of root tips per day. (d) Velocity of the tip of the main root. $n = 3–7$, mean value and standard error.

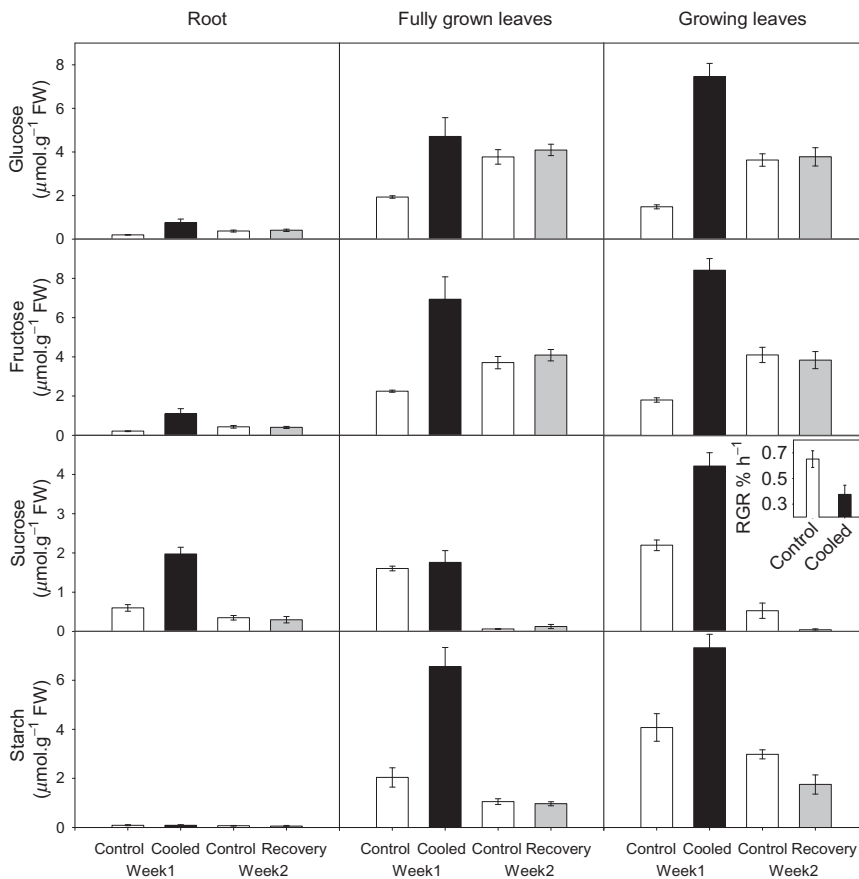


Figure 6. Starch and soluble sugar content of tissues from two populations of *Ricinus communis* plants exposed either to constant root-zone temperature throughout the experiment (control, $T = 20\text{ }^{\circ}\text{C}$) or to 5 d of cold root-zone temperature (cooled, $T = 10\text{ }^{\circ}\text{C}$) followed by a return to control temperature (recovery, $T = 20\text{ }^{\circ}\text{C}$). $n = 6\text{--}9$, mean value and standard error. The inset shows relative growth rate (RGR) of the total leaf area of the two populations during cold root-zone temperature ($n = 10$, mean value and standard error).

growing leaves. Sucrose and starch was significantly lower in recovered cooled leaves compared with control leaves ($P \leq 0.001$ and $P = 0.002$). During the cooled phase, leaf RGR of the sampled leaves was a factor of two lower compared with control leaves in the same developmental stage (inset of Fig. 6).

Transpiration rates of plants from cooled and control groups were comparable both during night and day, respectively (Fig. 7a), with even somewhat higher values in cooled plants compared with control plants. In contrast to this, xylem pressure of cooled plants was significantly lower ($P \leq 0.001$) compared with control plants: In the late afternoon, control plants reached values around -2.6 bar, whereas cooled plants had a xylem pressure of -3.8 bar (Fig. 7b).

We used ^{14}C tracer in experiment four to show the partitioning of carbohydrate between shoot and root, after export from a cotyledon. There was a dramatic increase in shoot/root partitioning, relative to controls, in plants with root-zones cooled from 20 to $10\text{ }^{\circ}\text{C}$ (Fig. 8). Two cases are shown, and the shoot/root partitioning increased threefold in one case, and fivefold in the other. The effect of cooling was reversible – when roots were returned to the warmer control temperature, partitioning immediately decreased, the shoots then receiving a smaller fraction of the carbon exported from the labelled cotyledons.

DISCUSSION

Growth reaction from day to day

Cold root temperatures resulted in decreased leaf growth of *Ricinus communis* (Fig. 2). The decrease was reversible and the acclimation to an altered root temperature occurred within days (Fig. 3). These results correspond to findings from monocot plants (Watts 1972; DeLucia *et al.* 1992) and they are consistent with the view that dicot leaf growth responds linearly to a range of atmospheric temperatures when analysed from day to day and not at a higher temporal resolution (Granier & Tardieu 1998). For the root itself, a similarly rapid and very intense reaction was found that was also reversible (Fig. 5). This finding is a logical consequence of the fact that root growth follows alterations in temperature almost linearly and immediately as long as temperature is within a physiological range (Pahlavanian & Silk 1988; Walter *et al.* 2002b; Hummel *et al.* 2007). The severe decrease of root elongation and ramification when temperature was decreased from 20 to $10\text{ }^{\circ}\text{C}$ indicates a strong loss of functionality that could then indirectly affect shoot growth, for example, via diminished transport of water or nutrients to the shoot, via diminished uptake of metabolites provided by the shoot or via altered exchange of signalling substances between shoot and root.

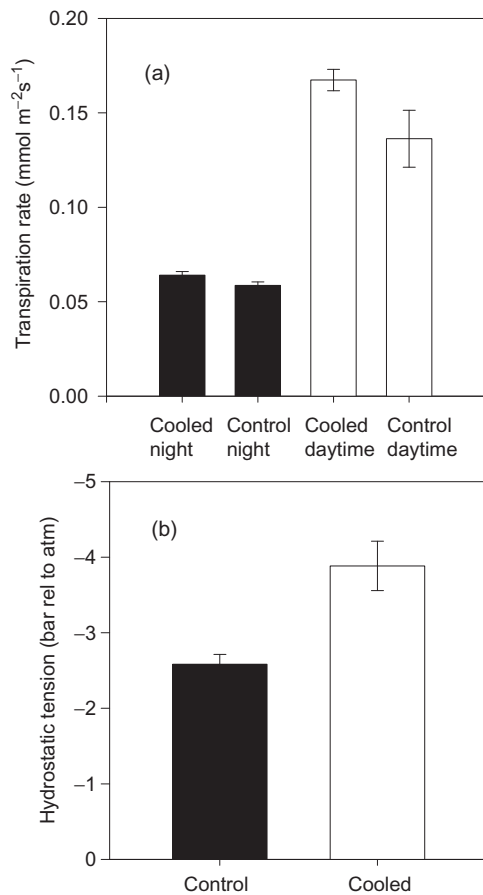


Figure 7. (a) Nocturnal and diurnal transpiration rates of two populations of *Ricinus communis* plants exposed to different root-zone temperatures (control: $T = 20\text{ }^{\circ}\text{C}$; cooled: $T = 10\text{ }^{\circ}\text{C}$). $n = 3\text{--}7$, mean value and standard error. (b) Diurnal xylem pressure values of both control and cooled *Ricinus communis* plants. $n = 3$, mean value and standard error.

Leaf growth reaction during the diel cycle

In contrast to the almost linear response of day-to-day leaf growth and biomass accumulation to root-zone temperature (Fig. 2), diel leaf growth patterns show a highly non-linear response (Fig. 4). Although nocturnal average growth rates scale well with the temperature, diurnal average growth rates are severely decreased to values close to zero and nocturnal maximal growth rates are more or less independent of root-zone temperature. This result is a cornerstone of our study. It demonstrates that leaf growth reaction to root-zone or root temperature alteration cannot simply be scaled between long-term and short-term responses, but that diel and probably circadian variations of regulating mechanisms have to be taken into account. As the molecular pathway, with which plants can perceive temperature, is just beginning to be unravelled, this is a valuable insight that can help to identify process regulation in the plant temperature signalling network. For example, low temperature has been shown to stall the rhythmic expression of LHY and TOC1, two central elements of the circadian clock

(Ramos *et al.* 2005). Because the circadian clock plays a prominent role to evoke physiological responses to alterations of the temperature (Penfield 2008) and because growth processes are also controlled by the circadian clock (Nozue *et al.* 2007; Harmer 2009), it will be important to clarify to which extent growth responses to temperature are affected by the circadian clock. Whereas LHY is an active component of the circadian clock in shoot and root, TOC1 is only involved in the circadian clock of the shoot (James *et al.* 2008), showing that differential regulation of temperature-induced processes in shoots and roots are likely. If, for example, the shoot–root temperature difference at a decisive ‘Zeitgeber’ time point of the circadian cycle is completely unnatural, growth might be disturbed for quite some time until physiological processes get back to a normal level again. Because the onset of light is such a Zeitgeber in a lot of situations (Harmer 2009), prominent morning differences between treatments with controlled, lowered root-zone temperature and uncontrolled root-zone temperature might be evoked as an output of the circadian clock.

The possible role of water relations

The reduction of leaf growth at reduced root-zone temperatures may also be caused by a reduction of water availability. Because the resistance to water uptake into roots increases at low temperature because of altered membrane fluidity and xylem conductivities (Holbrook *et al.* 2002; van Leperen 2007), leaves suffer from a restricted availability of water (Turner 1994). Nevertheless the same transpiration rate as in control plants was observed both night and day (Fig. 7). This means that water had to be withdrawn or

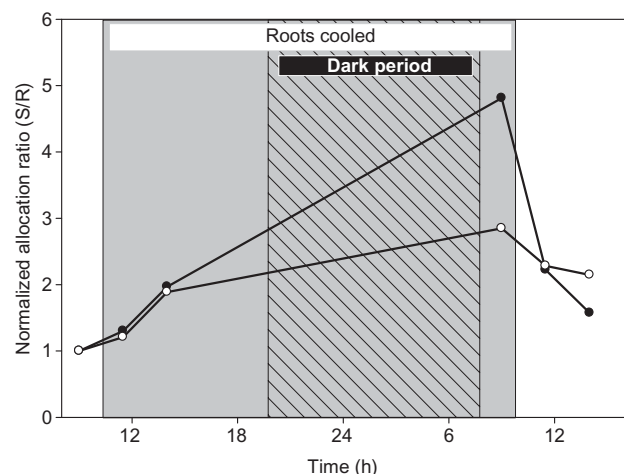


Figure 8. ¹⁴C gain data from two pairs of *Ricinus communis* plants exposed either to a constant root-zone temperature ($T = 20\text{ }^{\circ}\text{C}$) throughout the experiment or to a transient root-zone cooling event ($10\text{ }^{\circ}\text{C}$ during 24 h). Depicted values show shoot/root ratio of a cooled plant divided by shoot/root (S/R) ratio of the control plant (open symbols: pair one, closed symbols: pair two).

withheld from other process such as growth. Our observation of more negative xylem tension at lower root-zone temperature supports this suggestion, as it confirms that there was a high demand for water going into the transpiration stream in plants with cooled roots. Moreover, reduced xylem tension can correlate with reduced turgor, which decreases growth processes according to the Lockhart equation (Lockhart 1965). Because diurnal transpiration was much higher than nocturnal transpiration, root cooling would have caused a severe decline of the water available for growth processes by day and a less severe decline at night, which would fit with our observations.

The importance of the transpiration stream for coordinated dicot leaf growth was recently shown in the facultative crassulacean acid metabolism (CAM) plant *Clusia minor* (Walter *et al.* 2008): When *Clusia* is in the C₃ mode (stomata open at day), leaf growth increases during the night and is maximal at dawn. When the plant shifts to CAM (stomata open at night), nocturnal growth is strongly reduced and diurnal growth is enhanced. These effects might also be coupled to the altered carbohydrate metabolism when the plant shifts its mode of photosynthesis.

The possible role of carbohydrates

The significance of carbohydrate metabolism for the phasing of diel leaf growth cycles has recently been shown in a study on *Arabidopsis thaliana* (Wiese *et al.* 2007). There, starch-free mutants showed strongly reduced nocturnal but enhanced diurnal growth activity, demonstrating the importance of proper access to carbohydrate stores to facilitate nocturnal growth. Increased pools of carbohydrates, in turn, can support higher nocturnal leaf growth, which was shown in a study with tall fescue exposed to differing amounts of daily irradiance (Schnyder & Nelson 1988).

In our study, carbon accumulated during the day as transitory starch and plants with low root temperature even showed higher starch concentration than plants with high root temperature (Fig. 6). Hence, this transitory starch was used by the *Ricinus communis* plants of our study to utilize the full growth potential of the plants at night. The increased pools of carbohydrates stored in all investigated organs when roots were cooled were mobilized rapidly when plants recovered from cold root-zone temperature and provided essential substrates for leaf growth to recover again.

Also, our ¹⁴C analyses support the notion that root cooling increased carbohydrate supplies to the shoot, to the detriment of the root (Fig. 8). The reduction of carbon import after roots are cooled is likely caused by an increase in root carbohydrates after sink metabolism slows (Minchin, Farrar & Thorpe 1994). Cooling the roots also affects the phloem pathway itself, and so may have also caused an effect like that of cold girdling, reducing phloem solution flow into the roots, and thus increasing shoot carbohydrate concentrations (Peuke, Windt & Van As 2006).

Our results demonstrate that leaf and root carbohydrate content depends strongly on root temperature. Moreover, root temperature will not be uniform within a pot (Fig. 1) and will adjust to a steady-state temperature faster or slower, depending on the exact distribution of the root system within the soil of a pot and depending on the exposure of the pot to the irradiation within the growth chamber. Hence, root system architecture, exact location of the pot in an experimental setup, or simply the extent of the root system, can have a considerable effect not only on leaf growth but also on carbohydrate content of leaves.

CONCLUSION

In conclusion, this study of a dicot shows that leaf growth, plant water relations and carbohydrate transport and concentration depend strongly on root-zone temperature. Growing cells of the leaf have restricted access to water, especially during the day, when the transpiration is high, but the consequent growth decrease can be alleviated at night when water availability is improved and stored carbohydrate can be provided. Moreover, the distribution of the root system within pots is usually inhomogeneous and rarely known, and yet soil temperature can show strong gradients within a pot, and change dramatically in time. Therefore, the strong influence of root temperature on shoot physiology is likely to be the source of considerable variability in many laboratory and greenhouse experiments, and should be considered seriously as the variation will not be random.

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REFERENCES

- Ainsworth E.A., Walter A. & Schurr U. (2005) *Glycine max* leaflets lack a base-tip gradient in growth rate. *Journal of Plant Research* **118**, 343–346.
- Bigün J. & Granlund G.H. (1987) Optimal orientation detection of linear symmetry. *ICCV* **87**, 433–438.
- Bloom A.J., Zwieniecki M.A., Passioura J.B., Randall L.B., Holbrook N.M. & St Clair D.A. (2004) Water relations under root chilling in a sensitive and tolerant tomato species. *Plant, Cell & Environment* **27**, 971–979.
- Davies W.J. & Van Volkenburgh E. (1983) The influence of water deficit on the factors controlling the daily pattern of growth of *Phaseolus trifoliatus*. *Journal of Experimental Botany* **34**, 987–999.
- DeLucia E.H., Heckathorn S.A. & Day T.A. (1992) Effects of soil temperature on growth, biomass allocation and resource acquisition of *Andropogon gerardii* Vitman. *New Phytologist* **120**, 543–549.

