Cambial activity of Populus tremula x Populus alba clone 717-1B4 in hydroponic culture

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Abstract: *Populus tremula* Michx. × *Populus alba* L. clone 717-1B4 plants were grown in a hydroponic system in an effort to detect cambial activity in a changing nutrient environment. The secondary growth of the stem was determined by automated measurement of radial growth, as well as by histological study. This is the first time poplar cambial activity has been recorded in a hydroponic system. Further, we demonstrate that nutrient limitations can be tested with progressive deprivation of liquid medium. The system lends itself to measurements of stomatal conductance, primary stem growth, leaf growth, and radial stem growth. In this study we found that primary and secondary growth were affected by nutrient solution limitations. This hydroponic system will be valuable in elucidating the impact of environmental, physiological, and molecular factors on cambial activity and wood formation.

Résumé : Des plants de *Populus tremula* Michx. × *Populus alba* L. clone 717-1B4 ont été cultivés en système de culture hydroponique pour obtenir une activité cambiale dans un milieu nutritif variable. La croissance secondaire de la tige a été caractérisée par une mesure automatique de la croissance radiale et des observations histologiques. Les résultats montrent le premier enregistrement de l'activité cambiale en hydroponie chez le peuplier. En outre, nous démontrons que les carences nutritives peuvent être étudiées en privant progressivement les plants de solution nutritive. Le système se prête à la mesure de la conductance stomatique, de la croissance primaire de la tige, de la croissance foliaire et de la croissance radiale. Dans cette étude nous observons que les croissances primaire et secondaire sont touchées par les carences nutritives. Ce système de culture en hydroponie est exploitable pour l'élucidation de l'impact des facteurs environnementaux, physiologiques et moléculaires sur l'activité cambiale et la formation du bois.

Introduction

Poplars are among the fastest growing trees in temperate latitudes and produce a wood widely used by the forest industry (Dickmann et al. 1983). One of the major aims of a poplar breeding program is to introduce selected trees that will increase the wood productivity of managed plantations. Wood production results from cambial activity in the stem (Larson 1994). To understand how cambial activity affects wood productivity, it is essential to control the effect of environmental factors. Growth of poplar in the field or even in pots does not allow precise control of the nutritional parameters, and in vitro culture of woody plants has so far not led to wood formation. Moreover, sampling of growing tissue is limited in soil because of the concomitant wounding effect, which decreases the quality of samples for molecular biology analyses. Taking into account these criteria, we have developed an hydroponic culture method to monitor cambial activity in poplar and to ease tissue sampling (Raynal et al. 1985; Robertson et al. 1994; Dubos et al. 2003). As an example, we have studied the effect of progressive reduction of nutrient solution on cambial activity. This treatment mimics the reduction of water availability and nutrient absorption observed under natural conditions (Wimmer et al. 2002). It is well known that secondary growth is widely affected by water availability during the growing season (Zahner 1968; Kramer et al. 1995; Ortuno et al. 2004). Such an effect has been observed in *Picea glauca* (Moench) Voss (Barber et al. 2000) and *Thuja occidentalis* L. (Tardif et al. 2001) and is interpreted as being an adaptive response to drought (Chen et al. 1997).

The aim of this study was to obtain secondary growth under hydroponically controlled conditions and to demonstrate that a limiting the nutrient solution affects secondary growth. The hybrid poplar clone 717-1B4 was chosen because of its rapid growth and its use as a model species (Bradshaw et al. 2000).

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Material and methods

Hydroponic culture conditions

Plant preparation

Clone 717-1B4 of *Populus tremula* Michx. × *Populus alba* L., obtained at the Institut national de la recherche agronomique – Orléans, was used for hydroponic culture. Nodal segments, 15 cm long and with 11 ± 1 mm diameter and one axillary bud, were treated for 30 min with indole-3-butyric acid (1.2 $g \cdot L^{-1}$) and rooted in sand. After 1 month,

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48 rooted plants with a newly developed stem about 10 cm high and bearing 10 leaves were transferred to the hydroponic system (Kruse et al. 2003). Rooted cuttings were acclimated under hydroponic conditions for 15 days before the experiment as follows.

