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RESEARCH PAPER

Experimental analysis of the role of water and carbon in tree stem diameter variations

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Abstract

The variations of stem diameter as they can be accurately measured by Linear Variable Differential Transformer (LVDT) reflect the addition of four components: irreversible radial growth, reversible living-cell dehydration/rehydration, thermal expansion and contraction, and expansion of dead conducting elements due to the increase and relaxation of internal tensions. The correct interpretation of LVDT signals, with respect to the practical applications, should make an exact distinction between these four components. This paper describes a set of two experiments with potted hybrid walnut trees. Double girdling, water stress, and duration of the day versus night periods were used in the phytotron as experimental factors to induce variations of the carbon and water status of plant tissues. The latter were assessed, respectively, by water potential and transpiration, and by local stem respiration and carbohydrate content. The results are interpreted in terms of carbon or water limitation effects on stem diameter variations where radial growth and tissue elasticity could be distinguished. Moreover, they suggest no or very low involvement of CO_2 originating from a distance, i.e. carried by the transpirational flux of xylem sap, in the total stem CO_2 efflux rate.

Key words: Girdling, radial growth, stem diameter variation, stem respiration, water stress.

Introduction

Radial growth of woody perennial species is an indicator for plant health and growing conditions. Dendrochronology has been used for decades as a tool to analyse the inter-annual variability of diameter growth of individual trees as a result of the conjugated action of tree ageing, technical practices (fertilization, irrigation, pruning, etc) and climate variation. It has become a useful tool for long-term *a posteriori* evaluation of ecological stations status, forestry practices or climate effects. In forest conditions, in the absence of fertilization, the main factor which explains the inter-annual variations of radial growth is water balance, but such forestry operations as thinning appear very well marked in the series of annual rings (Becker *et al.*, 1994; Picard, 1995; Bréda and Granier, 1996).

When monitored with Linear Variable Differential Transformer (LVDTs) of sufficient resolution (1–10 μm), trunk or branch diameters exhibit variations which reflect the action of four components: irreversible radial growth, reversible shrinking and swelling in relation to changing levels of hydration and thermal expansion (Kozlowski, 1971, Klepper *et al*., 1971, McBurney and Costigan, 1984; Améglio and Cruiziat, 1992; Simonneau *et al.*, 1993; Zweifel *et al*., 2000; Cochard *et al*., 2001) and contraction and expansion of dead conducting elements due to the increase and relaxation of internal tensions (Irvine and Grace, 1997; Offenthaler *et al*., 2001; Sevanto *et al*., 2002). A fifth component, a tide effect related to lunar rhythm, has been invoked by Zürcher *et al*. (1998). LVDTs are commonly used to help farmers in their decision of irrigating orchards (Huguet, 1985; Garnier and Berger, 1986; Schoch *et al*., 1988; Li and Huguet, 1990; Huguet *et al.*, 1992; Goldhamer and Fereres, 2001), but the composite origin of measured variations makes their interpretation difficult in terms of tree water status only, as would be wished in the context of irrigation scheduling.

Irrigation scheduling is not the exclusive issue attainable through monitoring trunk diameter variations. Thus, LVDT sensors were used to measure continuously the plant response to stress caused by fungal pathogens (Cohen *et al*., 1997; Luque *et al.*, 1999) and by freezing temperature

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(Améglio *et al*., 2001, 2003). Continuous measurement of cambium production in field conditions would be of considerable physiological and practical interest, especially for forest management and wood production forecast. However, the use of LVDTs to access structural radial growth at a fine time-scale implies that the different components of the signal should be interpreted unambiguously, which could only be achieved via mechanistically modelling trunk diameter variations.

Most existing models of trunk diameter at a fine time-scale (*c*. 1 h) usually incorporate mechanistic descriptions of tissue elasticity and daily shrinkage in the plant stem related to difference in total water potential between the living cells of the bark and xylem vessels, but exclude or remain empirical with respect to structural growth, i.e. the incorporation of new carbon skeletons in the cell structures (So *et al*., 1979; Wronski *et al*., 1985; Archer *et al*., 1997; Genard *et al*., 2001). The objective of the present work was to propose an experimental analysis of the role of water and carbon in tree stem variations. The results of a specific set of phytotron experiments are presented, in which the water and carbon status of hybrid walnut trees (*Juglans nigra*× *J. regia*) were controlled, and the thermal expansion effect was discarded through isothermal conditions.

Materials and methods

Plant material and experimental conditions

Two series of experiments (A and B) were successively conducted in the phytotron in July and August 2001 on potted hybrid walnut trees (*Juglans nigra*×*J. regia*, cv. NG38, Payre nursery, France). Girdling, drought, or prolonged light or dark periods were used to manipulate the carbon and water status of the plants. The trees had been grown for three years in 35 l containers filled with 1/1 v/v mixture of Limagne soil (silt clay) and compost. Irrigation was applied daily. Ten experimental trees were selected for sufficient stem length (>0.75 m). At the moment of the measurements the mean height of these trees was *c*. 1.5 m, the mean leaf area was 1.2 ± 0.2 m^2 and the mean stem diameter at 50 ± 1 cm above ground level was *c*. 3 cm. The experiments were performed in the phytotron with controlled standard conditions: 12/12 h photoperiod, 300 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at mid-height of the trees, 25/25 °C air temperature, 65/65% relative humidity (RH), and daily non-limiting irrigation. Three individual trees were kept in the phytotron at the same time and were weighed continuously for measurement of their transpiration.