- Engels C. (1994) Effect of root and shoot meristem temperature on shoot to root dry matter partitioning and the internal concentrations of nitrogen and carbohydrates in maize and wheat. *Annals of Botany* **73**, 211–219.
- Füllner K. (2007) The influence of spatially heterogeneous soil temperatures on plant structure and functions. PhD thesis, *Heinrich-Heine Universität Düsseldorf*.
- Granier C. & Tardieu F. (1998) Is thermal time adequate for expressing the effects of temperature on sunflower leaf development? *Plant, Cell & Environment* **21**, 695–703.
- Harmer S.L. (2009) The circadian system in higher plants. *Annual Review of Plant Biology* **60**, 357–377.
- Hillel D. (1998) *Environmental Soil Physics: Fundamentals, Applications, and Environmental Considerations*. Academic, San Diego, CA, USA.
- Holbrook N.M., Shashidhar V.R., James R.A. & Munns R. (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* **53**, 1503–1514.
- Hummel G.M., Naumann M., Schurr U. & Walter A. (2007) Root growth dynamics of *Nicotiana attenuata* seedlings are affected by simulated herbivore attack. *Plant, Cell & Environment* **30**, 1326–1336.
- van Ieperen W. (2007) Ion-mediated changes of xylem hydraulic resistance in planta: fact or fiction? *Trends in Plant Science* **12**, 137–142.
- James A.B., Monreal J.A., Nimmo G.A., Kelly C.L., Herzyk P., Jenkins G.I. & Nimmo H.G. (2008) The circadian clock in arabidopsis roots is a simplified slave version of the clock in shoots. *Science* **322**, 1832–1835.
- Jones M.G.K., Outlaw W.H. Jr & Lowry O.H. (1977) Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. *Plant Physiology* **60**, 379–383.
- Lockhart J.A. (1965) An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology* **8**, 264–275.
- Markhart A.H., Fiscus E.L., Naylor A.W. & Kramer P.J. (1979) Effect of temperature on water and ion transport in soybean and broccoli systems. *Plant Physiology* **64**, 83–87.
- Matsubara S., Hurry V., Druart N., Benedict C., Janzik I., Chavarria-Krauser A., Walter A. & Schurr U. (2006) Nocturnal changes in leaf growth of *Populus deltoides* are controlled by cytoplasmic growth. *Planta* **223**, 1315–1328.
- Minchin P.E.H. & Thorpe M.R. (2003) Using the short-lived isotope C-11 in mechanistic studies of photosynthate transport. *Functional Plant Biology* **30**, 831–841.
- Minchin P.E.H., Farrar J.F. & Thorpe M.R. (1994) Partitioning of carbon in split root systems of barley: effect of temperature of the root. *Journal of Experimental Botany* **45**, 1103–1109.
- Nozue K., Covington M.F., Duek P.D., Lorrain S., Fankhauser C., Harmer S.L. & Maloof J.N. (2007) Rhythmic growth explained by coincidence between internal and external cues. *Nature* **448**, 358–361.
- Pahlavanian A.M. & Silk W.K. (1988) Effect of temperature on spatial and temporal aspects of growth in the primary maize root. *Plant Physiology* **87**, 529–532.
- Passioura J.B. (2006) The perils of pot experiments. *Functional Plant Biology* **33**, 1075–1079.
- Penfield S. (2008) Temperature perception and signal transduction in plants. *New Phytologist* **179**, 615–628.
- Peuke A.D., Windt C. & Van As H. (2006) Effects of cold-girdling on flows in the transport phloem in *Ricinus communis*: is mass flow inhibited? *Plant, Cell & Environment* **29**, 15–25.
- Ramos A., Pérez-Solís E., Ibáñez C., Casado R., Collada C., Gómez L., Aragoncillo C. & Allona I. (2005) Winter disruption of the circadian clock in chestnut. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 7037–7042.
- Schmundt D., Stitt M., Jähne B. & Schurr U. (1998) Quantitative analysis of the local rates of growth of dicot leaves at a high temporal and spatial resolution, using image sequence analysis. *The Plant Journal* **16**, 505–514.
- Schnyder H. & Nelson C.J. (1988) Diurnal growth of tall fescue leaf blades. *Plant Physiology* **86**, 1070–1076.
- Smith A.M. & Stitt M. (2007) Coordination of carbon supply and plant growth. *Plant, Cell & Environment* **30**, 1126–1149.
- Sulpice R., Pyl E.T., Ishihara H., *et al.* (2009) Starch as a major integrator in the regulation of plant growth. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 10348–10353.
- Thomas R.B. & Strain B.R. (1991) Root restriction as a factor in photosynthetic acclimation of cotton seedlings grown in elevated carbon dioxide. *Plant Physiology* **96**, 627–634.
- Turner N.C. (1994) Water relations, gas exchange and abscisic acid content of *Lupinus cosentinii* leaves in response to drying different proportions of the root system. *Journal of Experimental Botany* **45**, 909–918.
- Walter A., Feil R. & Schurr U. (2002a) Restriction of nyctinastic movements and application of tensile forces to leaves affects diurnal patterns of expansion growth. *Functional Plant Biology* **29**, 1247–1258.
- Walter A., Spies H., Terjung S., Kuesters R., Kirchgeßner N. & Schurr U. (2002b) Spatio-temporal dynamics of expansion growth in roots: automatic quantification of diurnal course and temperature response by digital image sequence processing. *Journal of Experimental Botany* **53**, 689–698.
- Walter A., Christ M.M., Rascher U., Schurr U. & Osmond B. (2008) Diel leaf growth cycles in *Clusia* spp. are related to changes between C3 photosynthesis and crassulacean acid metabolism during development and during water stress. *Plant, Cell & Environment* **31**, 484–491.
- Walter A., Silk W.K. & Schurr U. (2009) Environmental effects on spatial and temporal patterns of leaf and root growth. *Annual Review of Plant Biology* **60**, 279–304.
- Watts W.R. (1972) Leaf expansion in *zea mays* II – leaf extension in response to independent variation of the temperature of the apical meristem, of the air around the leaves, and of the root-zone. *Journal of Experimental Botany* **23**, 731–721.
- Wiese A., Christ M.M., Virnich O., Schurr U. & Walter A. (2007) Spatio-temporal leaf growth patterns of *Arabidopsis thaliana* and evidence for sugar control of the diel leaf growth cycle. *New Phytologist* **174**, 752–761.
- Zimmermann U., Schneider H., Wegner L.H. & Haase A. (2004) Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? *New Phytologist* **162**, 575–615.

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RESEARCH ARTICLE #II

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RESEARCH PAPER

Diel time-courses of leaf growth in monocot and dicot species: endogenous rhythms and temperature effects

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Abstract

Diel (24 h) leaf growth patterns were differently affected by temperature variations and the circadian clock in several plant species. In the monocotyledon *Zea mays*, leaf elongation rate closely followed changes in temperature. In the dicotyledons *Nicotiana tabacum*, *Ricinus communis*, and *Flaveria bidentis*, the effect of temperature regimes was less obvious and leaf growth exhibited a clear circadian oscillation. These differences were related neither to primary metabolism nor to altered carbohydrate availability for growth. The effect of endogenous rhythms on leaf growth was analysed under continuous light in *Arabidopsis thaliana*, *Ricinus communis*, *Zea mays*, and *Oryza sativa*. No rhythmic growth was observed under continuous light in the two monocotyledons, while growth rhythmicity persisted in the two dicotyledons. Based on model simulations it is concluded that diel leaf growth patterns in mono- and dicotyledons result from the additive effects of both circadian-clock-controlled processes and responses to environmental changes such as temperature and evaporative demand. Apparently very distinct diel leaf growth behaviour of monocotyledons and dicotyledons can thus be explained by the different degrees to which diel temperature variations affect leaf growth in the two groups of species which, in turn, depends on the extent of the leaf growth control by internal clocks.

Key words: Circadian clock, elongation, expansion, image analysis, photosynthesis, starch, sucrose.

Introduction

Leaf growth is controlled by a complex network of factors. Some of these factors are endogenous regulatory mechanisms that determine leaf shape (Wyrzykowska *et al.*, 2002; Rolland-Lagan *et al.*, 2003), the progression of the cell cycle (Tsukaya and Beemster, 2006), and the relationship of leaf growth to the circadian clock (Nozue and Maloof, 2006). Other factors can be regarded as external, such as the recurring changes of day and night, alterations in temperature, or further physical, chemical, or biotic parameters, which can increase or decrease growth at various time-scales (Granier and Tardieu, 2009; Walter *et al.*, 2009). All growth-controlling environmental effects regulate endogenous mechanisms, but to a different extent. Light quality and quantity are sensed by the phytochrome and cryptochrome photoreceptors (Somers *et al.*, 1998; Devlin and

Kay, 2000, 2001) and affect photosynthesis and plant primary metabolism, which is partly controlled by the circadian clock (Harmer *et al.*, 2000). Altered carbohydrate metabolism, in turn, leads to instantaneous and often very specific growth reactions in leaves (Wiese *et al.*, 2007; Sulpice *et al.*, 2009). Temperature changes also induce immediate and clear responses of growth, but it is still uncertain to what extent this is mediated via signalling cascades (Penfield, 2008; Franklin, 2009) or mere thermodynamic laws which affect the rate of all biochemical reactions (Parent *et al.*, 2010). It is well known that the duration of each phase in the plant life cycle is inversely related to temperature (Amir and Sinclair, 1991). This knowledge is at the base of a number of models predicting the progression of the plant life cycle in any climatic

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scenario (Keating *et al.*, 2003; Yan *et al.*, 2004). It also led to the definition of thermal time: a concept that can be used for the quantitative description of plant or even single leaf development (Granier and Tardieu, 1998; Granier *et al.*, 2002). It has been proposed recently that the rates of most developmental processes are co-ordinated in such a way that temperature-compensated rates and durations can be calculated (Parent *et al.*, 2010).

While, on a long time-scale, leaf growth of different species is tightly correlated to temperature, the response to short-term temperature alterations seems to vary strongly when growth reactions are investigated during a 24 h day (the diel cycle) as indicated by Walter *et al.* (2009). In monocotyledonous species, leaf elongation rate largely follows temperature alterations (Ben-Haj-Salah and Tardieu, 1995; Pietruszka *et al.*, 2007), whereas in dicotyledonous species, growth patterns do not seem to be related to the daily pattern of temperature variations. In the dicot model species *Arabidopsis thaliana*, leaf growth, as well as hypocotyl growth, seems to be controlled by the circadian clock (Dodd *et al.*, 2005; Nozue *et al.*, 2007).

The aim of this study was to investigate to what extent the 24 h pattern of leaf growth rate is linked to changes in temperature and/or to endogenous rhythms. Hence, leaf growth variations of several species in fluctuating temperature regimes were compared to (i) leaf growth patterns in a stable temperature and (ii) to the pattern which would be expected if short-term variations in leaf expansion rate followed the response curve to temperature established over longer periods. The involvement of the circadian clock in these patterns was investigated by analysing leaf growth in plants transferred from regular day–night regimes to continuous light. Investigations were done in two monocotyledons, *Zea mays* and *Oryza sativa*, and four dicotyledons, *Ricinus communis*, *Nicotiana tabacum*, *Arabidopsis thaliana*, and *Flaveria bidentis*. Two of these species are C₄ (*Z. mays* and *F. bidentis*); the others are C₃.

Materials and methods

Plant material, climate conditions, and experimental outline

Maize (*Zea mays*, hybrid Helix), flaveria (*Flaveria bidentis*), tobacco (*Nicotiana tabacum*, ecotype Samsun), and castor bean (*Ricinus communis*, ecotype Carmencita) plants were germinated and grown in the greenhouse for 3 weeks and were then moved to the growth chamber 1 week before experiments started. Plants were grown in pots (0.1 l) filled with a pre-fertilized soil (ED73, Balster Einheitserdewerk, Fröndenberg, Germany). Thale cress (*Arabidopsis thaliana*, ecotype Ler), rice (*Oryza sativa* cv. Azucena) and tobacco (*Nicotiana tabacum*, ecotype Samsun) plants were grown for specific experiments. During all experiments, soil water content was kept at retention capacity.

A growth chamber was set to 22 °C air temperature, 12/12 h day/night and 60% relative humidity. Plants were given 1 week to acclimate to those conditions before the experiments started. Unless specified otherwise, day 1 was the last day with constant air temperature. Between days 2 and 4, air temperature was switched to follow an approximately sine-shaped function centred on 22 °C with a minimum at 17 °C and a maximum at 27 °C. Minimum temperature was reached at the end of the night and maximum

temperature at the end of the day. In experiments with *A. thaliana* and *O. sativa*, only daylength was varied.

Light intensity measured with a quantum light sensor (LI-190SB; Li-Cor Biosciences GmbH, Bad Homburg Germany) was 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR). The light was provided by an array of high pressure sodium lamps (MASTER SON-T PIA Agro 400W; Phillips Deutschland GmbH, Hamburg, Germany). In experiments with *A. thaliana*, light intensity was 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and light was provided by fluorescent lamps (Osram; Fluora, Munich, Germany). Air temperature and relative humidity settings of the climate chamber were measured with a portable data logger placed at canopy level (Testo 175-H1; Testo AG, Lenzkirch, Germany). The experiment with rice was carried out in the *Phenodyn* phenotyping platform (Sadok *et al.*, 2007). Plants were first grown in the greenhouse under naturally fluctuating light. They were then transferred in a growth chamber under a continuous light intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf level, measured with a light sensor (LI-190SB, Li-Cor Quantum PAR, Lincoln, NE USA). The meristem temperature, measured with fine copper-constantan thermocouples inserted in the meristematic zones of four non-measured plants per treatment, was kept constant at 26 ± 2 °C.

Growth analysis

R. communis, *N. tabacum*, and *F. bidentis*: Investigated leaves were fixed in a stationary position (Walter *et al.*, 2002) by using a strip of Parafilm (Parafilm M, Pechiney Plastic Packaging Company) and kept flat with five nylon threads, clamped to the edge of the leaf using shortened hair clips and fabric tape to protect the leaf. Each of the threads was pulled with a weight (*R. communis*: 12 g; *N. tabacum*: 1.5 g; *F. bidentis*: 5 g) and spun over a metal ring surrounding the leaf (Fig. 1F), a procedure which does not affect temporal or spatial growth patterns (Walter *et al.*, 2002). Leaf images were acquired with high resolution, monochrome CCD cameras (Scorpion SCOR-20SO; Point Grey Research, Vancouver, BC, Canada), positioned above the plants and equipped with a standard objective lens (25 mm; Cosmicar/Pentax, The Imaging Source, Bremen, Germany) and an infrared interference filter (880 nm; Edmund Optics, Karlsruhe, Germany). Constant illumination throughout day and night was provided by six infrared diode clusters (880 nm; Conrad Electronics, Hirschau, Germany). Grey value images were taken every 180 s and saved in a multi-tiff

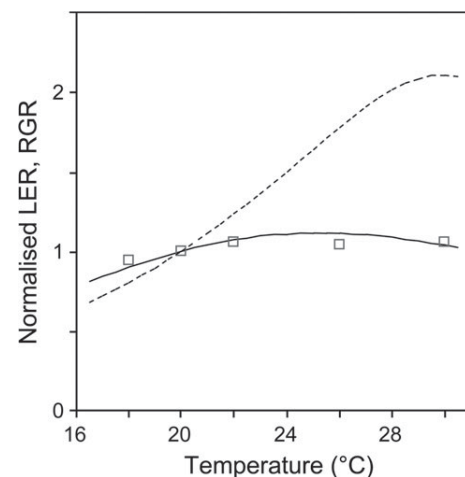


Fig. 1. Response curves of leaf expansion rate of *Z. mays* (dotted line) and *N. tabacum* (solid lines). Data originate from Parent *et al.* (2010) for maize and from Raper *et al.* (1976) for *N. tabacum*. Symbols show experimental data of Raper *et al.* (1976). LER, leaf elongation rate. RGR, relative growth rate. Values at 20 °C are by convention set to 1 for normalization (see Parent *et al.*, 2010).

format. Image sequences were evaluated with algorithms based on a structure tensor approach that calculates optical flow via the brightness constancy constraint equation (BCCE) (Bigün and Granlund, 1987; Schmundt *et al.*, 1998). Using the structure tensor approach, velocities of all visible and moving structures at the leaf surface within the image sequence of a growing leaf were calculated. Area relative growth rates (*RGR*) were calculated as the divergence of the estimated velocity field by selecting an area of interest (AOI) within the image and tracking the structure within this AOI with time. (For more details, see Walter *et al.*, 2002; Schmundt *et al.*, 1998; and Matsubara *et al.*, 2006.)

Z. mays, *O. sativa*, and *A. thaliana*: Leaf elongation rate of maize was measured with a rotation resistance transducer (RRT, low torque potentiometers Novotechnik, Ostfildern, Germany). A string was fixed to the tip of a growing leaf using shortened hair clips and fabric tape, spun over the pulley of the RRT sensor above the leaf and attached to a 25 g weight. Strings for the measurement of *A. thaliana* leaf length growth were glued (Pattex, Henkel, Düsseldorf, Germany) to the leaf tip of a growing leaf with *c.* 1 cm length and spun over the RRT sensor attached in length growth direction to a 1.8 g weight. RRT sensors were interfaced using a Multifunction Data Acquisition device (CompactDAQ National Instruments NI, Austin, Texas, USA) connected to a laptop PC. A custom made LabView 8.5 (National Instruments NI, Austin, Texas, USA) program allowed the wheel rotation to be measured and recorded every 10 s. In the rice leaf, elongation rate was continuously monitored with rotating displacement transducers (RDTs, 601–1045 Full 360 Smart Position Sensor; Spectrol Electronics, Ltd, Wiltshire, England), both in the greenhouse and in the growth chamber. Leaf elongation was transmitted to the sensor via a pulley attached to it, which carried a thread attached to the leaf tip and to a 20 g counterweight (Sadok *et al.*, 2007). Both elongation and climatic data were stored every 15 min in the Phenodyn database (<http://bioweb.supagro.inra.fr/phenodyn/>).