Standard culture

Plastic containers with 12 plants each were filled with 20 L of half-strength Murashige and Skoog culture medium (Murashige et al. 1962). A fish-tank pump provided aeration. Trees were grown in a growth chamber under environmentally controlled conditions: temperature was 21 ± 1 °C, relative water humidity was 75% ± 5%, and irradiance was 134 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot day^{-1}$.

Treatments

Day 0 is defined as the onset of radial growth measurement. Stress was applied at day 19 of the experiment.

Control conditions

The nutrient solution was refilled daily to the initial volume. Each week the solution was replaced with fresh solution. Twenty-four plants were cultured under control conditions throughout the experiment (39 days).

Nutrient solution limitation

During growth, leaf transpiration led to the decrease of nutrient solution in the tank. At day 19, we stopped daily replacement of the nutrient solution, thus imposing a gradient of water and nutrient limitation. This treatment was designated by "stress". Twenty-four plants were stressed for 20 days.

Water status measurement

We used a Scholander pressure chamber (Scholander et al. 1965) to measure predawn leaf water potential (Ψ_{wp}). The leaf relative water content (RWC) was calculated as follows:

$$[1] \qquad \text{RWC } (\%) = \frac{[(\text{fresh mass} - \text{dry mass}) \times 100]}{(\text{saturated mass} - \text{dry mass})}$$

The stem water content (WS) was calculated as follows:

$$[2] \qquad \text{WC } (\%) = \frac{[(\text{fresh mass} - \text{dry mass}) \times 100]}{\text{fresh mass}}$$

Stomatal conductance (g) was determined on both sides of poplar leaves with a steady state porometer (PMR-3, PP Systems, Hitchin, Hertfordshire, UK). Measurements were calculated as $g_{abaxial} + g_{adaxial}$ on a mature leaf (sixth leaf from the top of the plant).

Primary and secondary growth measurement

We determined leaf area increase ($cm^2 \cdot day^{-1}$) on 16 plants by using a binomial relationship between leaf maximum width and leaf area (Brignolas et al. 2000), with parameterization adapted to clone characteristics. The correlation was performed on 270 leaves collected from 10 different cuttings. We calculated the leaf area by drawing the leaf contour on a paper sheet (80 $g \cdot m^{-2}$), cutting out the form of the leaf, and weighing the paper. Each leaf width was manually measured. The formula used was

$$[3] \qquad L_a = (0.71 \times L_w^2) + (1.114 \times L_w)$$

Fig. 1. Radial growth measured with a linear motion sensor on the stem of *Populus tremula* × *Populus alba* clone 717-1B4 cultivated under hydroponic conditions.

where L_a is leaf area (cm^2) and L_w is leaf width (cm). The correlation coefficient was 0.98. Stem primary growth ($cm \cdot day^{-1}$) was estimated for 16 plants per treatment by measuring the length of the axillary stem.

Radial growth was measured every 15 min for 39 days with an automatic point dendrometer on eight plants per treatment (Fig. 1). A linear motion sensor (AB Electronics Ltd., Romford, Essex, UK) was adjusted on the tree nodal segment so that the increasing stem diameter mechanically impinged on it, forcing the electric tension out of the variable resistance of the sensor connected as a tension divider. The variable tension was digitized by an analog-to-digital converter (Adlink Technology Inc., Taiwan) plugged into a PC-based recording data system. The software piloting this device and storing the collected data was developed in Visual Basic for Applications and integrated in Excel's spreadsheet (Microsoft, Redmond, Washington, USA). It is available from the authors upon request.