Double girdling plus water stress

Experiment A: 2 July to 6 August 2001 (Day of Year (DOY) 183–218): Three trees (T1 as the control, T2 and T3 as treated) were maintained for the duration of the experiment in the 'standard' phytotron conditions. Two LVDT sensors were clamped onto the 3-year-old stem of the control tree T1, at *c*. 20 and 50 cm above ground level and three LVDTs onto each of the treated trees T2 and T3 (at 20 cm, 35 cm, and 50 cm height, all on the same 3-year-old growth unit). The stem respiration rate was also measured on tree T1 for a 10 cm long segment enclosed in a dark cylinder chamber clamped on the stem between the two LVDTs.

Double girdling: After a 10 d acclimation period (DOY 193), the treated trees were double girdled. Two 2 cm-wide rings of bark were carefully detached from the wood of trees T2 and T3 at the middle of the interval between LVDTs. The depth of the bark was approximately 3 mm and it was checked that no residual phloem tissue was left. Whereas double girdling is supposed not to affect the flow of xylem sap, it delimits three distinct zones with respect to phloem sap sucrose supply of the stem: the upper zone (U) still continues to receive current photoassimilates, the median zone (M) becomes completely isolated and can only use pre-existing local reserves; and the lower one (L) which is still allowed to communicate with the base of the tree and may benefit from an upward sugar supply from the roots.

Water stress: Irrigation was stopped on 26 July (DOY 207, i.e. 14 d after girdling) for tree T2, and on 30 July for tree T3. Tree T2 was rewatered on DOY 214.

Variation of the photoperiod with ungirdled trees

Experiment B: 7 August to 12 September 2001 (DOY 219–255): experiment B took place immediately after experiment A and made use of three identically treated new trees (T4, T5, T6). All three were equipped with two or three LVDT sensors each located at the same three heights (U, M, L) as in experiment A. After a 10 d acclimation period with a 12/12 h photoperiod, extended continuous light conditions started on 17 August and lasted for 8 d (DOY 229–236), then standard 12/12 h conditions were applied for 5 d (DOY 237–242). The experiment ended with a 13 d extended continuous dark period (DOY 243–255).

Measurement of stem diameter variations

The stem diameter variations were measured continuously with linear variable differential transformers (LVDT: model DF2.5; Solartron Metrology, Massy, France). LVDT transducers are very robust position sensors for use in industry and research. The LVDT used here was an unguided miniature displacement inductive transducer with infinite resolution, where the needle (the moving part of the measurement sensor) was applied with glue on the stem and separated from the body (bobbin). There was no physical contact between the sensing element providing position measurement and the needle (friction free movement).

LVDT sensors were mounted in specially designed Invar (an alloy composed of 65% Fe and 35% Ni that has minimal thermal expansion) frame (Huguet, 1985) and attached onto the stem. The sensors were connected to a data logger (model 21X, Campbell Scientific LTD, Logan, Utah, USA). Average measurements were recorded every 15 min. Daily growth (*DG*) and maximum daily shrinkage (*MDS*) were derived from a daily pattern. In order to compare growth dynamics in situations where absolute growth rate were very different, daily growth rates are expressed as percentage of the maximum observed in a given period.

Measurement of transpiration

Measurement of the transpiration rate was achieved through continuous weighing of the whole potted trees (electronic balance, model ID1, 10 g accuracy, 100 kg full range, Mettler, Viroflay, Switzerland). Decrease of the pot weight allowed the determination of the moving average of transpiration rates by periods of 3 h. The trees were rewatered twice a day (at predawn and midday), adjusting back to the initial weight.

Measurement of stem respiration

An open flow gas exchange system was designed by reusing parts of a Leaf Chamber Analyser system (LCA-2, ADC, Hoddesdon, Herts., UK) for measuring stem respiration. The mass flow air supply unit (ASUM) was used to drive a controlled flow rate Q of fresh air into a 1.0 l PVC respiration chamber clamped onto the stem of one of the trees. The analyser, used in differential mode, monitored the

difference in CO_2 concentration (ΔCO_2) between the inlet and outlet of the chamber. The respiration rate was calculated as $R=Q\times\Delta CO_2/A$ (μmol CO_2 m^{-2} s^{-1}) where A is the bark area inside the chamber.

Measurement of water potential

The predawn leaf water potential was measured with a pressure chamber (PMS, Corvallis, Oregon, USA) just before the light came on. The midday stem water potential was measured on non-transpiring leaves (transpiration was prevented for 6 h by enclosing the measured leaves within aluminium adhesive tape) just before rewatering.

Measurement of the carbohydrate content of bark

Before addressing the effect of girdling on bark carbohydrates, the effect of changing environment (transfer into the phytotron) was assessed by comparing the bark rings yielded by girdling on trees T2 and T3, after 10 d spent in the phytotron, with bark tissue pieces from the three levels U, M, and L, sampled from two control trees kept outdoors. The effect of the girdling treatment was then assessed from the carbohydrate status of the girdled trees T2 and T3 on DOY 205, i.e. 12 d after treatment, as compared with (i) the same trees T2 and T3 at girdling (DOY 193) and (ii) with the ungirdled control tree T1 on DOY 205.

The bark samples were assayed for glucose, fructose, sucrose, and starch by the enzymatic method of Boehringer (1984), after deep freezing at –196 °C, freeze-drying, and grinding to a 120 µm mesh powder. Results are expressed as average ±standard error.

Statistical analyses

Average and standard error values were computed for all repetitive measurements, and the significance of treatment effect was evaluated by Tukey Test and REGWQ (Ryan–Einot–Gabriel–Welsch). All statistical analyses were based on a 0.05 significance level and conducted with XLSTAT 6.1 (Addinsoft).