Modelling the temperature response

Leaf expansion rate under fluctuating temperature was compared to that expected from the response curves to temperature (Fig. 1). For maize, the response curve corresponding to several developmental processes was published by Parent *et al.* (2010). For *N. tabacum*, they originate from the study of Raper *et al.* (1976). All data were fitted to a Johnson *et al.* (1942) function, as in Parent *et al.* (2010).

$$LER(T) = \frac{A Te^{\left(\frac{\Delta H_A^\ddagger}{RT}\right)}}{1 + e^{\left[\frac{\Delta S_D}{R} \left(1 - \frac{\Delta H_D}{\Delta S_D T}\right)\right]}}$$

where *LER* is leaf expansion rate, *T* is temperature, *A* is a proportionality constant, ΔH_A^\ddagger (kJ mol⁻¹) is a fitted parameter representing the enthalpy of activation of the equivalent reaction. ΔH_D and ΔS_D are fitted parameters representing the enthalpy and entropy between the catalytically active and inactive states of the enzyme or enzymatic system. Parameters of response curves were calculated in three steps using the R language (R_Development_Core_Team, 2005). Data were first smoothed by a second order polynomial equation in a range of 5 °C at steps of 1 °C each. The inflexion point was determined where the slope of the linear regression on three consecutive points was maximum. This step was omitted for the tobacco data which lacked datapoints at low temperatures. The parameters of the numerator were determined by linear regression on transformed variables (ln (*J/T*), 1/*T*) in the range of temperatures lower than the inflexion point. The parameters of the denominator of equation 1 were determined by linearization in the range of temperatures above the inflection point following the method presented in Parent *et al.* (2010). The

resulting parameters were $\Delta H_A^\ddagger=3.71$ and 1.5, $\Delta H_D=285$ and 135, and $\Delta S_D=0.93$ and 0.46, for *Z. mays* and *N. tabacum*, respectively.

Carbohydrates

In the main experiment, leaves were harvested for carbohydrate analysis when the lamps were switched on and off (every 12 h), respectively. For each species, two plants were sampled and from each plant, two leaf discs (diameter 8 mm) were punched out of growing leaves (the same position as the leaves taken for growth analyses). Leaf discs were weighed, pooled, frozen in liquid nitrogen, and stored at -80 °C for further extraction. Soluble carbohydrates were extracted from frozen leaf material and glucose, fructose, sucrose, and starch concentrations were analysed in a coupled enzyme assay (Jones *et al.*, 1977) using a multiplate spectrophotometer (ht II; Anthos Mikrosysteme GmbH, Krefeld, Germany) as described in Walter *et al.* (2002) and Wiese *et al.* (2007).

Gas exchange

Gas exchange of growing leaves of each investigated species was measured using a portable, open path design, infrared gas-exchange system (Li-6400; Li-Cor Biosciences GmbH, Bad Homburg, Germany). The area of the leaf chamber was 6 cm². Light intensity and temperature inside the leaf chamber were allowed to follow the ambient conditions of the growth chamber. To avoid large fluctuations of the reference CO₂, the air that went to the analyser inside the growth chamber was buffered using a 50 l plastic tank linked to the greenhouse with a nozzle.

Results

Leaf expansion of dicotyledonous species did not follow temperature variations while leaf elongation of maize did

Similar diel leaf growth patterns occurred in tobacco plants regardless of the temperature regime (Fig. 2A–D). After having been grown at a constant temperature of 22 °C until the start of the experiment, plants were exposed on day 1 to a constant daytime temperature of 27 °C, followed by a night temperature of 17 °C. On day 2, temperature was set to a constant value of 22 °C again and on day 3, temperature was modulated between an afternoon peak temperature of 27 °C and a minimum of 17 °C at the end of the night. The average diel leaf growth pattern was almost identical during all three days (Fig. 2E).

In a second experiment; daily leaf growth cycles of four species were investigated with plants being exposed either to a constant temperature of 22 °C or to a fluctuating temperature regime with an afternoon maximum of 27 °C and a night minimum of 17 °C (Fig. 3). On day 1, with constant temperature, pronounced diel leaf growth variations were observed with maxima in the early morning or late at night for *N. tabacum*, *R. communis*, and *F. bidentis*. Despite variations between individuals, *RGR* varied significantly between the times of maximal and minimal growth activity during the diel cycle. Between days 2 and 4, plants were exposed to the fluctuating temperature regime. Diel growth cycles of the dicot species still reached maximal growth at the same time of the day, but the amplitude was damped and differences between maxima and minima were not significant any longer. By contrast, *Zea mays* leaves grew with a constant rate throughout the day and night when temperature was constant and developed a pronounced

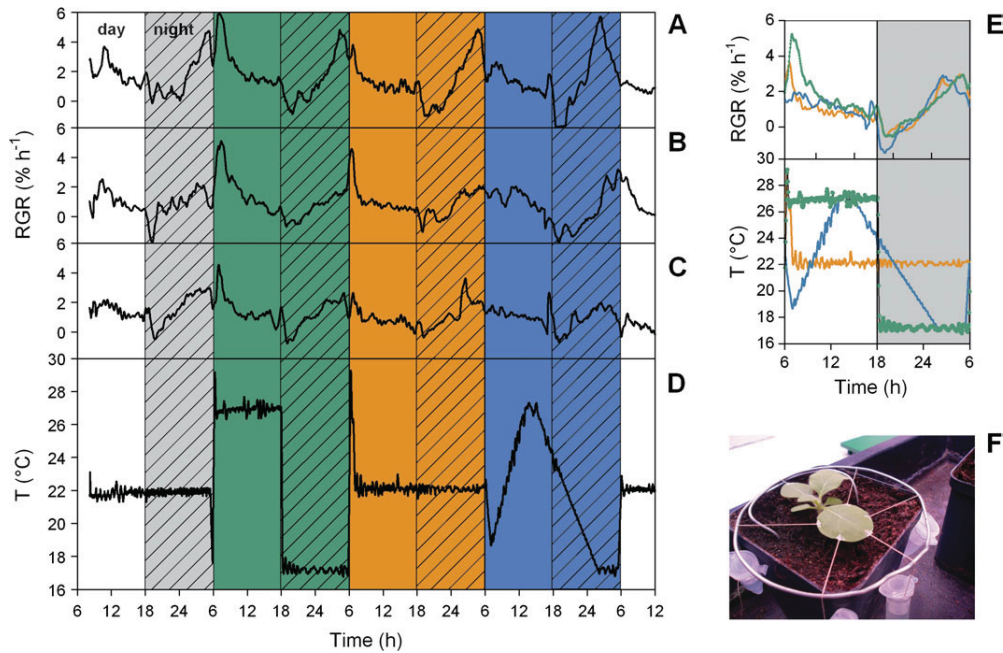


Fig. 2. Relative growth rates (*RGR*) of leaves of *N. tabacum* grown in different temperature regimes. (A, B, C) The *RGR* of three replicate plants, respectively. The temperature regime to which these plants were exposed is depicted in (D). Average values are shown in (E) with colours coding for the three different temperature regimes to which the plants were subsequently exposed. Positioning of the leaf for growth analysis is shown in (F).

variation in the fluctuating temperature regime. On day 1, leaf elongation rate only changed transiently when the light was switched on or off. On day 2, leaf elongation rate decreased sharply in the morning and increased throughout the day, reaching a maximum value in the early hours of the night, when temperature was still high. During the night, leaf elongation rate declined, reaching a plateau value at the end of the night, when temperature also reached a low plateau value. On day 3 and day 4, these patterns were repeated and the correlation between leaf elongation rate and temperature became even more prominent. Maximal leaf elongation occurred as soon as temperature reached its maximum value, without any appreciable delay, taking into account the temporal definition used in this experiment (10 s). When data from the two different temperature treatments was averaged (Fig. 3, right panels), it became obvious that growth patterns in the two dicot species were far less related to temperature than in *Z. mays*.

Fluctuations in carbohydrate availability and gas exchange did not account for differences in diel leaf expansion patterns between species

Gas exchange and carbohydrate analyses were performed in *Z. mays*, *F. bidentis*, *N. tabacum*, and *R. communis*. Assimilation rates of all the species studied were high ($20 \mu\text{mol m}^{-2} \text{s}^{-1} \text{CO}_2$ and more; Fig. 4) reflecting near optimal growth conditions and thus a good vegetative status. Carbohydrate analyses showed increasing contents of starch and sucrose in all species during the day and a decrease at night (Fig. 5). On day 1 (constant tempera-

ture) the nocturnal decrease in starch was similar in all species. On days 2, 3, and 4 (fluctuating temperature regime) starch fluctuations in *N. tabacum* and *F. bidentis* decreased. *Flaveria bidentis* has a very low overall soluble sugar concentration with values ranging between 0 and $1 \mu\text{mol g}^{-1} \text{FW}$, which do not fluctuate strongly throughout the day. For sucrose, the strongest fluctuations were seen in *Z. mays* and *R. communis*. Overall, no pronounced differences in carbohydrate fluctuations between the two different temperature regimes were observed for any of the species studied.

In continuous light, dicotyledonous species revealed diel oscillations of leaf expansion that were not observed in monocotyledonous species

To investigate the effect of the circadian clock on the rhythmicity of leaf growth patterns, the leaf elongation rates of the monocotyledonous species *Z. mays* and *O. sativa* and of the dicotyledonous species *R. communis* and *A. thaliana* were investigated in continuous light (Fig. 6). Plants that had been exposed to fluctuating light until the first day of analysis were transferred to constant light (LL) for at least 3 d. In *A. thaliana* and *R. communis*, the diel leaf growth cycle clearly continued. In *R. communis*, leaves from different plants were followed over a long time period (18 d) after transition. In *A. thaliana*, leaf growth was followed in the same leaves from the day before light transition throughout some days of LL, demonstrating a higher period length than 24 h. By contrast, leaf growth of *Z. mays* and of *O. sativa* did not oscillate markedly in a diel manner until the end of the experiment.

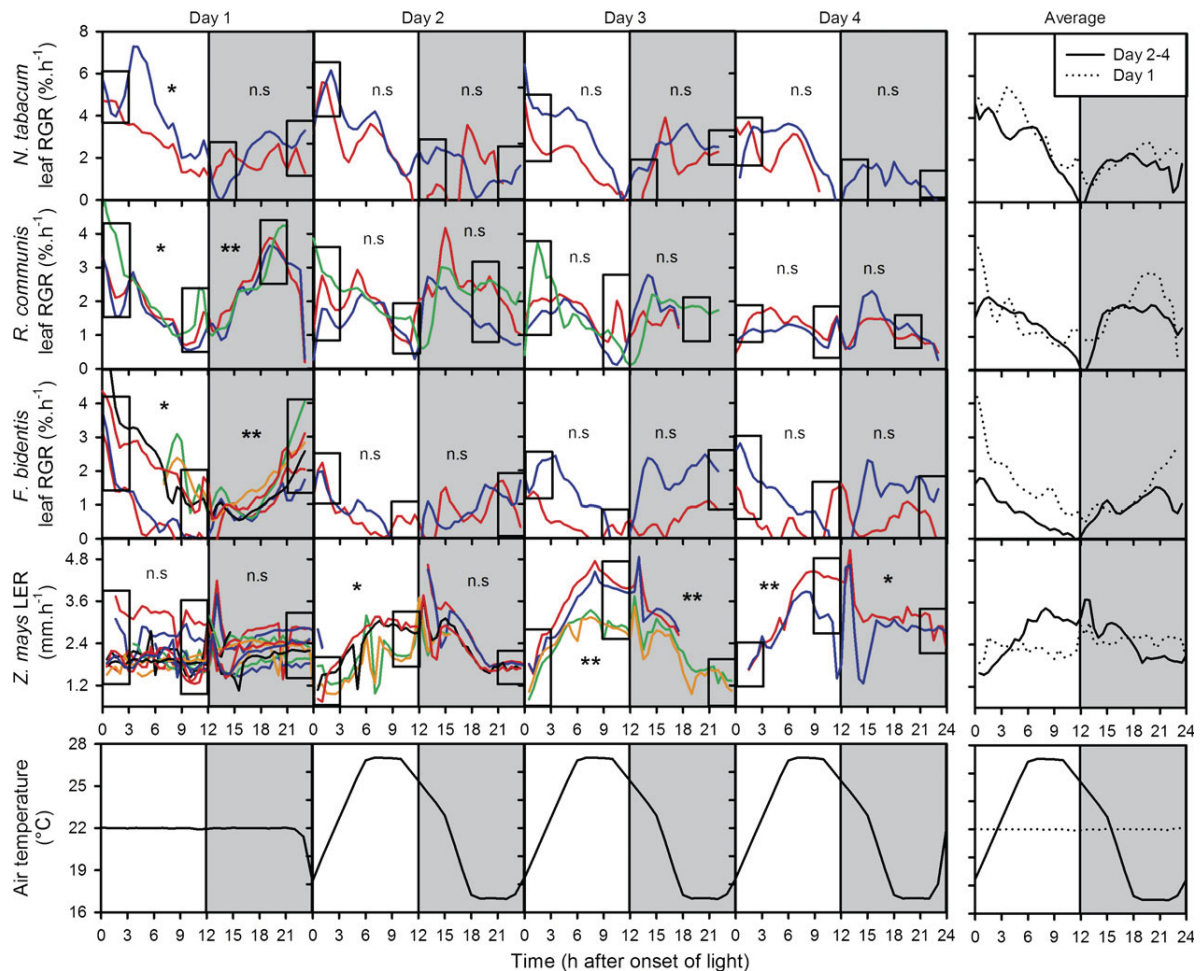


Fig. 3. Leaf growth of various plants from different species in different temperature regimes. Temperature regime was switched from constant to fluctuating at the beginning of day 2 (lower panels). For *N. tabacum*, *R. communis*, and *F. bidentis*, relative growth rates (RGR) were measured, while for *Z. mays*, leaf elongation rate (LER) was analysed. Right panels show average values; different line colours show different replicate plants. Asterisks indicate the significance level of growth differences in consecutive time intervals (framed in boxes; T-test): ** $P < 0.01$, * $P > 0.05$; n.s. not significant.

An addition of the effects of endogenous rhythms and temperature accounted for experimentally observed patterns in all studied species

Maize and *Nicotiana tabacum* were tested to find out to what extent the experimental patterns observed under fluctuating temperature (days 2–4; Fig. 3) could be accounted for by the additive effects of endogenous rhythms (day 1; Fig. 3) and temperature dependency of leaf growth (presented in Fig. 1). The endogenous rhythm was considered as flat in maize, consistent with Fig. 3 (day 1). The endogenous rhythm of *N. tabacum* was inferred from the mean pattern on day 1 (Fig. 3), with a minimum expansion rate at 12.00 h. Although maize and tobacco were subjected to the same temperature scenarios, simulated temporal patterns of leaf expansion rate deduced from the response curves presented in Fig. 1 differed markedly (Fig. 7). Because the response curve reached a plateau in tobacco at temperatures which still caused an increase in elongation rate of maize, the simulated pattern of tempera-

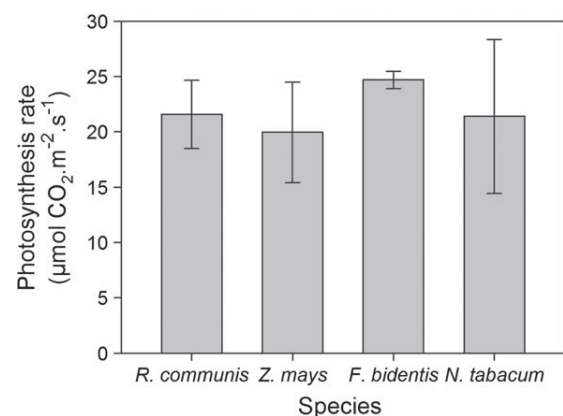


Fig. 4. Assimilation rate of growing leaves. Mean value and SD ($n=4$).

ture effect was much more pronounced in maize than in tobacco (Fig. 7B, D).