Histological description

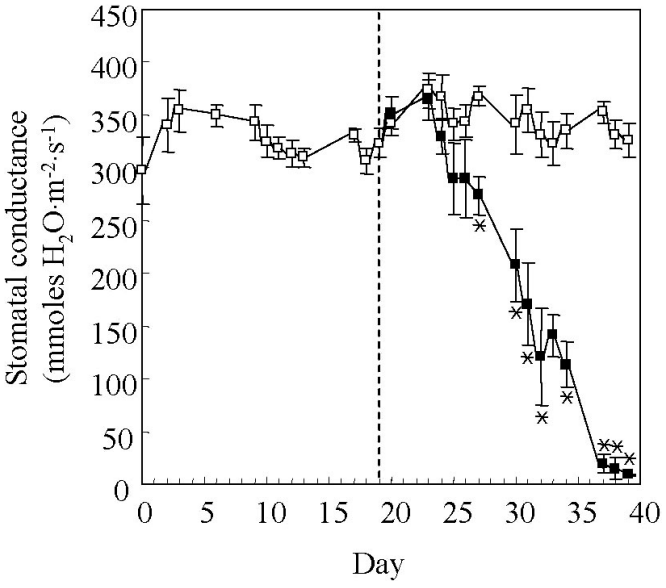
For the observation of the cambial zone, stem pieces below the radial growth sensor were collected at day 0 and day 39 on four different cuttings and stored in 70 (*v*/*v*) ethanol for 10 days at room temperature. Freehand sections were obtained with a razor blade and destained in 2.5% (*m*/*v*) NaClO aqueous solution for 6 min. Sections were abundantly rinsed with distilled water, partially dehydrated in 25% (*v*/*v*) and 50% (*v*/*v*) aqueous ethanol for 2 min each, stained with 1% (*m*/*v*) aqueous Safranin O for 3 min, subsequently stained

Table 1. Predawn leaf water potential, leaf relative water content, and stem water content of *Populus tremula* × *Populus alba* clone 717-1B4 cultivated in hydroponic conditions and submitted to control or nutrient solution limitation.

		Day 19	Day 30	Day 39
Predawn leaf Ψ_{wp} (MPa)	Control	–0.45±0.01	–0.43±0.04	–0.47±0.04
	Stress	—	–0.78±0.02*	–1.77±0.10*
Leaf RWC (%)	Control	81.1±7.9	82.9±2.1	80.0±3.1
	Stress	—	72.8±5.3*	66.5±3.5*
Stem water content (%)	Control	67.6±2.2	66.1±1.0	62.5±2.4
	Stress	—	65.3±3.5	57.3±1.8*

Note: Day 19 corresponds to the beginning of the stress period. *, significantly different at $P \leq 0.05$ ($n = 8$).

Fig. 2. Time course of stomatal conductance of *Populus tremula* × *Populus alba* clone 717-1B4 cultivated under hydroponic conditions and submitted to control conditions (□) or nutrient solution limitation (■). Day 19 corresponds to the beginning of the stress period. Vertical bars represent standard errors. Means for control and stressed cuttings ($n = 16$) were compared; asterisks indicate significant differences at $P = 0.05$.



with 1% (*m*/*v*) aqueous Astra Blue at room temperature for 5 min (Srebotnik et al. 1994), and rinsed in absolute ethanol.

Statistical methods

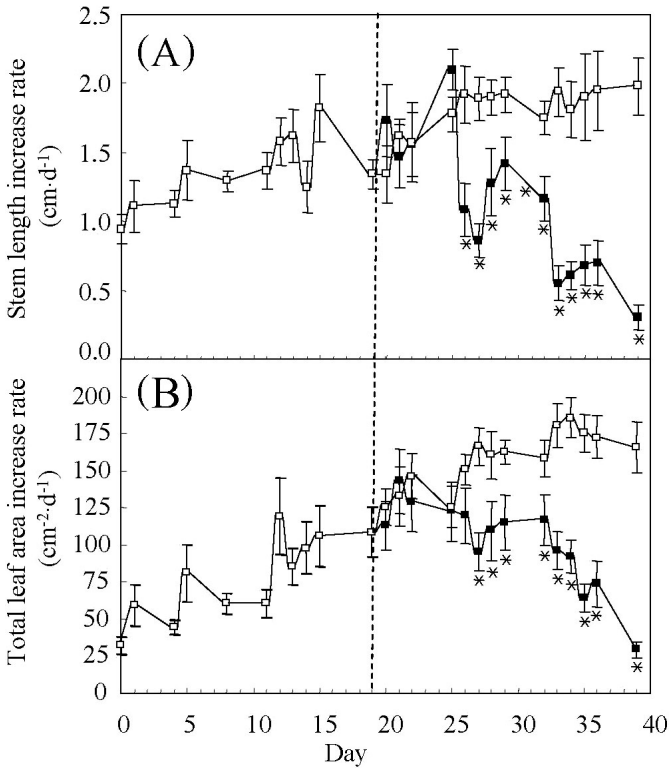
Means for control and stressed treatments were compared by using Statview software version 5.0.1 (SAS Institute Inc., Cary, North Carolina, USA). In each case the number of replicates (n) is indicated and the level of confidence defined at $P = 0.05$.