Results

Experiment A

Control tree: The time-course of stem diameter as measured by two LVDTs on the control tree T1 during the period DOY 190–205, is shown in Fig. 1A. The dynamics of stem diameter were fairly well repeated throughout the period, including in very fine detail (Fig. 2). On both curves, a daily variation of diameter with a similar maximal daily shrinkage (MDS) of 102±6 µm and 95±4 µm for the lower and upper positions, respectively, denoting elastic reversible shrinkage was clearly superimposed on a continuous increase. Note that the stem diameter at the lower location (L) grew faster (daily growth rate: DG=87±5 µm) than at the upper one (U: DG=65±2 µm) although the stem was the same age at both positions. The smoothed average transpiration rate as determined from continuous weighing of tree T1 is reported in Fig. 1B. The dark and light periods exhibited highly contrasting levels of transpiration and can be clearly identified, despite some residual between-day variability. These daily variations of transpiration in response to light–dark transitions induced changes in the plant water status, as can be seen in Fig. 1C on predawn (i.e. just before the onset of the lights) and midday (6 h later, just before rewatering) stem water potentials.

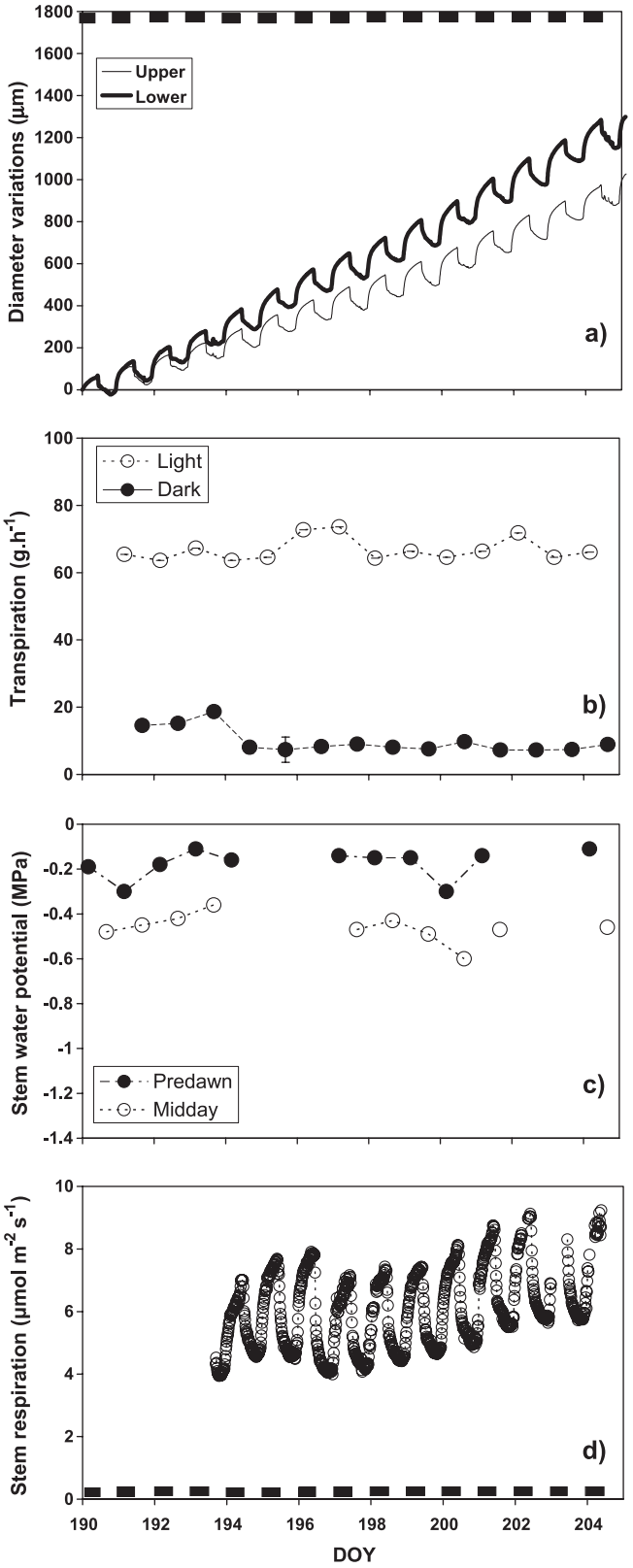

Fig. 1. Time-course of different variables for the control tree T1 in experiment A. (a) Stem diameter variation at locations U and L; (b) moving average of transpiration rate of the whole tree; (c) predawn and midday stem water potential; (d) stem respiration rate. Dark boxes show the nycto-periods.

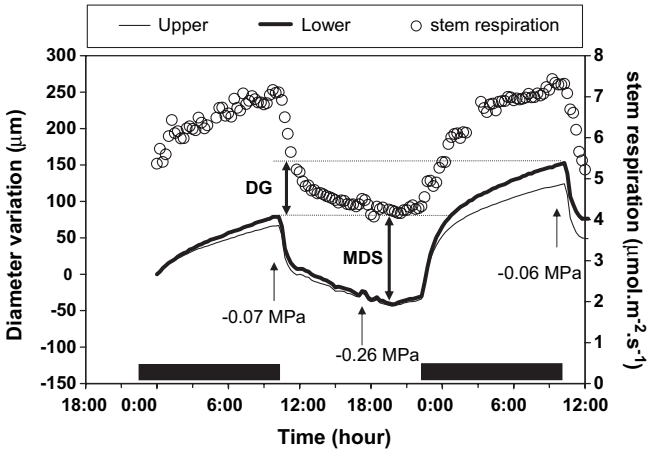

Fig. 2. Time-course of stem diameter variation at locations U and L and stem respiration rate for the control tree T1. Definitions of the daily growth rate (DG) and maximum daily shrinkage (MDS) are given by the two double arrows. Dark boxes show the nycto-periods.

The stem respiration rate of tree T1 could be measured continuously during the major fraction of the period as shown in Fig. 1D, exhibiting ample periodic daily variations. Light off coincided every day with a sharp increase in the stem diameter as well as in the stem respiration rate (Fig. 2).