In maize, the addition of the two effects correctly simulated the experimental pattern, with two exceptions: (i)

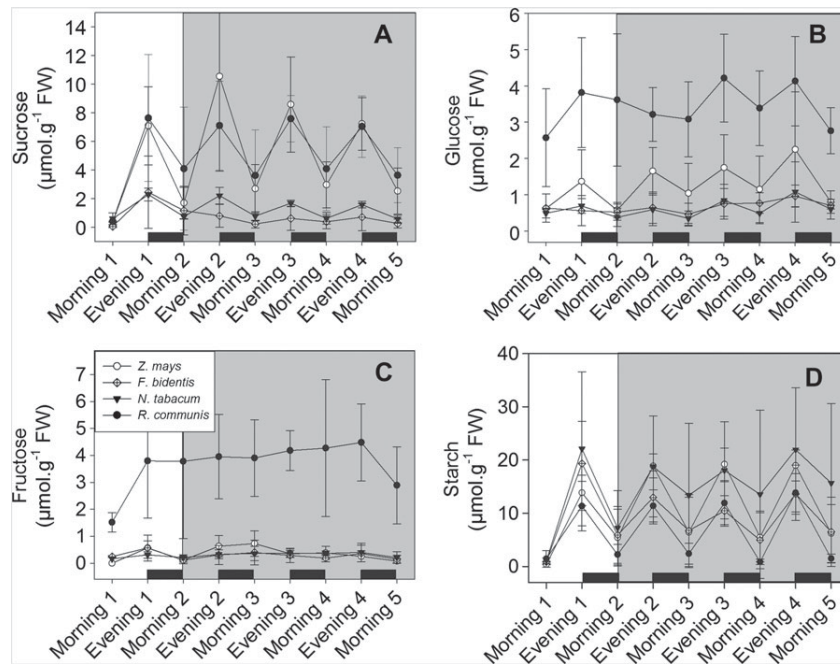


Fig. 5. Carbohydrate concentrations of growing leaves. (A) Sucrose, (B) glucose, (C) fructose, and (D) starch. During day 1, plants were exposed to constant temperature, thereafter (grey background) temperature fluctuated as shown in Fig. 2. Mean value and SD ($n=4$).

a marked peak of leaf elongation rate followed light extinction, (ii) observed elongation rates were lower than the simulated ones during the day, and higher during the night. These two exceptions are probably linked to the effect of evaporative demand which lowers maize leaf expansion rate during the day (Sadok *et al.*, 2007). In tobacco, the additive effects of endogenous rhythms and temperature also simulated the observed leaf growth pattern correctly. Observed and simulated patterns were consistent throughout the day, except for (i) a marked oscillation of *RGR* at 12 h, as in the case of maize, when the lights were turned off and (ii) an unexplained discrepancy 4 h after the lights were turned on. Overall, the additive effects of endogenous rhythms and temperature account for the observed leaf growth patterns, but a supplementary effect of evaporative demand was noted.

Discussion

The rhythmic variation of leaf growth in *A. thaliana* and *N. tabacum* with a slightly extending periodicity under continuous light (Fig. 3B) shows that this diel pattern is controlled by the circadian clock. This confirms findings on the overall growth performance of *Arabidopsis* plants (Dodd *et al.*, 2005) and on the circadian control of hypocotyl elongation (Dowson-Day and Millar, 1999; Nozue *et al.*, 2007). From an evolutionary point of view, a correct phasing between plant metabolism and forthcoming environmental conditions represents a crucial advantage compared with organisms with a coarser regulation (Dodd *et al.*, 2005; Kobayashi and Weigel, 2007; Resco *et al.*,

2009). Because temperature compensation is an essential feature of the circadian clock (Harmer, 2009), the periodicity and amplitude of rhythmic processes that are controlled by the circadian clock are affected only to a minor extent by temperature variations (Nakajima *et al.*, 2005). When measured leaf growth patterns are compared with patterns calculated from the effects of temperature on leaf growth at the longer time-scale (Fig. 4), it becomes clear that diel leaf growth variations in all species can be considered as the added effects of circadian-clock-controlled processes and temperature-related processes, with an additional effect of evaporative demand consistent with Sadok *et al.* (2007). The main difference between leaf growth in monocotyledonous and dicotyledonous species is that, in dicotyledons, circadian effects are much more pronounced than in monocotyledons, where they can be neglected. In addition, the temperature effect was lower in *N. tabacum* than in maize because of its lower sensitivity to temperature in the range considered. Although many processes throughout the plant kingdom are controlled by the circadian clock, the degree to which the circadian clock affects other processes differs between tissues, organs, and species (McClung, 2006; Hotta *et al.*, 2007; James *et al.*, 2008; Jones, 2009). More precisely, the circadian system is a network of more than one single clock inside the plant with independent oscillators in each cell (Jones, 2009) that can elucidate a multitude of output responses in a multicellular organism. Hence, it comes as no surprise that in a recent DNA-microarray study, roots were found to be much less affected by the circadian clock than shoots (James *et al.*, 2008).

A reason for the observed difference in the significance of the circadian clock for leaf growth processes in monocot

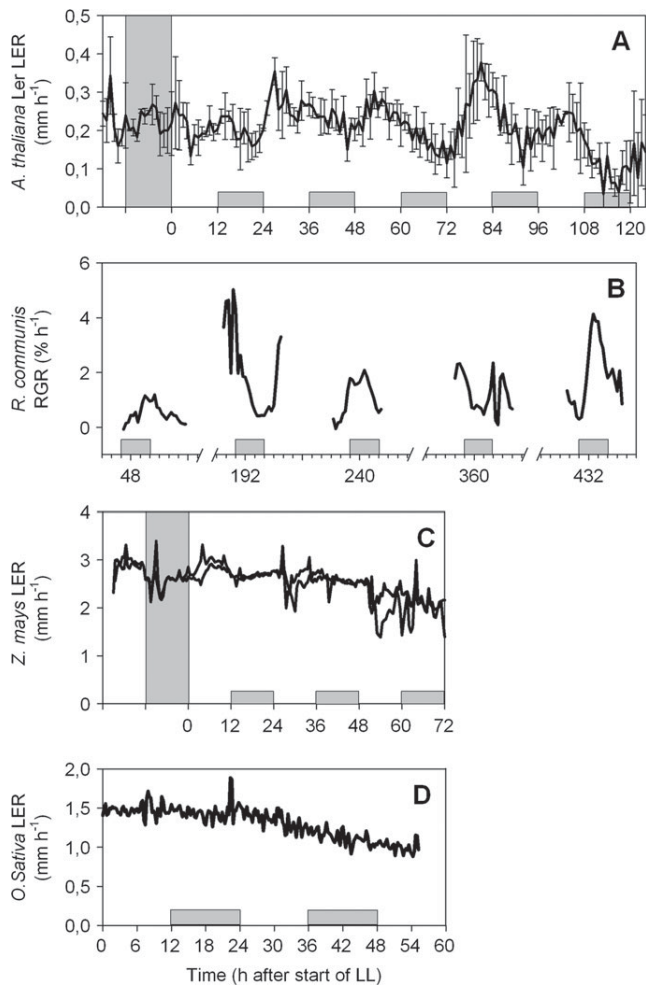


Fig. 6. Leaf growth of (A) *A. thaliana*, (B) *R. communis*, (C) *Z. mays*, and (D) *O. sativa* transferred from 12/12 h day/night to a continuous light regime. Hatched bars represent subjective night in the continuous light treatment. For *Z. mays*, replicate plants are shown with different lines; for *A. thaliana*, mean value and SD ($n=4$) are shown.

and dicot species might be found in the different organization and architectural location of their growth zones. The cellular organization of monocot leaf growth zones is very similar to that of roots, which is probably related to the similar constraints to which these tissues have been exposed in an evolutionary context (Walter *et al.*, 2009). In contrast to dicotyledonous leaf growth zones, root apices and growth zones of monocotyledonous leaves are protected from atmospheric temperature variations by being embedded in the soil or within the sheaths of older leaves, respectively. Moreover, the growth zones of roots and monocotyledonous leaves are not involved in photosynthesis, which is itself a process strongly controlled by the circadian clock (Harmer *et al.*, 2000).

Primary metabolism does not differ strongly between monocot and dicot species, which is reflected in prominent differences between starch and sucrose concentrations in the morning and evening in young leaves of both plant types

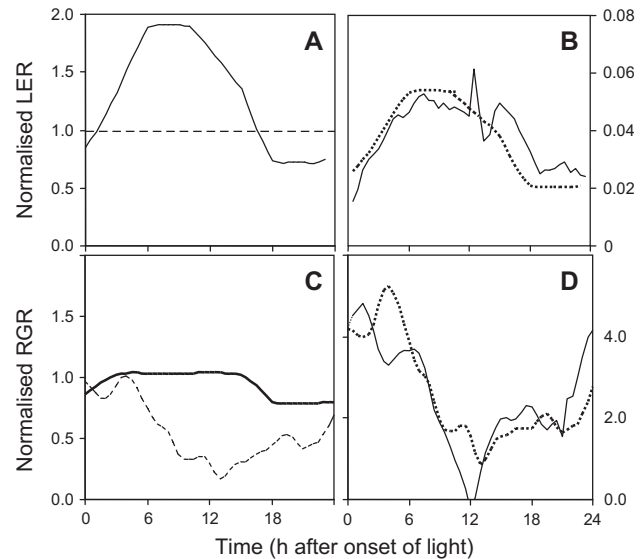


Fig. 7. Addition of the effects of temperature and endogenous rhythms in *Z. mays* (A, B) and *N. tabacum* (C, D). (A, C) Effect of temperature (solid line) and of endogenous rhythms (dotted line). (B, D) Experimental diel pattern (mean of experiments on days 2–4, solid lines) and simulation from the superposition of the two effects presented in (A) and (C).

under both temperature regimes. The periodic fluctuations of starch and sucrose in *Z. mays* in constant temperature suggest that light–dark cycles exert a stronger effect than available carbohydrates on growth patterns in monocotyledonous leaves.

The homologues of genes of the central oscillator of the plant circadian clock are conserved in monocots (Miwa *et al.*, 2006). Nevertheless, our data shows that maize and rice leaf growth proceeds constantly throughout the diel cycle when plants are transferred from a day–night growth regime into continuous light (Fig. 5A). The fact that leaf growth of the C_4 dicot species *F. bidentis* responds similarly to temperature alterations as the investigated C_3 dicot species indicates that differences between maize and the investigated dicot species are related to differences in the two families (possibly their growth zone organization) rather than to differences in photosynthetic metabolism. This is confirmed by the growth pattern of *O. sativa*, which as a C_3 monocot species does not show internal growth rhythms.

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References

- Amir J, Sinclair TR.** 1991. A model of the temperature and solar radiation effects on spring wheat growth and yield. *Field Crops Research* **28**, 47–58.
- Ben-Haj-Salah H, Tardieu F.** 1995. Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length: analysis of the coordination between cell division and cell expansion. *Plant Physiology* **109**, 861–870.
- Bigün J, Granlund GH.** 1987. Optimal orientation detection of linear symmetry. *International Conference on Computer Vision* 433–438.
- Devlin P, Kay S.** 2000. Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. *The Plant Cell Online* **12**, 2499–2510.
- Devlin P, Kay S.** 2001. Circadian photoperception. *Annual Review of Physiology* **63**, 677–694.
- Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ, Webb AAR.** 2005. Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* **309**, 630–633.
- Dowson-Day MJ, Millar AJ.** 1999. Circadian dysfunction causes aberrant hypocotyl elongation patterns in Arabidopsis. *The Plant Journal* **17**, 63–71.
- Franklin KA.** 2009. Light and temperature signal crosstalk in plant development. *Current Opinion in Plant Biology* **12**, 63–68.
- Granier C, Massonnet C, Turc O, Muller B, Chenu K, Tardieu F.** 2002. Individual leaf development in *Arabidopsis thaliana*: a stable thermal-time-based programme. *Annals of Botany* **89**, 595–604.
- Granier C, Tardieu F.** 1998. Is thermal time adequate for expressing the effects of temperature on sunflower leaf development? *Plant, Cell and Environment* **21**, 695–703.
- Granier C, Tardieu F.** 2009. Multi-scale phenotyping of leaf expansion in response to environmental changes: the whole is more than the sum of parts. *Plant, Cell and Environment* **32**, 1175–1184.
- Harmer SL.** 2009. The circadian system in higher plants. *Annual Review of Plant Biology* **60**, 357–377.
- Harmer SL, Hogenesch JB, Straume M, Chang H, Han B, Zhu T, Wang X, Kreps JA, Kay SA.** 2000. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science* **290**, 2110–2113.
- Hotta CT, Gardner MJ, Hubbard KE, Baek SJ, Dalchau N, Suhita D, Dodd AN, Webb AAR.** 2007. Modulation of environmental responses of plants by circadian clocks. *Plant, Cell and Environment* **30**, 333–349.
- James AB, Monreal JA, Nimmo GA, Kelly CL, Herzyk P, Jenkins GI, Nimmo HG.** 2008. The circadian clock in Arabidopsis roots is a simplified slave version of the clock in shoots. *Science* **322**, 1832–1835.
- Johnson FH, Eyring H, Williams RW.** 1942. The nature of enzyme inhibitions in bacterial luminescence: sulfanilamide, urethane, temperature and pressure. *Journal of Cellular and Comparative Physiology* **20**, 247–268.
- Jones MA.** 2009. Entrainment of the Arabidopsis circadian clock. *Journal of Plant Biology* **52**, 202–209.
- Jones MGK, Outlaw Jr WH, Lowry OH.** 1977. Enzymic assay of 10^{-7} to 10^{-14} moles of sucrose in plant tissues. *Plant Physiology* **60**, 379–383.
- Keating BA, Carberry PS, Hammer GL, et al.** 2003. An overview of APSIM, a model designed for farming systems simulation. *European Journal of Agronomy* **18**, 267–288.
- Kobayashi Y, Weigel D.** 2007. Move on up, it's time for change: mobile signals controlling photoperiod-dependent flowering. *Genes and Development* **21**, 2371–2384.
- Matsubara S, Hurry V, Druart N, Benedict C, Janzik I, Chavarria-Krauser A, Walter A, Schurr U.** 2006. Nocturnal changes in leaf growth of *Populus deltoides* are controlled by cytoplasmic growth. *Planta* **223**, 1315–1328.
- McClung CR.** 2006. Plant circadian rhythms. *The Plant Cell* **18**, 792–803.
- Miwa K, Serikawa M, Suzuki S, Kondo T, Oyama T.** 2006. Conserved expression profiles of circadian clock-related genes in two *Lemna* species showing long-day and short-day photoperiodic flowering responses. *Plant and Cell Physiology* **47**, 601–612.
- Nakajima M, Imai K, Ito H, Nishiwaki T, Murayama Y, Iwasaki H, Oyama T, Kondo T.** 2005. Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation *in vitro*. *Science* **308**, 414–415.
- Nozue K, Covington MF, Duek PD, Lorrain S, Fankhauser C, Harmer SL, Maloof JN.** 2007. Rhythmic growth explained by coincidence between internal and external cues. *Nature* **448**, 358–361.
- Nozue K, Maloof JN.** 2006. Diurnal regulation of plant growth. *Plant, Cell and Environment* **29**, 396–408.
- Parent B, Turc O, Gibon Y, Stitt M, Tardieu F.** 2010. Modelling temperature-compensated physiological rates, based on the coordination of responses to temperature of developmental processes. *Journal of Experimental Botany* doi:10.1093/jxb/erq003.
- Penfield S.** 2008. Temperature perception and signaltransduction in plants. *New Phytologist* **179**, 615–628.
- Pietruszka M, Lewicka S, Pazurkiewicz-Kocot K.** 2007. Temperature and the growth of plant cells. *Journal of Plant Growth Regulation* **26**, 15–25.
- R Development Core Team.** 2005. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
- Raper CD, Weeks WW, Wann M.** 1976. Temperatures in early post-transplant growth: influence on carbohydrate and nitrogen utilization and distribution in tobacco. *Crop Science* **16**, 753–757.
- Resco V, Hartwell J, Hall A.** 2009. Ecological implications of plants' ability to tell the time. *Ecology Letters* **12**, 583–592.
- Rolland-Lagan A, Bangham JA, Coen E.** 2003. Growth dynamics underlying petal shape and asymmetry. *Nature* **422**, 161–163.
- Sadok W, Naudin P, Boussuge B, Muller B, Welcker C, Tardieu F.** 2007. Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. *Plant, Cell and Environment* **30**, 135–146.

- Schmundt D, Stitt M, Jähne B, Schurr U.** 1998. Quantitative analysis of the local rates of growth of dicot leaves at a high temporal and spatial resolution, using image sequence analysis. *The Plant Journal* **16**, 505–514.
- Somers DE, Devlin PF, Kay SA.** 1998. Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. *Science* **282**, 1488–1490.
- Sulpice R, Pyl ET, Ishihara H, et al.** 2009. Starch as a major integrator in the regulation of plant growth. *Proceedings of the National Academy of Sciences, USA* **106**, 10348–10353.
- Tsakaya H, Beemster GTS.** 2006. Genetics, cell cycle and cell expansion in organogenesis in plants. *Journal of Plant Research* **119**, 1–4.
- Walter A, Feil R, Schurr U.** 2002. Restriction of nyctinastic movements and application of tensile forces to leaves affects diurnal patterns of expansion growth. *Functional Plant Biology* **29**, 1247–1258.
- Walter A, Silk WK, Schurr U.** 2009. Environmental effects on spatial and temporal patterns of leaf and root growth. *Annual Review of Plant Biology* **60**, 279–304.
- Wiese A, Christ MM, Virnich O, Schurr U, Walter A.** 2007. Spatio-temporal leaf growth patterns of *Arabidopsis thaliana* and evidence for sugar control of the diel leaf growth cycle. *New Phytologist* **174**, 752–761.
- Wyrzykowska J, Pien S, Hui Shen W, Fleming AJ.** 2002. Manipulation of leaf shape by modulation of cell division. *Development* **129**, 957–964.
- Yan HP, Kang MZ, Reffye P, Dingkun M.** 2004. A dynamic, architectural plant model simulating resource-dependent growth. *Annals of Botany* **93**, 591–602.