Results

Water status

Predawn leaf Ψ_{wp}, leaf RWC, and stem WC (Table 1) of control plants were –0.45 MPa, 81.1%, and 67.6%, respectively. These values did not vary significantly during the experiment. For the stressed plants, predawn leaf Ψ_{wp} decreased to –0.78 and –1.77 MPa after 11 days (day 30) and 20 days (day 39), respectively. Leaf RWC significantly decreased to 72.8% and 66.5% after 11 days (day 30) and 20 days (day 39) of nutrient solution deprivation, respectively. This stress significantly affected stem WC only by day 39, when it reached 57.3% (Table 1). Stomatal conductance of the control plants remained stable at 300–370 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$ throughout the experiment (Fig. 2). For stressed plants, stomatal conductance was not significantly affected before day 27 (8 days of stress). From day 27 to day 39, the stomatal conductance of stressed cuttings decreased to 10 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$.

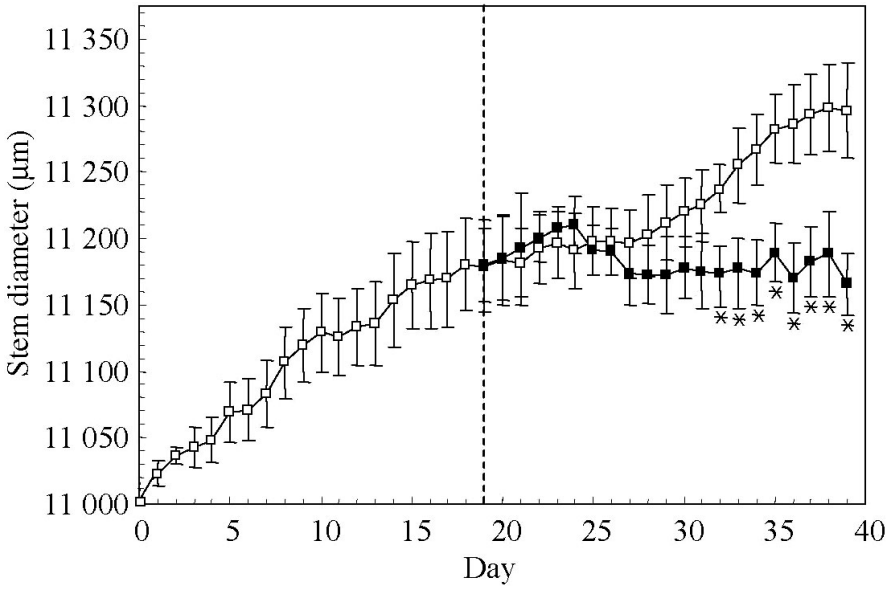
Fig. 3. Time course of stem length increase (A) and leaf area increase (B) of *Populus tremula* × *Populus alba* clone 717-1B4 cultivated in hydroponic conditions and submitted to control conditions (□) or nutrient solution limitation (■). Day 19 corresponds to the beginning of the stress period. Vertical bars represent standard errors. Means for control and stressed cuttings ($n = 16$) were compared; asterisks indicate significant differences at $P = 0.05$.