During the dark period the stem diameter continued to increase gradually as the respiration rate did. The maximum stem diameter occurred at the end of the dark period (Fig. 2); onset of lights was immediately followed by a sharp decrease in stem diameter and respiration rate; both continued to decrease gently until 2 h before the end of the light period. During these two h, a very short increase, certainly due to the daily rewatering of the container, could be observed.

Effect of double girdling and subsequent water stress: The time-courses of stem diameter of the girdled and water-stressed trees T2 and T3 from DOY 190–218 are displayed in Fig. 3A and B. The three distinct zones (U, M, L), as delimited by girdling, immediately displayed contrasting diameter variations. Both trees exhibited comparable features, although variability in the individual response was present. (i) Radial growth rate at level U on trees T2 and T3 (Fig. 4A), was notably accelerated after girdling (*DG*= 64±7 and 101±13 μm for the 3 d before girdling, versus 89±6 and 157±15 μm for the 3 d after girdling, respectively, for T2 and T3). However, it continued to increase for tree T3 (*DG*=122±9 μm, reaching a cumulative diameter increment of 2260 μm before the application of water stress), whereas it eventually slowed down for T2 (*DG*=62±8 μm, reaching 1500 μm only). (ii) Level M (Fig. 4B) displayed an almost complete cessation of radial growth for both trees after 3 d and until DOY 202, but this cessation was followed by an apparent recovery. (iii) Level L displayed either the same behaviour as level M (tree T3) or intermediate between levels U and M (tree T2), but the radial growth of both trees never completely stopped.

The general trends of T2 and T3 stem diameter responses to water stress were similar, despite individual differences, probably attributable to differences in leaf area and in soil water supply. Water shortage induced a synchronous and sharp shrinkage of stem diameter at the three levels for tree T2 (Fig. 3A). The diameter was reduced by *c.* 500 μm within 5 d at level U and by 570 μm and 620 μm at levels M and L, respectively. After this rapid shrinkage, the stem diameter remained relatively stable until rewatering on DOY 214, which led to an almost complete recovery within 1 d. A marked increase in the amplitude of daily shrinkage was noticeable during the shrinking phase, with a sharp maximum on DOY 209: *c.* 440 μm for levels M and L and 250 μm at level U. The effect of water shortage on stem diameter between DOY 211 and DOY 218 was less pronounced for tree T3 which shrank by *c.* 370 μm at levels M and L and by 140 μm at level U (Fig. 3B). The amplitude of daily shrinkage at levels M and L reached a maximum of *c.* 100 μm during the water-stressed period; it was significantly less at level U.

The mean transpiration rate during the light- and dark-periods, as displayed on Fig. 3E, could be measured on tree T3 only. After an initial period before girdling when the daytime transpiration presented an average of 106±4 g, the daytime transpiration rate decreased continuously to an average of 64±1 g. Drought resulted in a marked decrease of T3 day and night transpiration.

Before girdling, the stem water potential of both trees displayed daily variations similar to the control tree T1, with maximal predawn values of *c.* −0.15 MPa and minimal midday values of *c.* −0.5 MPa. After girdling, the daily amplitude of stem water potential variations was significantly decreased, which is consistent with the observed decreased T3 daytime transpiration (Fig. 3E).

During the drought periods, the water potential reached values of *c.* −1.4 MPa. Complete recovery of an optimal water status was achieved shortly after rewatering tree T2 (Fig. 3C).

Carbohydrates: The starch and soluble sugars contents were statistically uniform along the stem of control trees, whether before or after transfer into phytotron, so that any difference, either between girdled and ungirdled trees or between height levels within girdled trees, could be assigned to the girdling treatment.

The amount of soluble sugars (data not shown) in the bark did not significantly change with either the location, date or treatments, with a common value of 52±3 mg.g^{-1} DM.

The mean starch content of the bark of the transferred trees (Table 1) decreased from 24% during the 10 d period they spent in the phytotron before girdling, and 12 d after double girdling, the starch content of the bark of the treated trees significantly depended on the location with respect to