RESEARCH ARTICLE #III

Katrine Kjaer, Richard Poiré, Carl-Otto Ottosen & Achim Walter (2010 - submitted) Changes in time-of-day application of light, day length and night temperature lead to a rapid adjustment in chrysanthemum leaf growth pattern and carbohydrate turnover. *Plant, Cell & Environment*.

1 **Changes in time-of-day application of light, day length and night**
2 **temperature lead to a rapid adjustment in chrysanthemum leaf**
3 **growth pattern and carbohydrate turnover**

4
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14

15 **Keywords:** image processing, starch, relative growth rate, circadian rhythm,

16

17 **ABSTRACT**

18 Leaf growth occurs in an ever-changing environment. It is unclear, how rapid alterations of
19 light and temperature, usually interconnected in the field, lead to alterations of leaf growth
20 patterns due to physiological parameters restricting leaf growth. In this study, chrysanthemum
21 plants were exposed to a change in the time-of-day application of light, to shorter day length
22 and to lower night temperature (LNT). We observed a clear shift in the diel (24 h) cycle of
23 leaf relative growth rate (RGR) indicating a resetting of the circadian clock with a zeitgeber in

24 the early morning. In the treatment with LNT we observed a decrease in overall leaf growth
25 during the acclimation phase. Furthermore, overall leaf starch and diurnal sucrose contents
26 were affected by the light and temperature treatment, indicating that dynamic alterations of
27 light and temperature can lead to a non-linear alteration of leaf carbohydrate contents. This in
28 turn affects leaf growth dynamics in a characteristic manner. These results shed new light on
29 the dynamic processes during acclimation towards altered environmental responses of plants
30 in fluctuating climates.

31

32 **INTRODUCTION**

33 Plants are adapted to sense day length in order to schedule developmental events, such as
34 flowering, to coincide with particular environmental conditions (Jackson, 2009). In dicot
35 species this accurate sensing is based on the circadian clock, which has evolved as an
36 endogenous 24-hour oscillator present for most biochemical and physiological processes such
37 as CO₂ assimilation, carbohydrate availability and plant growth (Kreps & Kay 1997; Harmer
38 et al. 2000; Nozue & Maloof 2006; Poiré et al. 2010). Plants of the dicot model species
39 *Arabidopsis thaliana* sense day length and shift their pattern in starch accumulation during the
40 day and starch degradation during the night within the first 24 h after transferring the plants
41 from one day length to another (Gibon et al. 2004; Lu, Gehan & Sharkey 2005; Smith & Stitt
42 2007). Leaf and hypocotyl growth seem to be controlled by the circadian clock indicating that
43 plants with a clock period corresponding to the environment perform better than plants with
44 circadian clocks differing from the environment (Dodd et al. 2005; Nozue et al. 2007).
45 Furthermore, it was recently evidenced that this reduction in growth was caused by reduced
46 carbohydrate utilisation at night (Graf et al. 2010).

47 Short days (SD) promote a decrease in dry weight of plants that are otherwise grown in long
48 days (LD), even when the daily light integral (DLI) is the same (Adams & Langton 2005).

49 This phenomenon is partly explained by a decreased light use efficiency under high light
50 intensities in SD conditions. Furthermore, SD conditions with higher light intensities are often
51 accompanied by a decrease in individual leaf size, possibly due to formation of leaves with a
52 thicker palisade tissue and a larger mesophyll surface area, similar to sun leaves (Björkman
53 1981; Adams & Langton 2005). Even though leaf areas are smaller when compared to plants
54 grown in low light intensities and LD, the short-term acclimation patterns in leaf expansion to
55 a change in light intensity or day length have to our knowledge not been studied. However, it
56 has been suggested that the absolute leaf size is determined shortly after bud break, when
57 growth is maximum (Granier & Tardieu 1999; Cookson, Van Lijsebettens & Granier 2005).
58 This suggests that the light signal sensed by mature leaves for changes in the anatomical
59 differentiation of growing leaves must be perceived at a specific stage and very early in leaf
60 development (Yano & Terashima 2001; Kim et al. 2005).

61 A link between leaf expansion rate and carbohydrate metabolism has been suggested, but only
62 during early development stages (Granier & Tardieu 1999). This was confirmed by Nagel,
63 Schurr & Walter (2006), showing that a change in light intensity, and thereby a change in
64 carbohydrate availability had a minor effect on leaf growth. In contrast, Wiese et al. (2007)
65 showed that the starch-free mutant of *Arabidopsis thaliana* changed temporal growth activity
66 strongly in relation to altered carbohydrate availability. Thus, alterations in carbohydrate
67 availability impose significant effects on the circadian regulated activities of growth, even
68 though this may not influence final leaf area.

69 In the field, alterations in day length or light intensity are linked to alterations in temperature,
70 which, in turn, also exert characteristic effects on the circadian clock (Harmer 2009) which
71 can induce non-linear alterations of leaf growth (Walter, Silk & Schurr 2009; Poiré et al.
72 2010). As plants are adapted to adjust growth and metabolism to combined alterations of
73 temperature and light, it is important to adjust experimental protocols for this fact. The

74 manipulation of climatic parameters allows studies of the relation between carbohydrate
75 metabolism and growth activity in plants with a normally functioning circadian clock.
76 Nowadays, several methods of high-resolution growth analysis (for a review see Walter et al.
77 2009) allow us to measure the tiniest changes in the growth patterns of single leaves and roots
78 non-invasively.

79 The aim of this study was to monitor diel patterns of leaf growth and carbohydrate content in
80 response to combinations of altered light and temperature using, *Chrysanthemum x*
81 *morifolium* an appropriate dicotyledonous species since it retains vegetative growth as long as
82 day length is above 12 h. Moreover, it is an important horticultural plant for which it is of
83 interest to realize a most efficient use of light and energy.

84

85 **MATERIALS AND METHODS**

86 **Plant material, climatic conditions and experimental set up**

87 Cuttings of *Chrysanthemum x morifolium* cultivar ‘Coral Charm’ were propagated in soil
88 under long day conditions in a greenhouse with 18/6 h day/night. After two weeks, plants
89 were pinched back to three leaves, and after a total of five weeks, 50 plants were moved to a
90 climate chamber with 18/6 h day/night regime and day/night temperature of 20/18°C.

91 After four days of acclimation to climate chamber conditions the plants were exposed to one
92 of three different treatments for four days; LD: LD conditions of 18/6 h day/night, SD: a shift
93 to SD conditions after day one by an extended night of 6 h followed by 12/12 h day/night or
94 SDLNT: a shift to SD conditions and low night temperature (LNT) by extended night of 6 h
95 followed by 12/12 h day/night with 20/12°C day/night temperature. For each treatment,
96 different plants at the same developmental stage were used. Each treatment was repeated three
97 times with several replicate plants analysed per population.

98 Illumination within the chamber was provided by two sets of lamps to allow for comparable
99 light integrals in LD and SD. In LD, the light source was changed every other hour throughout
100 the 18 h photoperiod. During the first hour of each day, the light source was metal halide
101 lamps (Master HPI-T Plus; Phillips, Eindhoven, The Netherlands) and during the second hour
102 the light source were high pressure sodium lamps (Master SON-T PIA Agro; Phillips,
103 Eindhoven, The Netherlands).

104 The shift to SD conditions was accompanied by a shift to higher light intensity by jointly
105 using both sets of lamps to maintain the DLI during the 12 h day (SD) in relation to the 18 h
106 day (LD). The photosynthetic photon flux density (PPFD) was measured continuously
107 throughout the experiment in combination with measurements of leaf photosynthesis using the
108 external light sensor of an infrared gas-exchange system (LI-6400; LI-COR Lincoln,
109 Nebraska, USA). Temperature and humidity were logged every minute throughout the
110 experiments by a data logger placed at canopy level (Testo 175-H2, Testo AG, Lenzkirch,
111 Germany). Figure 1 illustrates the climatic conditions during the first three days.

112

113 **Growth analysis**

114 Young leaves of four plants were selected for growth analysis. The 3rd or 4th youngest leaf
115 was used being less than 50% of the size of a fully grown leaf. The leaves were mechanically
116 fixed (Walter, Feil & Schurr 2002) by using a strip of parafilm (Parafilm M, Pechiney Plastic
117 Packaging Company), and kept flat with five nylon threads clamped to the edge of the leaf,
118 using shortened hair clips and fabric tape to protect the leaf. Each of the threads was pulled
119 with a weight of 5 g and spun over a metal ring surrounding the whole leaf. Leaf fixation with
120 appropriate weights does not affect temporal and spatial growth patterns, as shown previously
121 in *Ricinus communis* (Walter et al. 2002) and in *Arabidopsis thaliana* (Wiese et al. 2007). To
122 establish the leaf expansion analysis procedure for *Chrysanthemum x morifolium*, the

123 influence of different weights (ranged from 0.5 g to 10 g) had been analysed showing that
124 fixation with five weights of 5 g each per leaf was sufficient to keep the leaf in an optical
125 plane of the camera and did not affect leaf expansion.

126 Images of fixed leaves were acquired with CCD cameras (Sony XC55 or XC75; Sony, Köln,
127 Germany and Scorpion SCOR-20SO, Point Grey Research, Vancouver BC, Canada)
128 positioned above the plants and equipped with a standard objective lens (25 mm;
129 Cosmiar/Pentax; The Imaging Source, Bremen, Germany) and an infrared interference filter
130 (880 nm; Edmund Optics, Karlsruhe, Germany). Constant illumination throughout the day and
131 night without affecting plant growth and photosynthesis was provided by infrared diodes (880
132 nm; Conrad Electronics, Hirschau, Germany). Grey value images were acquired every 180 s
133 and were saved in multi-tiff format. Image sequences were evaluated for average RGR of the
134 leaf lamina by the structure tensor approach (Schmundt et al. 1998; Walter et al. 2002; Wiese
135 et al. 2007). As a result of shading by the camera and infrared diodes, light intensity reaching
136 the analysed leaf was reduced to about half of the ambient light intensity at plant level.

137

138 **Carbohydrate analysis**

139 For the carbohydrate analysis, leaf discs with a diameter of seven mm were punched out of the
140 interveinal tissue of the tip of four leaves selected using the same criteria as for the growth
141 measurements. The leaf discs were sampled at 00:00, 06:00, 12:00 and 18:00 h each day
142 during the three days in each treatment. Then, after fresh weight (FW) had been determined,
143 they were frozen in liquid nitrogen and stored at -80°C for further extraction. Soluble
144 carbohydrates were extracted from frozen leaf material and glucose, fructose and sucrose
145 concentrations were analysed in a coupled enzyme assay (Jones, Outlaw & Lowry 1977)
146 using a multiplate spectrophotometer (ht II; Anthos Mikrosysteme GmbH, Krefeld, Germany)
147 as described in Walter et al. (2002). The remaining leaf material was prepared for starch

148 analysis. The pellets were dried and then gelatinised in an autoclave at 120°C for 90 min.
149 After incubation with Na-acetate for 16 h, the supernatant was analysed for glucose by the
150 enzymatic assay described above.

151

152 **Physiological measurements**

153 Gas exchange of growing leaves was measured using a portable open path design infrared
154 gas-exchange system (LI-6400, LI-COR Lincoln, Nebraska USA). The third fully-expanded
155 leaf of each plant was clamped into a leaf cuvette (6 cm²) with controlled humidity.

156 Measurements lasted for three days after the start of experiment. PPFD, CO₂ concentration
157 and leaf temperature inside the chamber was set to track the ambient conditions in the climate
158 chamber. To reduce fluctuations in the reference CO₂, the air was buffered using a 50 l plastic
159 tank linked to a nozzle placed outside the growth chamber at the top of the greenhouse.

160 Chlorophyll fluorescence was measured with a MiniPam (Heinz Walz GmbH, Pulheim,
161 Germany) at 07:00 h each day. In total 15 leaves of individual plants were dark-adapted for 30
162 min with leaf clips followed by measurement of the maximum quantum efficiency of PSII
163 (F_v/F_m) expressed as: $F_v/F_m = (F_w - F_0)/F_w$ (Maxwell & Johnson 2000).

164

165 **Data analysis**

166 Statistical analyses were carried out using the R-language 'stat' package, release 2.10.0
167 (<http://www.r-project.org>). The effect of day and treatment on weekly means of RGR values
168 and leaf carbohydrates were analysed by a linear mixed effects model allowing for nested
169 random effects taking into consideration that different plants, with all individuals being at the
170 same developmental stage, were used in the treatments. This allowed results from day one to

171 be pooled across treatments. Means of the different treatments on day two, three and four
172 were separated by a pairwise t-test using the holms correction (95% confidence intervals).

173 **RESULTS**

174 Young expanding leaves of *Chrysanthemum x morifolium* showed diel leaf growth patterns
175 with maximum growth activity in the early morning when grown in an 18/6 h day/night
176 regime and 20/18°C day/night temperature (Fig. 2). Minimal growth activity was reached
177 some hours before onset of night, leading to roughly 12 h of increasing RGR and 12 h of
178 decreasing RGR within a diel cycle. Overall, RGR declined from day to day with the observed
179 leaf reaching maturity. When the plants were exposed to a change in the time-of day
180 application of light followed by SD, the plants displayed a distinct shift in leaf growth pattern
181 (Fig. 3). This shift was characterised by a resetting of the circadian clock towards the
182 zeitgeber in the early morning. In the first extended night, RGR did not decrease from
183 midnight to 06:00 h while in the second night there was a pronounced increase in growth
184 towards the early morning. This was repeated in the third night although RGR was overall
185 much lower due to the decrease in growth activity with ongoing development of the leaf.
186 When the SD treatment was combined with LNT, the daily average leaf growth activity
187 decreased significantly ($p < 0.05$) on day three (Fig. 4). The leaf growth patterns were similar
188 in the SD and SD LNT treatment showing that temperature was a less important zeitgeber for
189 the adjustment of leaf growth compared to a change in the time-of-day application of light.
190 As the growth maximum was shifted by some hours towards 6 o'clock (instead midnight) and
191 as overall, the entire 24-h-curve was shifted by six hours (Figs 2 and 3), it can be stated that
192 the onset of day - and not the onset of night - is the decisive point in time to which the phase
193 of the growth cycle is adjusted.

194 The starch content in growing leaves of the chrysanthemum plants was relatively high at all
195 time points and in all treatments (Fig. 6a). Furthermore, only a weak diurnal pattern of starch

196 accumulation during the day and starch degradation during the night could be detected. Also,
197 the daily average in leaf starch content was similar for all three days in the LD treatment (Fig.
198 6e). When the plants were subjected to SD, leaf starch contents generally increased. The daily
199 average in leaf starch contents was higher on both days two and three in SD compared to LD.
200 When the change in day length was combined with a low night temperature, leaf starch
201 contents generally decreased during the following two days and the daily average was lower
202 in the SD LNT treatment on day three compared to the LD treatment.

203 Diurnal changes in leaf sugars (glucose, fructose and sucrose) were not very pronounced but
204 present during the first day of the experiment, with weak maxima at 12:00 or 18:00 (Fig. 6b-
205 d). The daily average in glucose, fructose and sucrose was comparable during all days in the
206 LD treatment (Fig 5f-h). In SD, the temporal patterns became more unclear and the maxima of
207 the distributions did not show a clear temporal shift as it was the case for RGR maxima. The
208 two clearest features of the SD and SD LNT treatments were that i) the SD LNT treatment
209 increased leaf fructose contents significantly, but not glucose and sucrose and ii) for sucrose, a
210 marked decrease was seen during the extended 6 h night (24:00 until 06:00) in both SD
211 treatments, whereas no differences were seen in daily average values.

212 The chrysanthemum plantlets were grown under higher light intensities in the climate
213 chambers compared to the greenhouse environment. Therefore, their net photosynthesis (P_n)
214 was near saturation in the LD treatment during all three days in the climate chambers. For
215 these plants this value was $6.8 \pm 0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ at an average light intensity of 468 ± 3.7
216 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 7a). The higher light intensity during SD did not increase P_n and when SD
217 was combined with low night temperature, P_n decreased resulting in a significantly lower
218 daily carbon gain in this treatment compared to the SD and LD treatment (Fig 6b).

219 In the greenhouse environment the plants had a maximum capacity of PSII (F_v/F_m) at $0.82 \pm$
220 0.014 , thus almost reaching the maximal values of 0.83 of maximum capacity of PSII (Fig. 7)

221 (Johnson et al. 1993). In the growth chamber F_v/F_m was reduced to 0.77 ± 0.004 in the LD
222 treatment due to effects of increased light level, while F_v/F_m was significantly reduced to 0.72
223 ± 0.01 in the SD LNT treatment on day three ($p < 0.05$).