Primary growth

From day 0 until day 39 the stem length increase rate (designated by "stem growth") of control poplar cuttings

Fig. 4. Time course of radial growth increase of *Populus tremula* × *Populus alba* clone 717-1B4 cultivated in hydroponic conditions and submitted to control conditions (□) or nutrient solution limitation (■). Day 19 corresponds to the beginning of the stress period. Vertical bars represent standard errors. Means for control and stressed cuttings (n = 16) were compared; asterisks indicate significant differences at P = 0.05.



approximately doubled (Fig. 3A), to 1.99 cm·day^{-1} from 0.94 cm·day^{-1}. During the same period, the total leaf area increase rate (designated by "leaf growth") of control plants increased to 183 cm^2·day^{-1}, about six times the original value (29 cm^2·day^{-1}) (Fig. 3B). Before we applied stress, stem growth increased to 1.36 cm·day^{-1} from 0.94 cm·day^{-1}, and leaf growth increased to 120 cm^2·day^{-1} from 29 cm^2·day^{-1}. Between day 19 and day 25, stem growth and leaf growth did not significantly differ between control and stressed plants. After day 26 stem growth decreased significantly in stressed plants to 0.3 cm·day^{-1} from 1.09 cm·day^{-1}, and leaf growth decreased to 31 cm^2·day^{-1} from 132 cm^2·day^{-1}. At the end of the stress treatment (day 39), stem growth and leaf growth were, respectively, eight and six times lower in stressed plants than in control plants.

Secondary growth

For control conditions, stem diameter increased at a steady rate of 7.5 µm·day^{-1} (Fig. 4) to 11 298 µm from 11 002 µm. Between the start of the experiment and the onset of stress (day 19), stem diameter increased to 11 178 µm. from 11 002 µm. During the period of stress, no differences were observed between control and stressed cuttings until day 31 (corresponding to 12 days of stress). After day 31, the stem diameter of control plants increased to 11 298 µm from 11 196 µm, whereas that of stressed plants never exceeded on average 11 189 µm until the end of the experiment.

Histology

Tissue anatomy was observed at the onset of the culture (day 0) and at day 39 (Fig. 5). Cambial cells (CZ) could be observed at day 0 (Fig. 5B) between phloem (P) and xylem formed before the experiment (Xf), without any new xylem formed yet. In control plants (Fig. 5A), cambium was composed of at least two times more cells, and the xylem produced during the experiment (Xc) showed the largest increment. A lowered production of xylem (Xs) occurred under stressed conditions (Fig. 5C). Under control conditions (Fig. 5A), the xylem increment was estimated to be 360 µm during the 39 days of experiment, whereas only 175 µm of xylem was produced at the same time in stressed plants.