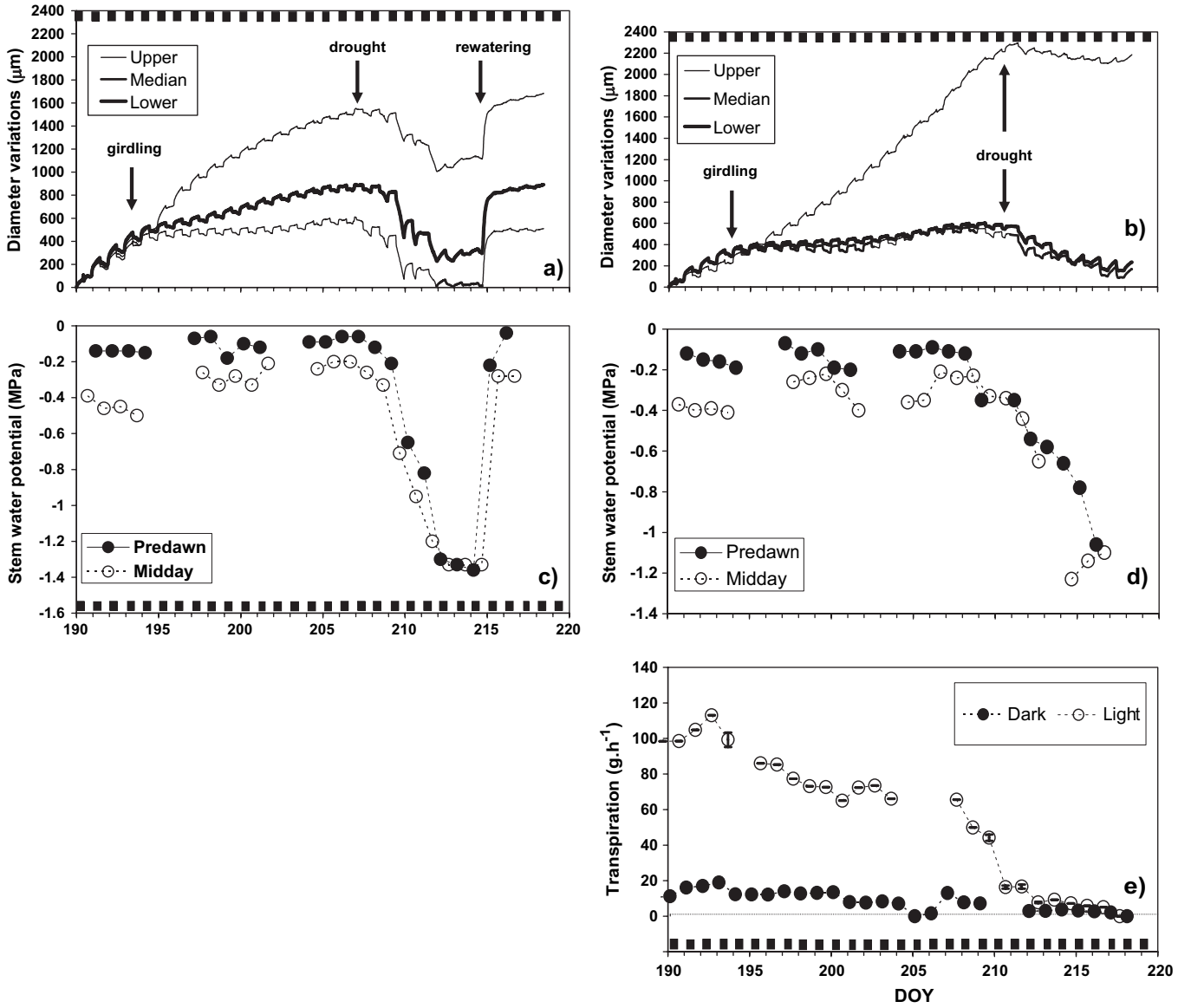

Fig. 3. Time-course of different variables for the treated trees in experiment A. (a, b) Stem diameter variations at locations U, M, and L along the stem of trees T2 and T3, respectively; (c, d) predawn and midday stem water potential; (e) mean transpiration rate of tree T3 during the successive hemero- and nycto-periods. Tree T2 was double-girdled on day 193, irrigation was stopped on DOY 207 and resumed on DOY 214. Tree T3 was double-girdled on day 193 and irrigation was stopped on DOY 210.

girdling. Location M exhibited a starch content similar to that of the ungirdled control tree T1; the mean starch content was higher at location U and lower at location L.

Experiment B

Despite individual variation affecting absolute levels, trees T4, T5, and T6 exhibited similar behaviour, so that the individual results of experiment B are given in Fig. 5 for tree T4 only. Figure 6 summarizes the variations of relative daily growth rate of the three trees.

Variations of the stem diameter of tree T4 at levels U and L, as continuously measured for 45 d are shown in Fig 5A. (i) Daily reversible variation of stem diameter was superimposed onto a continuous increase during the two 12/12 h light/dark periods as in experiment A (Fig. 1A) and the daily growth rates, before and after the extended continuous light period, were, respectively, 21 ± 2 µm and 17 ± 2 µm at level U versus 45 ± 3 µm and 40 ± 4 µm at level L. (ii) Continuous diametric growth occurred throughout the extended continuous light period, but the daily growth rate slowed down within the first 48 h before finally reaching a constant value of *c.* 9 ± 1 µm d^{-1} at level U versus 30 ± 3 µm d^{-1} at level L. (iii) Residual, slowing down growth occurred during the final continuous dark period.

For all trees the growth rate at level L was at least twice that at levels U or M and the amplitude of daily shrinkage in