224

225 **DISCUSSION**

226 Chrysanthemum leaves show a pronounced diel growth cycle with maximum growth activity
227 in the early morning and a minimum 12 h later when grown under a day length of 18 h.
228 Similar 12 h growth patterns have been reported for *Nicotiana tabacum*, *Ricinus communis*
229 and *Arabidopsis thaliana* (Wiese et al. 2007; Walter & Schurr 2005; Walter et al. 2002;
230 Walter et al. 2009). The present results suggest that both day length and temperature have
231 minor influence on the phasing of the leaf growth pattern, whereas the time-of-day application
232 of light seems to be the zeitgeber to reset the circadian clock in the early morning. In
233 *Nicotiana tabacum*, circadian oscillations of leaf growth were found to continue for up to two
234 weeks after plants were transferred from a 12 h photoperiod to continuous light (Walter &
235 Schurr 2005; Poiré et al. 2010), confirming that the circadian clock controls the oscillations in
236 leaf growth.

237 The change in the time-of-day application of light followed by SD induced a phase-shift in
238 leaf growth pattern in the chrysanthemum plants, confirming that growth activity in plants is
239 regulated by an interaction between light-dependent factors such as the phytochrome system
240 and the internal circadian clock (Dodd et al. 2005; Nozue et al. 2007). When plants were
241 exposed to LNT of 12°C from the beginning of the first 12 h night, this phase-shift was
242 accompanied by significantly lower growth activity. Lower leaf growth activity is often
243 coinciding with decreased glucose/fructose ratio that might be correlated with altered
244 hexokinase and phosphoglucose isomerase activities (Walter & Schurr 2005), which is also
245 the case here. Low temperatures can stall the expression of genes related to the circadian

246 clock (Ramos et al. 2005) and reset the clock followed by a new rhythm in circadian regulated
247 growth activities (Nozue et al. 2007). In the present experiment it was not possible to
248 distinguish the effect of the LNT, which confirms that temperature variations have only minor
249 effects on the periodicity and amplitude of leaf growth pattern and other rhythmic processes
250 controlled by the circadian clock, at least when temperature variations are followed by the
251 light-dark cycles (Poiré et al. 2010; Nakajima et al. 2005).

252 Circadian rhythm effects on leaf growth are pronounced in dicotyledons, but can be largely
253 neglected in monocotyledons, possibly due to the difference in the cellular organization and
254 architectural location of growth zones (Poiré et al. 2010). Also, light-dark cycles exert a
255 stronger effect on growth patterns than available carbohydrates. The rapid adjustment in leaf
256 growth in chrysanthemum was followed by a less clear change in temporal patterns of
257 carbohydrate concentrations. Yet, the lower growth activity of plants in the LNT treatment
258 coincided with decreased leaf starch content and glucose/fructose ratio. Furthermore, a
259 distinct decrease in leaf sucrose contents occurred during the 6 h extended night in the SD
260 treatment, where growth activity and hence growth-related demand for carbohydrates was at
261 its highest. This suggests a direct correlation between sucrose availability and diel leaf growth
262 patterns (Wiese et al. 2007).

263 Arabidopsis and several other plant species show prominent differences in leaf carbohydrate
264 contents in the morning and evening when grown in various day lengths (Chatterton & Silvius
265 1979; Gibon et al. 2009; Poiré et al. 2010). This effect was not pronounced due to high light
266 conditions resulting in carbohydrate accumulation, which itself affects the turnover of
267 photosynthates (Kuehny et al. 1991; Makino et al. 1997).

268 The chlorophyll fluorescence result indicates slight photoinhibition due to the change in light
269 level. During the following days the maximum capacity of PSII remained stable, showing that
270 the photosynthetic apparatus had adapted to the new light conditions. When the conditions

271 were shifted towards short day, there was only a small reduction in the photosynthetic
272 efficiency. However, the photosynthetic efficiency values decreased significantly, when plants
273 were exposed to short day and low night temperature. The decrease in total carbon gain was
274 due to a decrease in P_n . Leaf photosynthesis have earlier been shown to be negatively
275 correlated with leaf starch contents in chrysanthemum plants grown at low night temperatures
276 (Kjaer et al. 2007). However, the decrease in photosynthetic efficiency together with low leaf
277 temperatures in the morning possibly explained the lower P_n in the LNT experiment.
278 The results have a significant value in describing plant responses to an ever-changing
279 environment occurring in greenhouse production and as shown in this study, daily changes in
280 the timing of light may induce continuous phase-shifts in the rhythmic periodicity of leaf
281 growth.

282

283 **CONCLUSIONS**

284 The manipulation of the circadian clock induced by a change in the time-of day application of
285 light was useful as a tool in showing a phase-shift in the clock-regulated leaf growth pattern of
286 chrysanthemum plants with a normally functioning circadian clock. The response of leaf
287 carbohydrate contents to a change in day length illustrates that shifts in carbohydrate turnover
288 are affected by light conditions and that periodicity and amplitude in leaf growth patterns are
289 controlled by the interaction between the circadian clock and carbohydrate availability.

290

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297

298 REFERENCES

299 Adams S.R. & Langton F.A. (2005) Photoperiod and plant growth: a review. *Journal of*
300 *Horticultural Science & Biotechnology* 80, 2-10.

301 Björkman O. (1981) Responses to different quantum flux densities. In *Encyclopedia of Plant*
302 *Physiology I* New series (eds O.L. Lange, C.B. Osmond & H. Ziegler), pp. 57-107.
303 Springer-Verlag, Berlin, Germany.

304 Chatterton N.J. & Silvius J.E. (1979) Photosynthate partitioning into starch in soybean leaves.
305 1. Effects of photoperiod versus photosynthetic period duration. *Plant Physiology* 64, 749-
306 753.

307 Cookson S.J., Van Lijsebettens M. & Granier C. (2005) Correlation between leaf growth
308 variables suggest intrinsic and early controls of leaf size in *Arabidopsis thaliana*. *Plant*
309 *Cell and Environment* 28, 1355-1366.

310 Dodd A.N., Salathia N., Hall A., Kevei E., Toth R., Nagy F., Hibberd J.M., Millar A.J. &
311 Webb A.A.R. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and
312 competitive advantage. *Science* 309, 630-633.

313 Gibon Y., Blasing O.E., Palacios-Rojas N., Pankovic D., Hendriks J.H.M., Fisahn J., Höhne
314 M., Gunther M. & Stitt M. (2004) Adjustment of diurnal starch turnover to short days:
315 depletion of sugar during the night leads to a temporary inhibition of carbohydrate
316 utilization, accumulation of sugars and post-translational activation of ADP-glucose
317 pyrophosphorylase in the following light period. *Plant Journal* 39, 847-862.

318 Gibon Y., Pyl E.T., Sulpice R., Lunn J.E., Höhne M., Günther M. & Stitt M. (2009)
319 Adjustment of growth, starch turnover, protein content and central metabolism to a

320 decrease of the carbon supply when Arabidopsis is grown in very short photoperiods.
321 *Plant, Cell & Environment* 32, 859–874.

322 Graf A., Schlereth A., Stitt M. & Smith A.M. (2010) Circadian control of carbohydrate
323 availability for growth in Arabidopsis at night. *Proceedings of the National Academy of*
324 *Sciences* 107, 9458-9463.

325 Granier C. & Tardieu F. (1999) Leaf expansion and cell division are affected by reducing
326 absorbed light before but not after the decline in cell division rate in the sunflower leaf.
327 *Plant, Cell and Environment* 22, 1365-1376.

328 Harmer S.L. (2009) The circadian system in higher plants. *Annual Review of Plant Biology* 60,
329 357 – 377.

330 Harmer S.L., Hogenesch L.B., Straume M., Chang H.S., Han B., Zhu T., Wang X., Kreps J.A.
331 & Kay S.A (2000) Orchestrated transcription of key pathways in Arabidopsis by the
332 circadian clock. *Science* 290, 2110-2113.

333 Jackson S.D. (2009) Plant responses to photoperiod. *New Phytologist* 60, 379-383

334 Jones M.G.K., Outlaw W.H. & Lowry O.H. (1977) Enzymatic assay of 10^{-7} to 10^{-14} moles of
335 sucrose in plant tissues. *Plant Physiology* 60, 379-383.

336

337 Johnson G.N., Young A.J., Scholes J.D. & Horton P. (1993) The dissipation of excess
338 excitation-energy in british plant-species. *Plant, Cell and Environment* 16, 673-679.

339

340 Kim G.T, Yano S., Kozuka T. & Tsukaya H. (2005) Photomorphogenesis of leaves: shade-
341 avoidance and differentiation of sun and shade leaves. *Photochemical & Photobiological*
342 *Sciences* 4, 770-774.

343 Kjaer K.H., Thorup-Kristensen K., Rosenqvist E. & Aaslyng J.M. (2007) Low night
344 temperatures change whole-plant physiology and increase starch accumulation in

345 *Chrysanthemum morifolium*. *Journal of Horticultural Science and Biotechnology* 82, 867-
346 874.

347 Kreps J.A. & Kay S.A. (1997) Coordination of plant metabolism and development by the
348 circadian clock. *Plant Cell* 9, 1235-1244.

349 Kuehny J.S., Peet M.M., Nelson P.V. & Willits D.H. (1991) Nutrient dilution by starch in
350 CO₂-enriched *Chrysanthemum*. *Journal of Experimental Botany* 42, 711-716.

351 Lu Y., Gehan J.P. & Sharkey T.D. (2005) Day length and circadian effects on starch
352 degradation and maltose metabolism. *Plant Physiology* 138, 2280-2291.

353 Makino A., Sato T., Nakano H. & Mae T. (1997) Leaf photosynthesis, plant growth and
354 nitrogen allocation in rice under different irradiances. *Planta* 203, 390-398.

355 Maxwell K. & Johnson G.N. (2000) Chlorophyll fluorescence – a practical guide. *Journal of*
356 *Experimental Botany* 51, 139-162.

357 Nagel K.A., Schurr U. & Walter A. (2006) Dynamics of root growth stimulation in *Nicotiana*
358 *tabacum* in increasing light intensity. *Plant, Cell and Environment* 29, 1936–1945

359 Nakajima M., Imai K., Ito H., Nishiwaki T., Murayama Y., Iwasaki H., Oyama T. & Kondo
360 T. (2005) Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation
361 *in vitro*. *Science* 308, 414-415.

362 Nozue K. & Maloof J.N. (2006) Diurnal regulation of plant growth. *Plant Cell and*
363 *Environment* 29, 396-408.

364 Nozue K., Covington M.F., Duek P.D., Lorrain S., Fankhauser C., Harmer S.L. & Maloof J.N.
365 (2007) Rhythmic growth explained by coincidence between internal and external cues.
366 *Nature* 448, 358-361.

367 Poiré R., Wiese-Klinkenberg A., Parent B., Mielewczik M., Schurr U., Tardieu F. & Walter
368 A. (2010) Diel time-courses of leaf growth in monocot and dicot species: endogenous
369 rhythms and temperature effects. *Journal of Experimental Botany* 61, 1751-1759.

370 Ramos A., Perez-Solis E., Ibanez C., Casado R., Collada C., Gomez L., Aragoncillo C. &
371 Allona I. (2005) Winter disruption of the circadian clock in chestnut. *Proceedings of the*
372 *National Academy of Sciences of the United States of America* 102, 7037-7042.

373 Schmundt D., Stitt M., Jahne B. & Schurr U. (1998) Quantitative analysis of the local rates of
374 growth of dicot leaves at a high temporal and spatial resolution, using image sequence
375 analysis. *Plant Journal* 16, 505-514.

376 Smith A.M. & Stitt M. (2007) Coordination of carbon supply and plant growth. *Plant Cell*
377 *and Environment* 30, 1126-1149.

378 Walter A., Feil R. & Schurr U. (2002) Restriction of nyctinastic movements and application
379 of tensile forces to leaves affects diurnal patterns of expansion growth. *Functional Plant*
380 *Biology* 29, 1247-1258.

381 Walter A. & Schurr U. (2005) Dynamics of leaf and root growth: Endogenous control versus
382 environmental impact. *Annals of Botany* 95, 891-900.

383 Walter A., Silk W.K. & Schurr U. (2009) Environmental effects on spatial and temporal
384 patterns of leaf and root growth. *Annual Review of Plant Biology* 60, 279–304.

385 Wiese A., Christ M.M., Virnich O., Schurr U. & Walter A. (2007) Spatio-temporal leaf
386 growth patterns of *Arabidopsis thaliana* and evidence for sugar control of the diel leaf
387 growth cycle. *New Phytologist* 174, 752-761.

388 Yano S. & Terashima I. (2001) Separate localization of light signal perception for sun or
389 shade type chloroplast and palisade tissue differentiation in *Chenopodium album*. *Plant*
390 *and Cell Physiology* 42, 1303-1310.

391

392 **Legends:**

393 **Figure 1.** Light intensity and temperature in treatments: Long day (black line), short day (red
394 line) and short day in combination with low night temperature. A. light intensity and B.
395 temperature in the climate chamber on day 1 and day 2-3. Mean value (n=4).

396

397 **Figure 2.** Leaf growth of *Chrysanthemum x morifolium* grown in long day conditions with
398 18/6 h light/ dark at 20/18°C temperature. Grey areas illustrate dark periods. Mean value
399 (n=3).

400

401 **Figure 3.** Leaf growth in *Chrysanthemum x morifolium* exposed to a change in the time-of-
402 day application of light on day two and a change in temperature during the first extended
403 night. Long day (black line), short day from day two (red line) and short day from day two
404 and low temperature from the beginning of the first night (blue line). Grey areas illustrate dark
405 periods. LD mean value (n=11), SD mean value (n=7), SD LNT mean value (n =12)

406

407 **Figure 4.** Average values of leaf growth during four days in three treatments. LD (black bars),
408 SD (grey bars) and SD LNT (white bars). On day one the average values for LD are shown for
409 three treatments (n=3). For day 2 to 4 the average values are from the three separate
410 treatments: LD (n=11), SD (n = 7), SD LNT (n = 12). B. Error bars are SE

411

412 **Figure 5.** (a-d) Diurnal patterns and (e-h) daily average values of leaf carbohydrate contents
413 in plants grown in three treatments. For diurnal patterns, the LD plants are shown as black
414 symbols, short day plants (red symbols) and short day and low night temperature (blue
415 symbols). Grey areas illustrate dark periods. On day 1, average values for all three treatments

416 are shown (n = 3). On day 2 and 3, values are only shown for each treatment (n=6). Error bars
417 are SE.

418 The daily values were averaged over three time points during each day (06:00, 12:00 and
419 18:00 h). LD plants (black bars), SD plants (grey bars), SD and LNT (white bars). Average
420 values are shown for all three treatments on day 1 (n =3), and for each treatment on day 2 and
421 3 (n = 18). Error bars are SE.