Discussion

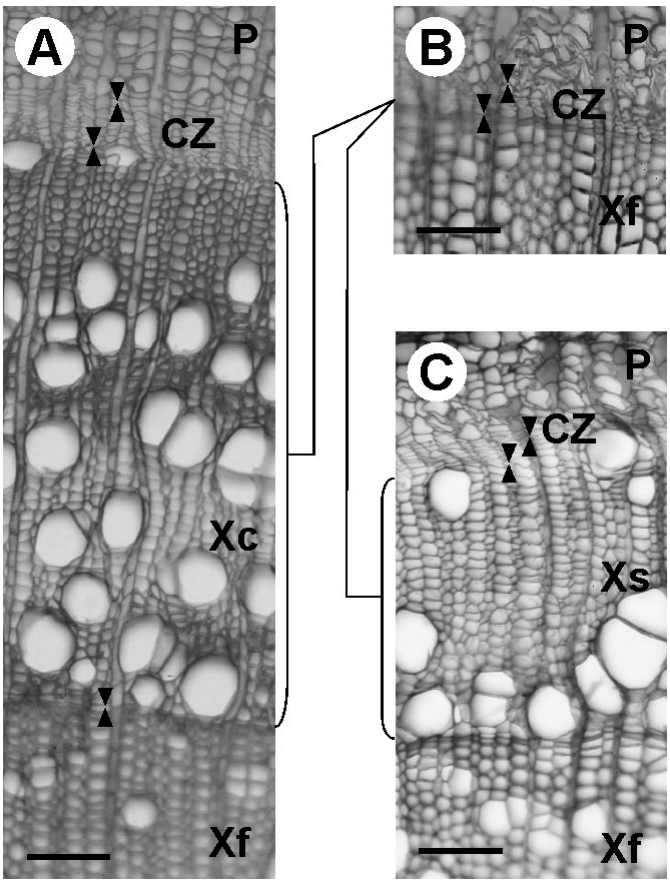
Characterization of water stress

In *Populus euramericana* (Dole) Guinier, growing in soil and submitted to 11 days of water withholding (Caruso et al. 2002), the decrease in predawn leaf Ψ_{wp} was associated with stomatal closure and the decrease in leaf RWC. In *P. tremula* × *P. alba* clone 717-1B4 cultivated under hydroponic conditions, we obtained the same results after 20 days of stress (Table 1), when approximately two-thirds of the root system was no longer in contact with the nutritive liquid medium (data not shown). Such results have also been observed in leaves of *Lotus corniculatus* L. stressed by water shortage in hydroponic culture. This drought was obtained by totally withdrawing the nutrient solution in contact with the roots for 8 h (Borsani et al. 2001). Under hydroponic conditions, leaf RWC measured under progressive nutrient solution deprivation (Table 1) was affected before stem WC was. From these measurements, we can estimate a maximum delay of 9 days between primary and secondary growth impacts of nutrient solution deprivation.

Radial growth

Under control conditions, we observed an increase in the radial diameter of the cuttings as a result of the cambial activity. The xylem width increment measured on tissue sections corresponded to the radial enlargement measured by the point dendrometer. Indeed, the histological study con-

Fig. 5. Anatomical characterization of secondary growth on *Populus tremula* × *Populus alba* clone 717-1B4 cultivated in hydroponic conditions and submitted to control conditions or nutrient solution limitation. (A) Full secondary growth of control plant at day 39. (B) Initial observation of cambial zone before secondary growth (day 0). (C) Full secondary growth of stressed plant at day 39. CZ, cambial zone; P, phloem; Xf, final wood of the former xylem; Xc, control xylem increment; Xs, xylem increment during nutrient solution limitation. Bar represents 50 µm. Double arrowheads show tissue limits on the section. Lines between pictures show the xylem growth increment estimated for the section.



firmed that this diameter increase was mainly caused by the production of the xylem. The rate of phloem tissue production by the cambium is about 10% of the xylem production rate for most species (Larson 1994). Under hydroponic conditions, the stem diameter measurement showed that cambial growth was significantly arrested 12 days after the limitation of nutrient solution began.

Relationship between nutrient solution limitation and radial growth

Stomatal conductance decreases with increasing water stress (Comstock 2002). We observed the cessation of radial growth when stomatal conductance was <110 mmol $H_2O \cdot m^{-2}$ s^{-1}. Plant responses to water withholding can be interpreted as being the result of progressive root desiccation (Davies et al. 1994), obtained in our case by withholding nutrient solution. Vascular cambium activity slowdown, impacting xylem production, has been shown to be a consequence of water deficit (Zahner et al. 1964; Rozenberg et al. 2002). From our results, stomatal conductance was reduced 8 days after water shortage began, whereas radial growth was significantly arrested 12 days after water shortage began. Therefore, we measured a 5-day delay between primary and secondary growth retardation. Histological observations confirmed the cambial activity slowdown: fewer xylem cells were produced under nutrient solution limitation than under control conditions. The observed 5-day delay could be interpreted as the time required for poplar cuttings cultured hydroponically to adjust their cambium activity to water shortage.

To our knowledge, this is the first report of cambial activity in hydroponic culture. This paper also emphasizes the observation of water-stress-like response at the cambial level in rooted cuttings cultured under hydroponic conditions. This system, in which the level of nutrient solution can be decreased more or less rapidly, could be used in the future to detect the adaptative and non-adaptative drought responses in cambial tissue involved in wood formation under limited water availability. This ability to control cambial activity by water availability or nutrient changes (heavy metals, salts) under hydroponic conditions makes this system a good model for studying cambial activity and wood formation.

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