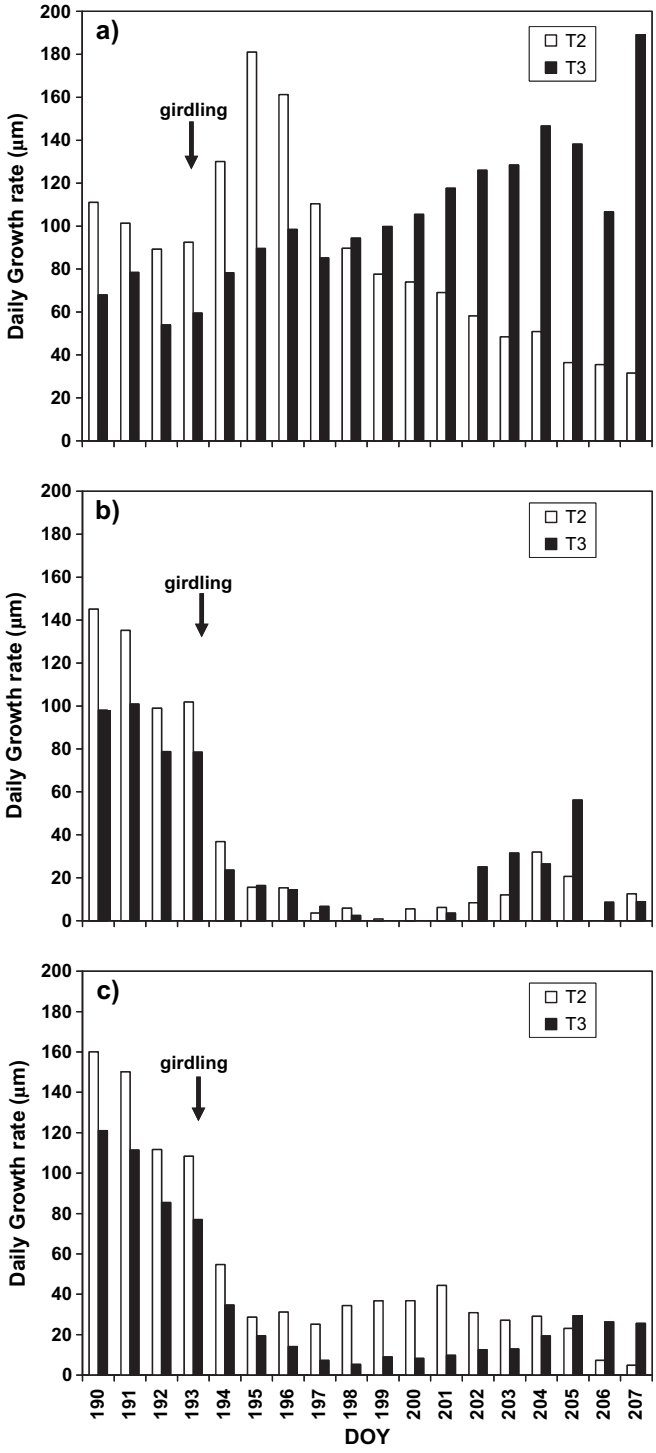

Fig. 4. Time-course of daily growth rate for trees T2 and T3. (a) Upper level, (b) median level, (c) lower level.

the 12/12 h photoperiod was in the range 60–100 µm, depending on the size of the tree and the location of the sensors.

It is remarkable that although continuous light potentially resulted in doubling the daily supply of photoassimilates, the daily radial growth rate of the stem was not enhanced compared with the 12/12 h conditions. The mean stem diameter growth rate at level U was approximately reduced by 25% in continuous light and reduced by 45% at the beginning of the extended continuous dark period. Daily growth rate vanished almost completely within 5 d of continuous dark, but zero growth was observed only after several days of continuous dark (15 d in level U versus 25 d in level L). Apparent negative radial growth could be observed in level U from DOY 256 while it remained slightly positive in level L, supporting the hypothesis of poor but not null remobilization of carbon reserves for radial growth in the absence of current photoassimilates.

Table 1. *Variation of the starch content in the bark of walnut tree stems in experiment A (double girdling) at different dates and locations (U, M, L, see text)*

Position or average	Starch (mg g^{-1} DM±SE)			
	Outdoor control (DOY 183)	Indoor control (DOY 193)	After girdling (DOY 205)	Ungirdled control (DOY 205)
U			87±30	
M	145±13	110±21	63±1	62±1
L			50±5	

The daily mean transpiration rate of tree T4 as derived from pot weight is shown in Fig. 5B. Dark-to-light transitions greatly affected transpiration as in experiment A. In continuous prolonged light, tree T4 exhibited transpiration rates *c.* 30–50% lower than the mean daytime rate observed in the preceding and subsequent 12/12 h periods. Continuous dark corresponded with fairly constant residual transpiration (more likely soil evaporation only).

The time-course of stem water potential of tree T4 is displayed in Fig. 5C. Permanent and relatively low values of stem water potential (−0.6 to −0.75 MPa) were exhibited in continuous light, similar to the midday water potential observed in 12/12 h dark/light conditions. After a quick recovery at the beginning of the second 12/12 h dark/light period, high water potential was maintained until the end of continuous dark.

Variations of the stem respiration rate and of the air temperature in the phytotron are displayed in Fig. 5D. Significant traits can easily be outlined. (i) During the first 12/12 h dark/light period the daytime respiration rate was lower than dark respiration, as in experiment A in Fig. 1D, but an obvious superimposed decreasing tendency was now present which could denote the adaptation of the tree from outdoor to phytotron conditions (lower light resulting in lower carbohydrate content in the tissues and lower respiration rate). (ii) During the subsequent continuous light period, the respiration rate tended to increase; residual variations could mainly be explained by uncontrolled variations of air temperature. (iii) The second 12/12 h dark/light period started with 3 d of faulty temperature