422

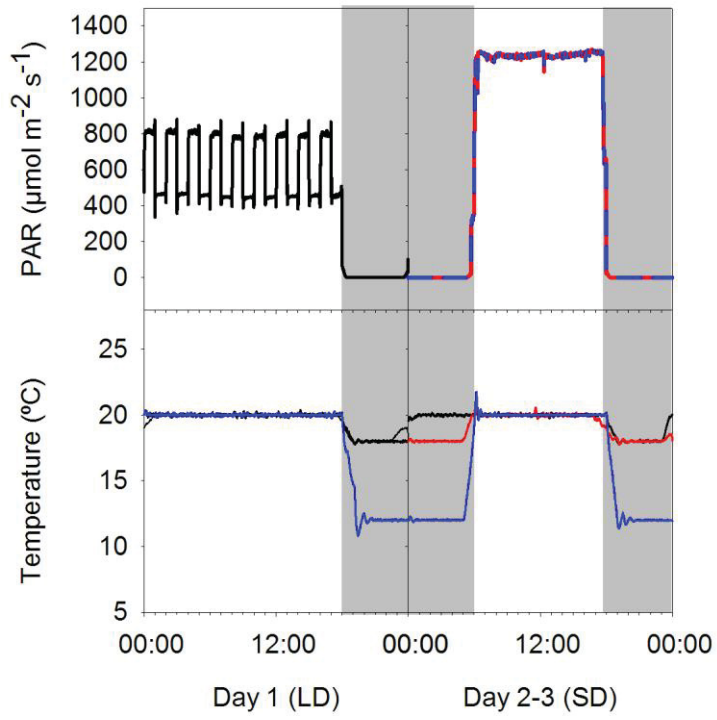
423 **Figure 6.** (a) Leaf photosynthesis (P_n , $\mu\text{mol m}^{-2} \text{s}^{-1}$), (b) daily carbon gain (DCG, mmol m^{-2}
424 day^{-1}) and (c) Daily light integral (DLI, $\text{mol m}^{-2} \text{day}^{-1}$) in three treatments. Long day (black
425 bars), short day (grey bars) and short day with low night temperature (white bars). On day 1
426 the average value are shown for all three treatments (n = 3), for day 2 – 3 the average values
427 are from the separate treatments (n = 2 for each treatment). Error bars are SE

428

429 **Figure 7.** F_v/F_m values on day 0 (in greenhouse), day 1 (after four days in climate chamber)
430 and the following days in the three treatments. Long day (black bars), short day (grey bars)
431 and short day with low night temperature (white bars). Mean values (n = 15). Error bars are
432 SE. D

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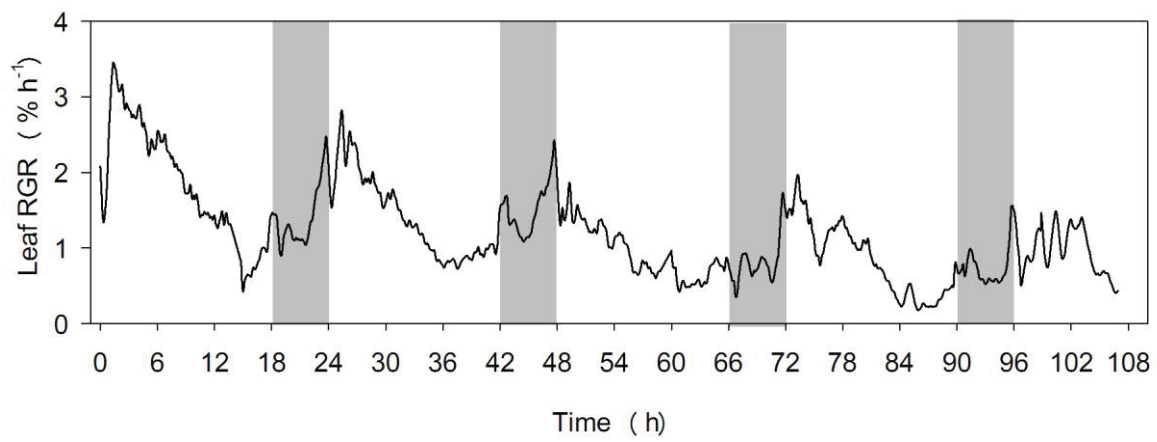
434 Fig 1:



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437 Fig 2



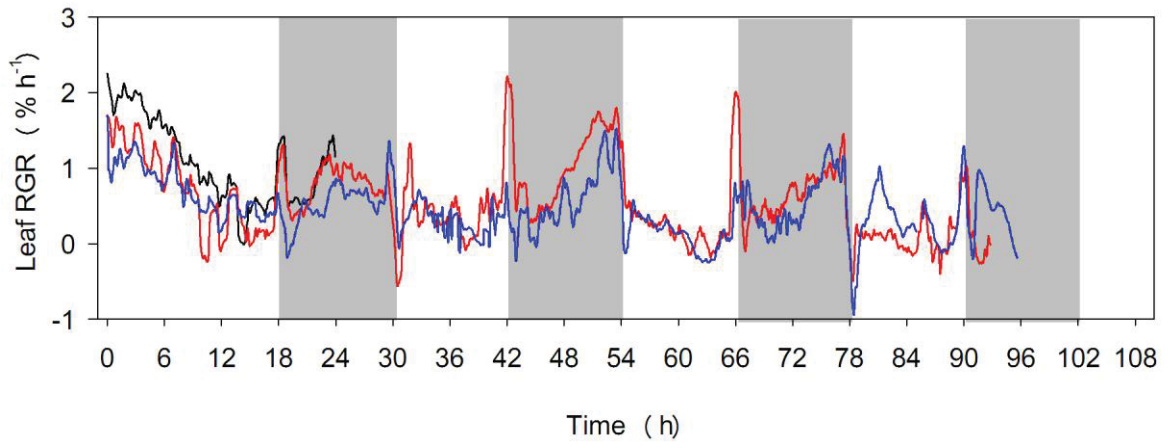
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441 Fig 3;

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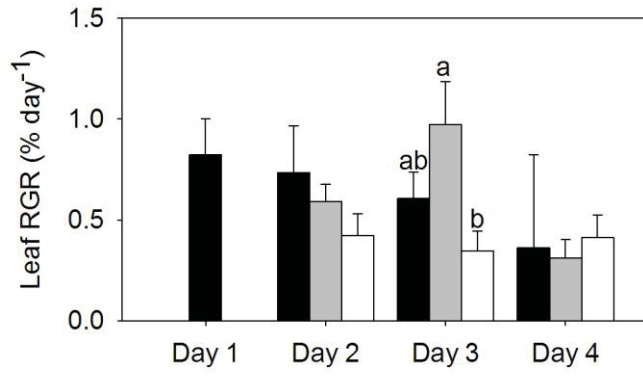


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446 Fig 4:

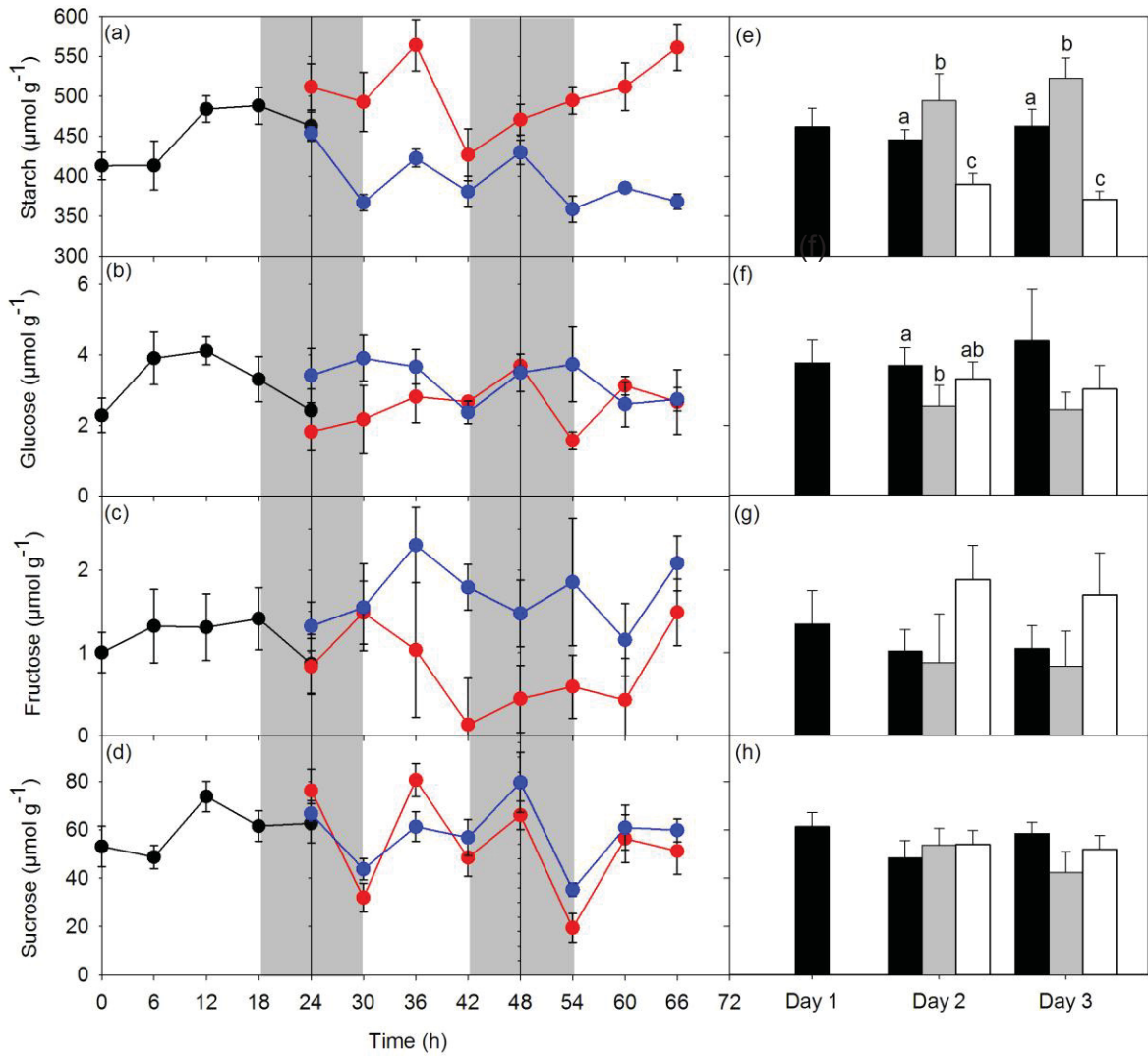


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449 Fig 5:

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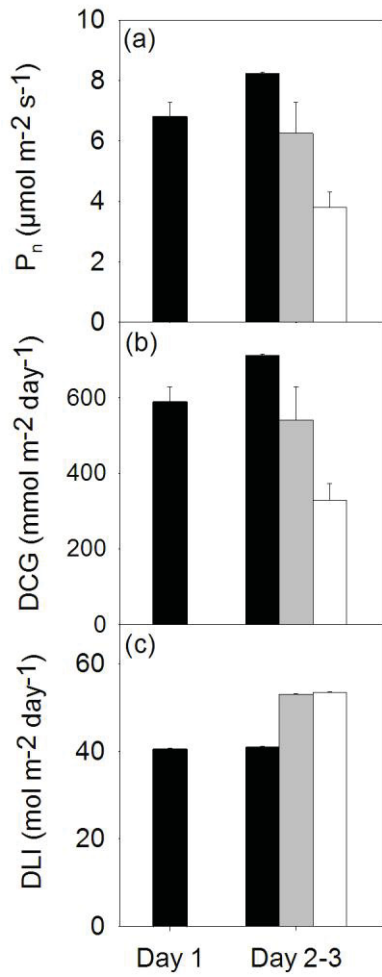


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454 Fig. 6



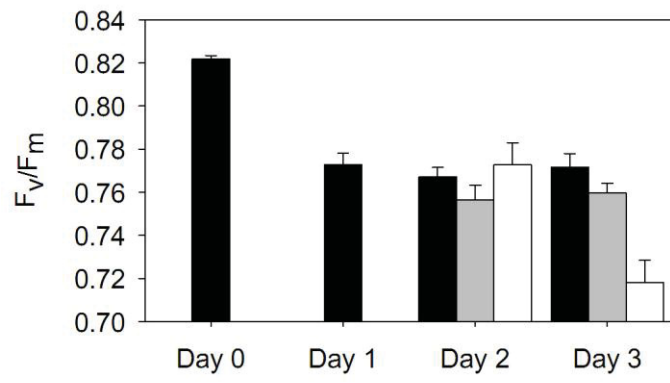
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458 **Fig. 7**

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ABSTRACT

Environmental cues have a major impact on plant growth and development. Plants have to make the best use of the available resources to achieve their full potential and maximum yield. When environmental conditions are suboptimal for given species then they have to invest more resources to survive resulting into lower yield. The focal point of this work was to broaden the physiological knowledge about the effects of temperature and light intensity on leaf growth dynamics. Many current scientific studies are based on the assumption that laboratory conditions are comparable with field conditions and that one can directly transpose the knowledge gained from the lab to the field. Field conditions are complex to reproduce artificially and laboratory conditions are often unrealistic. Those climatic discrepancies can lead to erroneous statements about plant response toward its environment.

It is one aim of this study to investigate *Ricinus communis* responses to artificial soil and air temperature scenarios. This work relies on the extensive use of Digital Image Sequence Processing (DISP). DISP allows the acquisition of time lapse movies of the growing tissues and calculating its relative growth rate. This imaging technique associated with accurate control and monitoring of the environmental conditions allows characterising plant responses to specific environmental parameters. Different levels of steady-state temperatures were applied to investigate the potential growth limitation while variable temperature scenarios were helped to understand the dynamics of those limitations. When the root-zone temperature in *Ricinus communis* was decreased, leaf growth occurred preferentially at night and was strongly inhibited during the day. Overall, leaf expansion, shoot biomass growth, root elongation and ramification decreased rapidly, carbon fluxes from shoot to root were diminished and carbohydrate contents of both root and shoot increased. Low root temperature increased hydrostatic tensions in shoot xylem. When root temperature was increased again, xylem tension reduced, leaf growth recovered rapidly, carbon fluxes from shoot to root increased, and carbohydrate pools were depleted.

The circadian clock is known to have pleiotropic effect on plant metabolisms underlying plant growth. Depending on the plant species, leaf growth patterns are differently affected by temperature variations and the circadian clock. We investigated the effect of continuous light and air temperature scenarios on monocotyledons and dicotyledonous species with different photosynthesis types. Dicotyledon species displayed rhythmicity in leaf growth and carbohydrate content regardless of the external light and temperature conditions. Monocotyledonous plants, in turn, did not retain any rhythm when grown under constant temperature. Photosynthesis type did not influence the phasing of the growth pattern. Based on model simulations it is concluded that diel leaf growth patterns in mono- and dicotyledons result from the additive effects of both circadian-clock-controlled processes and responses to environmental changes such as temperature and evaporative demand.

Leaf growth occurs in an ever-changing environment. It is unclear, how rapid alterations of light and temperature, usually interconnected in the field, lead to alterations of leaf growth patterns due to physiological parameters restricting leaf growth. In this study, chrysanthemum plants were exposed to a change in the time-of-day application of light, to shorter day length and to lower night temperature (LNT). We observed a clear shift in the diel (24 h) cycle of leaf relative growth rate (RGR) indicating a resetting of the circadian clock with a zeitgeber in the early morning. In the treatment with LNT we observed a decrease in overall leaf growth during the acclimation phase. The manipulation of the circadian clock induced by a change in the time-of day application of light was useful as a tool in showing a phase-shift in the clock-regulated leaf growth pattern of chrysanthemum plants with a normally functioning circadian clock. The response of leaf carbohydrate contents to a change in day length illustrates that shifts in carbohydrate turnover are affected by light conditions and that periodicity and amplitude in leaf growth patterns are controlled by the interaction between the circadian clock and carbohydrate availability.

There is a need to bridge the gap between lab-based phenotyping and field breeding. Modern phenomics strategies are aimed toward this goal. We should remain aware of the climatic differences between the field and the lab.

Mankind will be facing new agricultural challenges in the future and we will always need to improve our understanding of plant physiology. Our agriculture has to be more sustainable by using crops that make a better use of the available resources.

ZUSAMMENFASSUNG

Umwelt-Bedingungen haben einen großen Einfluss auf das Pflanzenwachstum und -entwicklung. Pflanzen haben, um die bestmögliche Nutzung der verfügbaren Ressourcen, um ihr volles Potential und maximalen Ertrag zu erzielen. Wenn Umgebungsbedingungen suboptimal sind für bestimmte Arten dann müssen diese mehr Mittel investieren, um zu überleben was zu geringerer Ausbeute führt. Diese Arbeit soll das Wissen über die Auswirkungen von Temperatur und Licht in Labor-Experimenten auf pflanzliche Wachstumsdynamik erhöhen. Viele aktuelle wissenschaftliche Studien basieren auf der Annahme, dass Laborbedingungen vergleichbar mit Feldbedingungen sind und dass eine direkte Umsetzung der gewonnenen Erkenntnisse aus dem Labor auf das Feld möglich ist. Feldbedingungen sind nur sehr schwer künstlich zu reproduzieren und Laborbedingungen sind oft unrealistisch. Die klimatischen Unterschiede können zu falschen Aussagen über pflanzliche Reaktionen auf ihre Umwelt führen.

Es ist ein Ziel dieser Studie, Antworten von *Ricinus communis* auf künstliche Boden- und Lufttemperatur Szenarien zu untersuchen. Diese Arbeit stützt sich auf die extensive Nutzung von Bildanalyse Verfahren (digital image sequence processing DISP). DISP erlaubt den Erwerb von Zeitraffer-Filmen der wachsenden Gewebe und der Berechnung ihrer relativen Zuwachsraten. Dieses bildgebende Verfahren mit präziser Steuerung und Überwachung der Umweltbedingungen erlaubt, pflanzliche Reaktionen auf Veränderungen bestimmter Umwelt-Parameter zu untersuchen. Unterschiedliche, konstante Temperaturen wurden angewandt, um potenzielle Wachstums-Einschränkungen zu untersuchen, während variable Temperatur-Szenarien angewandt wurden, um die Dynamik dieser Einschränkungen zu verstehen. Wenn die Wurzel-Zonen-Temperatur in *Ricinus communis* gesenkt war, kam Blattwachstum bevorzugt nachts zustande und war während des Tages stark gehemmt. Insgesamt wurden Blatt Expansion, Wachstum der Biomasse-, Wurzel-Ausdehnung und Verzweigung rasch reduziert; Kohlenstoffflüsse zur Wurzel waren vermindert und Kohlenhydratgehalte von Wurzel und Spross waren erhöht. Niedrige Temperatur Wurzel erhöhten hydrostatischen Spannungen in schießen Xylem. Als Wurzel-Temperatur wieder erhöht wurde, Xylem Spannung reduziert, Blattwachstum erholte sich rasch, Kohlenstoffflüsse von Aufnahme zu erhöhten, Wurzel- und Kohlenhydrat-Pools wurden reduziert.