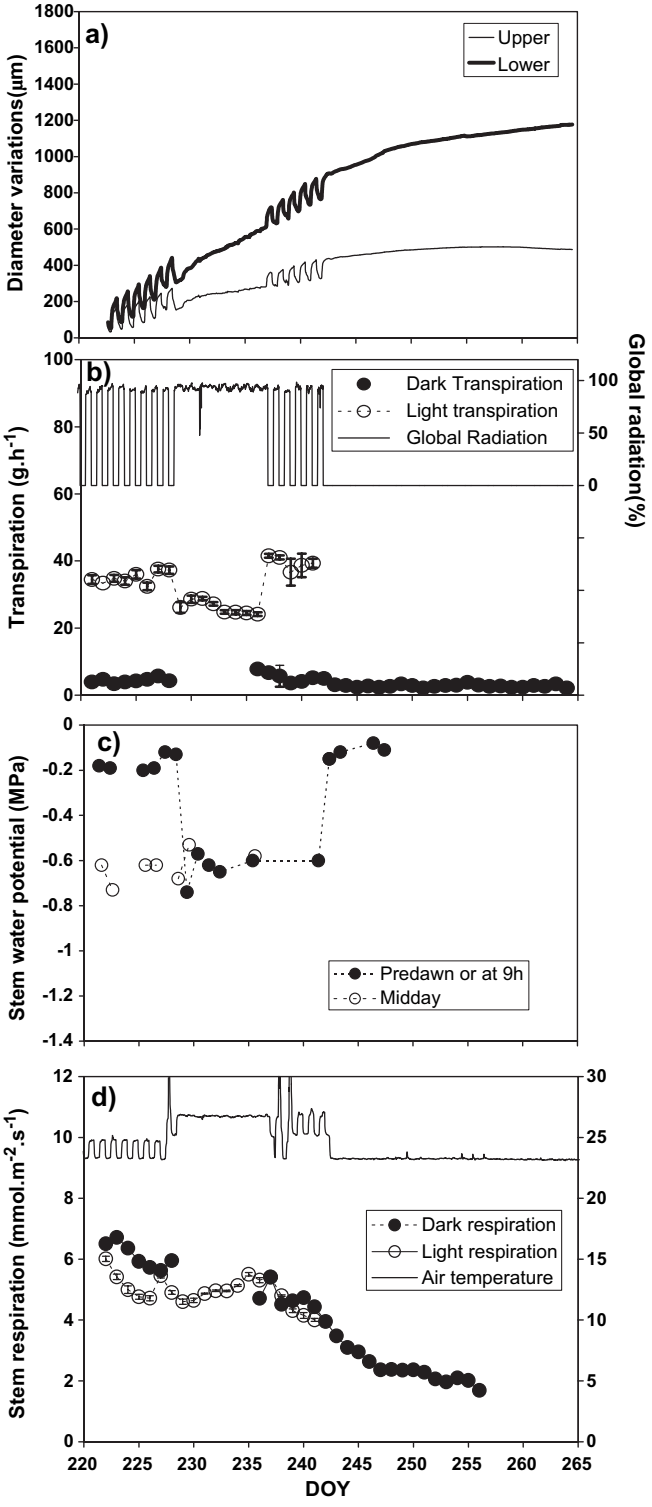

Fig. 5. Time-course of different variables for the tree T4 in experiment B with four distinct periods (12/12 h, prolonged light, 12/12 h, prolonged dark). (a) Stem diameter variation at locations U and L; (b) transpiration rate and global radiation; (c) predawn and midday stem water potential; d) stem respiration rate and air temperature.

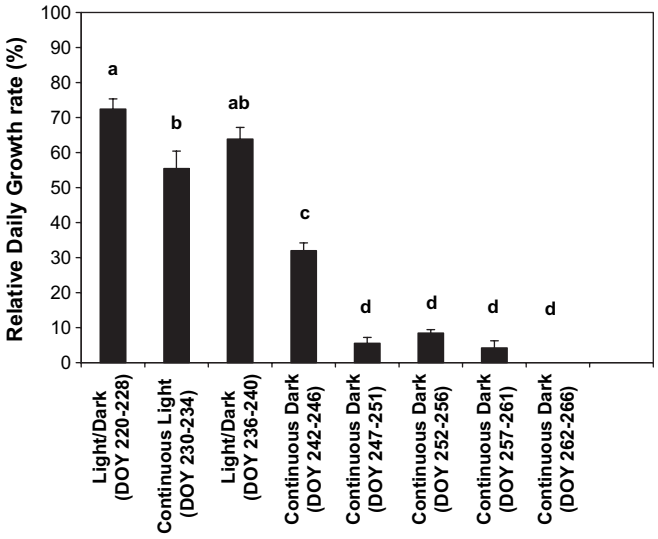

Fig. 6. Variation of the mean of relative daily growth rate (daily growth rates are expressed as the percentage of the maximum observed in a given period) for the three trees (T4, T5, and T6) and three levels (U, M, and L) in experiment B (n=8±SE). Significant differences (P <0.05) between periods are indicated by different letters.

regulation, resulting in difficult interpretation of the respiration rate; it ended with more even temperature conditions, exhibiting again periodic daily variations. (iv) The final continuous dark period exhibited a continuous decrease of the stem respiration rate.

Figure 6 shows the mean relative daily growth rate as measured by the eight sensors (2 T4, 3 T5, and 3 T6) during each period (first 12/12 h dark/light, continuous light, second 12/12 h dark/light, continuous dark). Only two days with a transient dark/light regime were not used for the statistical analysis. The mean relative daily growth rate of the three trees exhibited trends similar to tree T4 as displayed in Fig. 5. Relative daily growth rate was not enhanced by continuous light with respect to 12/12 h dark/light standard conditions and was reduced in average by *c.* 50% only during the first 5 d of continuous dark, vanishing almost completely afterwards.

Discussion and conclusion

Experiments A and B aimed to 'manipulate' the water and carbon status of the stem independently, through girdling, water shortage, and duration of the dark and light periods. The reported results are now interpreted through an analysis of the roles of water and carbon in the variations of stem diameter.

The role of carbon

Carbon is involved in radial growth as structural material and as the source for metabolic energy. Both functions can be approached more or less directly through the observed

changes in stem diameter and in the respiration rate, respectively.

Double-girdling differentially limited or enhanced the carbon supply to the different stem segments (U, M, and L). As a result, 12 d after girdling, starch accumulation could be observed in the bark of segment U, whereas the starch content did not change in M and decreased in L to the likely benefit of roots (Table 1); note that these changes in the starch content did not affect the concentration of soluble sugars. Girdling resulted in an almost immediate cessation of radial growth in the medium (M) segment (experiment A: Fig. 3A, B) where axial phloem communications were impossible, whereas the growth of the lower segment (L) was either markedly depressed (T2) or stopped (T3). By contrast, the upper segment (U) exhibited significantly enhanced growth. These observations show the importance of current photoassimilate flux for active radial growth. The behaviour of M segments shows that local stem reserves could only marginally contribute to radial growth (complete cessation of radial growth within 3 d after girdling). However, the significant recovery of radial growth observed 9 d after girdling suggests that a sharp shortage in assimilate supply can induce, within a few days, significant mobilization of local xylem parenchyma reserves, not excluding new axial phloem connection.

Residual growth was observed during the final continuous dark period in experiment B showing that growth can continue in level L even in the absence of photosynthesis. This observation supports the assumption that more distant reserves (e.g. coming from the roots) could be mobilized more readily for the radial growth of the whole stem.