Die circadiane Uhr ist bekannt, pleiotrope Effekte auf den dem Pflanzenwachstum zugrunde liegenden Stoffwechsel zu haben. Je nach Pflanzenart sind Blattwachstumsmuster unterschiedlich durch Temperaturschwankungen und die circadiane Uhr betroffen. Wir untersuchten die Wirkung von Dauerlicht- und Lufttemperatur Szenarien auf Monokotyledonen und dikotyle Arten mit unterschiedlichen Photosynthese-Typen. Dicotyle Arten zeigten Rhythmik von Blattwachstum und Kohlenhydratgehalten, die weitgehend unabhängig von den externen Licht- und Temperaturbedingungen waren. Monocotyle Pflanzen wiederum veränderten ihren Rhythmus, wenn sie unter veränderten Temperaturbedingungen wuchsen. Der Typ der Photosynthese hatte keinen Einfluss auf die Veränderung der Wachstumsmuster. Basierend auf Modellrechnungen wurde der Schluss gezogen, dass diurnale Blattwachstumsmuster in Mono- und Dikotyledonen sich aus der additiven Wirkung von circadianen Uhr-gesteuerten Prozessen und Reaktionen auf Veränderungen der Umwelt wie Temperatur und Verdunstung zustande kommen.

Blattwachstum spielt sich in einer sich ständig verändernden Umwelt ab. Es ist unklar, wie schnell Veränderungen von Licht und Temperatur, die in der Regel miteinander verbunden sind, zu Veränderungen der Blattwachstumsmuster aufgrund physiologischer Prozesse führen. In dieser Studie wurden Chrysanthempflanzen einer veränderten Verteilung von Licht, und einer verringerten Nachttemperatur (LNT) ausgesetzt. Wir beobachteten eine deutliche Verschiebung des diurnalen Wachstumszyklus der relativen Blattwachstumsrate (relative growth rate, RGR), was eine auf eine Rückstellung der circadianen Uhr mit einem

Zeitgeber am frühen Morgen zurückgeführt werden konnte. Bei der Behandlung mit LNT beobachteten wir einen Rückgang des Blattwachstums während der Akklimatisierungsphase.

Um die Lücke zwischen Labor-basierter Phänotypisierung und Feld-basierter Züchtung zu überbrücken, müssen verstärkte Forschungsanstrengungen unternommen werden. Moderne Phenomics-Strategien sind auf dieses Ziel ausgerichtet. Wir sollten die klimatischen Unterschiede zwischen Feld und Labor stets im Blick behalten. Die Menschheit wird sich neuen Herausforderungen der Landwirtschaft in Zukunft stellen müssen, wofür es essenziell ist, unser Verständnis der Physiologie der Pflanzen zu verbessern. Unsere Landwirtschaft muss nachhaltiger werden mit Hilfe von Pflanzen, die eine bessere Nutzung der verfügbaren Ressourcen erlauben.

REFERENCES

- Aaslyng JM, Lund JB, Ehler N & Rosenqvist E (2003) IntelliGrow: a greenhouse component-based climate control system. *Environmental Modelling & Software* **18**: 657-666.
- Ainsworth EA, Walter A & Schurr U (2005) *Glycine max* leaflets lack a base-tip gradient in growth rate. *Journal of Plant Research* **118**: 343-346.
- Ben-Haj-Salah H & Tardieu F (1995) Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length. Analysis of the coordination between cell division and cell expansion. *Plant Physiology* **109**: 861-870.
- Biskup B, Scharr H, Schurr U & Rascher U (2007) A stereo imaging system for measuring structural parameters of plant canopies. *Plant, Cell & Environment* **30**: 1299-1308.
- Bowler C, Van Montagu M & Inzé D (1992) Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 83-116.
- Breidenbach RW, Wade NL & Lyons JM (1974) Effect of chilling temperatures on the activities of glyoxysomal and mitochondrial enzymes from castor bean seedlings. *Plant Physiology* **54**: 324-327.
- Chaerle L, Leinonen I, Jones HG & Van Der Straeten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. *Journal of Experimental Botany* **58**: 773-784.
- Chenu K, Rey H, Dauzat J, Guilioni L & Lecoq J (2008) Estimation of light interception in research environments: a joint approach using directional light sensors and 3D virtual plants applied to sunflower (*Helianthus annuus*) and *Arabidopsis thaliana* in natural and artificial conditions. *Functional Plant Biology* **35**: 850-866.
- Cordell D, Drangert J-O & White S (2009) The story of phosphorus: Global food security and food for thought. *Global Environmental Change* **19**: 292-305.
- Davies WJ & Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**: 55-76.
- DeLucia EH, Heckathorn SA & Day TA (1992) Effects of soil temperature on growth, biomass allocation and resource acquisition of *Andropogon gerardii* Vitman. *New Phytologist* **120**: 543-549.
- Demple B & Harrison L (1994) Repair of oxidative damage to DNA: enzymology and biology. *Annual review of biochemistry* **63**: 915-948.
- Dennis ES & Peacock JW (2009) Vernalization in cereals. *Journal of Biology* **8**: 57.
- Devlin PF, Robson PRH, Patel SR, Goosey L, Sharrock RA & Whitelam GC (1999) Phytochrome D acts in the shade-avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. *Plant Physiology* **119**: 909-915.
- Dodd AN, Salathia N, Hall A, Kévei E, Tóth R, Nagy F, Hibberd JM, Millar AJ & Webb AAR (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* **309**: 630-633.
- FAO (Food and Agriculture Organization of the United Nations) (2010) *The state of food and agriculture 2009 – Livestock in the balance*. Rome, Italy, 140 pages. ISBN: 978-92-5-106215-9, ISSN: 0081-4539.

- Farquhar GD, von Caemmerer S & Berry JA (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* **149**: 78-90.
- Franklin KA (2009) Light and temperature signal crosstalk in plant development. *Current Opinion in Plant Biology* **12**: 63-68.
- Franklin KA & Whitelam GC (2005) Phytochromes and shade-avoidance responses in plants. *Annals of Botany* **96**: 169-175.
- Füllner K (2007) *The influence of spatially heterogeneous soil temperatures on plant structure and function*. Inaugural-Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität Düsseldorf.
- Furbank RT (2009) Plant phenomics: from gene to form and function. *Functional Plant Biology* **36**: v-vi.
- Granier C, Massonnet C, Turc O, Muller B, Chenu K & Tardieu F (2002) Individual leaf development in *Arabidopsis thaliana*: a stable thermal-time-based programme. *Annals of Botany* **89**: 595-604.
- Granier C & Tardieu F (1998) Is thermal time adequate for expressing the effects of temperature on sunflower leaf development? *Plant, Cell & Environment* **21**: 695-703.
- Grant NM (2010) *Thermogenesis in plants: the mode of heating and regulation in hot flowers*. Doctor of Philosophy thesis, School of Biological Sciences, Faculty of Science, University of Wollongong.
- Guy CL (2003) Freezing tolerance of plants: current understanding and selected emerging concepts. *Canadian Journal of Botany* **81**: 1216-1223.
- Harmer SL (2009) The circadian system in higher plants. *Annual Review of Plant Biology* **60**: 357-377.
- Hillel D (1998) *Environmental soil physics: Fundamentals, applications, and environmental considerations*. Academic Press, San Diego, CA, USA, 771 pages. ISBN-10: 0123485258.
- Holbrook NM, Shashidhar VR, James RA & Munns R (2002) Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. *Journal of Experimental Botany* **53**: 1503-1514.
- Jobbágy EG & Jackson RB (2001) The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry* **53**: 51-77.
- Kjaer K, Poiré R, Ottosen C-O & Walter A (2010 - submitted) Changes in time-of-day application of light, day length and night temperature lead to a rapid adjustment in chrysanthemum leaf growth pattern and carbohydrate turnover. *Plant, Cell & Environment*.
- Kumar SV & Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140**: 136-147.
- Lecoœur J, Poiré-Lassus R, Christophe A, Casadebaig P, Debaeke P, Vear F & Guilioni L (2010 - submitted and in revision) SUNFLO: A joint phenotyping and modelling approach to analyse and predict differences in yield potential of sunflower genotypes. *Functional Plant Biology*.
- Lockhart JA (1965) An analysis of irreversible plant cell elongation. *Journal of Theoretical Biology* **8**: 264-275.

- Long SP & Bernacchi CJ (2003) Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of Experimental Botany* **54**: 2393-2401.
- Ma Y, Li B, Zhan Z, Guo Y, Luquet D, de Reffye P & Dingkuhn M (2007) Parameter stability of the functional-structural plant model GREENLAB as affected by variation within populations, among seasons and among growth stages. *Annals of Botany* **99**: 61-73.
- Ma Y, Wen M, Guo Y, Li B, Cournède P-H & de Reffye P (2008) Parameter optimization and field validation of the functional-structural model GREENLAB for maize at different population densities. *Annals of Botany* **101**: 1185-1194.
- Massonnet C, Vile D, Fabre J, Hannah MA, Caldana C, Lisee J, Beemster GT, Meyer RC, Messerli G, Gronlund JT, Perkovic J, Wigmore E, May S, Bevan MW, Meyer C, Rubio-Díaz S, Weigel D, Micol JL, Buchanan-Wollaston V, Fiorani F, Walsh S, Rinn B, Gruissem W, Hilson P, Hennig L, Willmitzer L & Granier C (2010) Probing the reproducibility of leaf growth and molecular phenotypes: a comparison of three Arabidopsis accessions cultivated in ten laboratories. *Plant Physiology* **152**: 2142-2157.
- McClung CR (2006) Plant circadian rhythms. *The Plant Cell* **18**: 792-803.
- Menzel MI, Tittmann S, Bühler J, Preis S, Wolters N, Jahnke S, Walter A, Chlubek A, Leon A, Hermes N, Offenhäuser A, Gilmer F, Blümler P, Schurr U & Krause H-J (2009) Non-invasive determination of plant biomass with microwave resonators. *Plant, Cell & Environment* **32**: 368-379.
- Minchin PEH & Thorpe MR (2003) Using the short-lived isotope ¹¹C in mechanistic studies of photosynthate transport. *Functional Plant Biology* **30**: 831-841.
- Mott KA & Parkhurst DF (1991) Stomatal responses to humidity in air and helox. *Plant, Cell & Environment* **14**: 509-515.
- Nagel KA, Kastenholz B, Jahnke S, van Dusschoten D, Aach T, Mühlich M, Truhn D, Scharr H, Terjung S, Walter A & Schurr U (2009) Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. *Functional Plant Biology* **36**: 947-959.
- Nakajima M, Imai K, Ito H, Nishiwaki T, Murayama Y, Iwasaki H, Oyama T & Kondo T (2005) Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* **308**: 414-415.
- Passioura JB (2006) The perils of pot experiments. *Functional Plant Biology* **33**: 1075-1079.
- Poiré R, Schneider H, Thorpe MR, Kuhn AJ, Schurr U & Walter A (2010) Root cooling strongly affects diel leaf growth dynamics, water and carbohydrate relations in *Ricinus communis*. *Plant, Cell & Environment* **33**: 408-417.
- Prusinkiewicz P & Barbier de Reuille P (2010) Constraints of space in plant development. *Journal of Experimental Botany* **61**: 2117-2129.
- Richards RA, Rebetzke GJ, Watt M, Condon AG, Spielmeyer W & Dolferus R (2010) Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. *Functional Plant Biology* **37**: 85-97.
- Sadok W, Naudin P, Boussuge B, Muller B, Welcker C & Tardieu F (2007) Leaf growth rate per unit thermal time follows QTL-dependent daily patterns in hundreds of maize lines under naturally fluctuating conditions. *Plant, Cell & Environment* **30**: 135-146.

- Sage RF (2004) The Evolution of C₄ photosynthesis. *New Phytologist* **161**: 341-370.
- Schmundt D, Stitt M, Jähne B & Schurr U (1998) Quantitative analysis of the local rates of growth of dicot leaves at a high temporal and spatial resolution, using image sequence analysis. *The Plant Journal* **16**: 505-514.
- Sharkey TD & Zhang R (2010) High temperature effects on electron and proton circuits of photosynthesis. *Journal of Integrative Plant Biology* **52**: 712-722.
- Sinclair TR, Tanner CB & Bennett JM (1984) Water-use efficiency in crop production. *BioScience* **34**: 36-40.
- Smith AM & Stitt M (2007) Coordination of carbon supply and plant growth. *Plant, Cell & Environment* **30**: 1126-1149.
- Sticklen MB (2008) Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. *Nature Reviews Genetics* **9**: 433-443.
- Sulpice R, Pyl ET, Ishihara H, Trenkamp S, Steinfath M, Witucka-Wall H, Gibon Y, Usadel B, Poree F, Piques MC, Von Korff M, Steinhauser MC, Keurentjes JJB, Guenther M, Hoehne M, Selbig J, Fernie AR, Altmann T & Stitt M (2009) Starch as a major integrator in the regulation of plant growth. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 10348-10353.
- Tittmann S, Albert A, Bühler J, Schurr U & Walter A (2010 - submitted) Growth response to UV-B radiation interacts with pronouncedly differing diel water status fluctuations in tomato and tobacco plants. *Plant, Cell & Environment*.
- Vandiver R & Goriely A (2008) Tissue tension and axial growth of cylindrical structures in plants and elastic tissues *Europhysics Letters* **84**: 58004.
- Walter A, Christ MM, Rascher U, Schurr U & Osmond B (2008) Diel leaf growth cycles in *Clusia* spp. are related to changes between C₃ photosynthesis and crassulacean acid metabolism during development and during water stress. *Plant, Cell & Environment* **31**: 484-491.
- Walter A, Feil R & Schurr U (2002a) Restriction of nyctinastic movements and application of tensile forces to leaves affects diurnal patterns of expansion growth. *Functional Plant Biology* **29**: 1247-1258.
- Walter A, Silk WK & Schurr U (2009) Environmental effects on spatial and temporal patterns of leaf and root growth. *Annual Review of Plant Biology* **60**: 279-304.
- Walter A, Spies H, Terjung S, Küsters R, Kirchgeßner N & Schurr U (2002b) Spatio-temporal dynamics of expansion growth in roots: automatic quantification of diurnal course and temperature response by digital image sequence processing. *Journal of Experimental Botany* **53**: 689-698.
- Zhu X-G, Long SP & Ort DR (2010) Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology* **61**: 235-261.
- Zimmermann D, Reuss R, Westhoff M, Geßner P, Bauer W, Bamberg E, Bentrup F-W & Zimmermann U (2008) A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. *Journal of Experimental Botany* **59**: 3157-3167.
- Zimmermann U, Schneider H, Wegner LH & Haase A (2004) Water ascent in tall trees: does evolution of land plants rely on a highly metastable state? *New Phytologist* **162**: 575-615.

LIST OF ABBREVIATIONS

BCCE	Brightness Constancy Constrain Equation
°C	degree Celsius
C₃	Photosynthesis metabolism starting with 3 carbons molecule
C₃-C₄	Intermediate photosynthesis metabolism (e.g. in some Flaveria members)
C₄	Photosynthesis metabolism starting with 4 carbons molecule
CMOS	Complementary Metal–Oxide–Semiconductor
d	day
DISP	Digital Image Sequence Processing
DLI	Daily Light Integral
DNA	Deoxyribonucleic acid
Fig.	Figure
h	hour
ha	hectare
HT	High Throughput
IR	Infrared. IR wavelength starts at 700 nm.
K	Kelvin
LED	Light-Emitting Diode
LER	Leaf Expansion Rate
LHC	Light Harvesting Complex
LIDAR	Light Detection And Ranging
LNT	Low Night Temperature
LPCP	Leaf Patch Clamp Pressure (probe)
LVDT	Linear Variable Differential Transformer
MeV	Megaelectronvolt
min	minute
mm	millimetre

PAR	Photosynthetic Active Radiation. PAR wavelength ranges from 400 to 700 nm.
PUE	Phosphorus Use Efficiency
RGB	Red, Green and Blue colour model
RGR	Relative Growth Rate
ROS	Reactive Oxygen Species
RRT	Rotation Resistance Transducer
RuBisCO	Ribulose-1,5-bisphosphate carboxylase oxygenase
RZT	Root Zone Temperature
SLR	Single Lens Reflex
UV	Ultraviolet. UV wavelength ranges from 10 to 400 nm
UV-A	Ultraviolet A (long wave). UVA wavelength ranges from 315 to 400 nm.
UV-B	Ultraviolet B (medium wave). UVB wavelength ranges from 280 to 315 nm.
VPD	Vapour Pressure Deficit
WUE	Water Use Efficiency
XRCT	X-Ray Computed Tomography

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