The stem respiration pattern (Figs 1D, 5D), with maximal respiration rate occurring at night when transpiration was low, does not support the hypothesis of a significant efflux of CO_2 originating from a distance, i.e. carried from underground or the lower part of the plant by the transpirational flux of xylem sap, as proposed by Teskey and McGuire (2002). Note also that since the stem respiration chamber was opaque, bark photosynthesis could not explain the observed variations of stem respiration in conditions of constant air temperature. Therefore, these variations of the efflux of CO_2 clearly reflect variations of the local respiration rate and must be attributed to metabolic changes.

According to the common conception as formalized 30 years ago by Thornley (1970) and McCree (1970), the respiration rate of tissues reflects the production of metabolic energy for growth and maintenance. The ample daily variation of stem respiration that was observed in experiments A (Fig. 1D) and B (during both 12/12 h dark/light periods, data not shown) was closely correlated with changes in stem diameter, strongly suggesting that structural radial growth (with its specific energy requirement) occurred mainly during the night-time, in relation to an improved water status and consequently higher turgor pressure, as expected according to Lockhart's (1965) model.

It was hypothesized that growth respiration would respond quickly, as could the growth rate itself, to water limitations; by contrast, maintenance respiration would mainly respond to rather slow changes in the carbohydrate content of tissues, as determined by changes in the whole plant carbon balance. In this view, the initial decrease of stem respiration in the first 12/12 h dark/light period of experiment B could be an effect of the decreased whole tree carbohydrate content (as observed on control trees in experiment A) as a consequence of relatively low photosynthesis in the phytotron with respect to previous outdoor conditions. The subsequent increase in respiration rate in prolonged light, although the radial growth rate was lower (as limited by relatively low water potential), could be attributed to an increased carbohydrate content as a consequence of increased photosynthesis. Water limitation of growth would be less severe during the second 12/12 h dark/light period as displayed in Fig. 5C, resulting in the recovery of the daily variation of respiration superimposed on a decreasing tendency (Fig. 5D) in response to decreased carbohydrate content. The continuous decrease of the stem respiration rate as observed during the final continuous dark period could reflect the decline of the metabolic activity of tissues (maintenance respiration) in response to decreasing sugar content. Similar conclusions were reached by Hölgberg *et al*. (2001), as trunk girdling significantly decreased the respiratory activities of plant roots.

The role of water

The variations in stem water potential in experiments A and B were a consequence of variations in the transpiration rate and in the soil water content. In the absence of soil water limitation, the reversible daily shrinkage, as observed in experiments A and B, was a clear consequence of changes in the tree water status that resulted from changes in the transpiration rate. The minimum stem diameter occurred just before the end of the light period after rewatering each day (Fig. 2), and the maximum at the end of the dark period when optimal tissue water content was restored. Rapid, reversible variations of stem diameter occurred immediately after light-on and light-off, and were followed by slower changes that could reflect the progressive achievement of a new water equilibrium. This dynamic behaviour suggests the involvement of two kinds of water reservoir: (i) the first one, in direct connection with the transpiration flow, could correspond to apoplastic water; and (ii) the second one, more slowly involved through higher resistances (plasma membrane?) and physiological mechanisms, could correspond to symplastic water.

The light-to-dark transitions in experiments A and B lead to changes in the stem water potential (Figs 1C; 3C, D; 5C), as normally expected from the Ohm-like transfer of water in the plant: the greater the transpiration flow rate, the lower the stem water potential. In this view, the continuous light

period in experiment B looks partially anomalous since relatively low water potential was observed in conjunction with low transpiration, although relatively high soil water content was maintained.

A reduced transpiration during continuous light while water potential remained unchanged suggested a reduction in hydraulic conductance (stomatal and/or at soil–root interface). At such stem water potential values, xylem embolism is unlikely for walnut (Tyree *et al*., 1993; Cochard *et al*., 2002). It could be that rewatering twice a day to constant weight did not allow the maintenance of an optimal plant water status for such permanently transpiring plants, leading to increased stomatal closure. Nevertheless, cumulative transpiration over 1 d under continuous light (627 ± 2 g d^{-1}) was higher than during a 12/12 h dark/light alternation (473 ± 2 g d^{-1}); this can be expected to result in at least a proportionately higher C gain under continuous than under alternating light conditions, and possibly even higher if the water-use efficiency was improved as commonly observed in similar situations.

Water-stress-induced reduction of radial growth is well known (Kozlowski, 1971) and clearly occurred in the present work. The question arises whether such growth limitation is a direct consequence of the altered water status, through alteration of the functions that are involved in tissue enlargement (cell division and/or expansion), and/or (not mutually exclusive alternatives) indirect through the impact on the carbon balance via stomatal limitation and reduced photosynthesis.

As outlined above, the stem respiration measurements (experiments A and B) and the observed reduced growth during the continuous light period in experiment B support the first alternative. The similarity between the daily variation of stem respiration and of the tissue water content, support the common assumption that hydratation expansion and structural growth could occur concurrently and mostly during the night, in relation to an improved water status and higher turgor pressure. Despite potentially increased carbon resources, the growth rate during the continuous light period in experiment B was only *c.* 75% of that observed in the 12/12 h dark/light photoperiod (Fig. 6), and this reduced radial growth could be related to an alteration of the water status of the plant as revealed by water potential measurements (Fig. 5C).

Finally, the present experimental data and discussion support the common conception that the daily variation in plant stem, related to the difference in total water potential between living bark cells and xylem vessels, and structural radial growth are the two main components of the observed daily variations of stem diameter and that carbon and water limitations can occur concurrently in the process of radial growth.

To conclude, any mechanistic model of stem diameter variations should therefore integrate the role of the water and carbon status of the plant.

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