RESEARCH PAPER

# Evaluation of drought response of two poplar clones (*Populus×canadensis* Mönch 'I-214' and *P. deltoides* Marsh. 'Dvina') through high resolution analysis of stem growth

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## Abstract

**Different irrigation effects on stem radius variation ($\Delta R$) and maximum daily shrinkage (MDS) in *Populus deltoides* 'Dvina' and *Populus×canadensis* 'I-214' were studied to assess differences in drought tolerance between clones. One-year-old trees growing in concrete tanks were submitted to two irrigation regimes (natural rainfall and irrigation) from 24 June to 10 August, and $\Delta R$ was monitored by automatic point dendrometers. Independently of the irrigation regime, 'Dvina' showed a higher stem radial increment than 'I-214'. In both clones, the first response to changed soil water content was a significant increase in MDS, whilst $\Delta R$ decreased about 20 d later when pre-dawn leaf water potential ($\Psi$pd) dropped below –0.4 MPa. However, they displayed different strategies to overcome drought. 'Dvina' maintained a positive $\Delta R$ for longer than 'I-214', which had lower leaf $\Psi$pd and greater leaf abscission at the end of the drought period. After irrigation resumed, 'Dvina' showed a higher capacity to restore stem growth. 'I-214' was probably unable to recover secondary growth because of higher leaf abscission during drought stress and the production of newly expanded leaves during recovery. It is concluded that the larger radial growth of 'Dvina' derived from a better water use (carbon uptake versus water loss) than 'I-214' under limited water availability.**

## Introduction

The success of intensive poplar cultivation is partly due to the great versatility of its wood and its properties of lightness, clear colour, homogeneity, and ease of working (peeling, bonding, and finishing), but also to the normally very fast growth of the species on flood plains and fertile soils. The high wood production of poplars is strictly linked to soil water availability, which is normally assured by irrigation in intensive upland plantations with recurrent soil water deficit. Increasing irrigation costs and water shortages worldwide have led to the development of irrigation methods that minimize water use (Jones, 2004) and to the definition of integrative criteria for assessing drought tolerance in poplar (Tschaplinski *et al.*, 1998; Marron *et al.*, 2003). Poplar clones display very varied drought tolerance and water use efficiency according to genotype (Marron *et al.*, 2005; Monclus *et al.*, 2005), but the effects of drought on stem growth pattern and characteristics of merchantable stems is rarely taken into account (Arend and Fromm, 2007).

Wood characteristics are due to cambium division, cell expansion, and secondary wall production. Radial growth requires an increase in cell volume, which depends on maintenance of high cell turgor pressure, with an

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irreversible influence on cell extension and wall polymer deposition (Proseus and Boyer, 2005). Drought affects several growth features such as xylem anatomy and radial increment (Abe *et al.*, 2003; Corcuera *et al.*, 2004; Arend and Fromm, 2007). Many environmental, genetic, and physiological factors drive the growth response of plants to water deficit (Chaves *et al.*, 2003), and, to gain new insights, growth has to be monitored with a high temporal resolution (Zweifel *et al.*, 2006). Daily stem variation has long been used in order to study tree water use as well as radial growth (Kozlowski and Winget, 1964; Wronski *et al.*, 1985; Herzog *et al.*, 1995). Information on plant response to water deficit has more recently been obtained through the use of automatic dendrometers (Downes *et al.*, 1999; Zweifel *et al.*, 2005) providing daily patterns of stem radius variation ($\Delta R$) and maximum daily shrinkage (MDS). MDS was highly sensitive to changes in water status in peach orchards (Conejero *et al.*, 2007), indicating a fast response of the plant. However, few studies have been done on the temporal and quantitative response of $\Delta R$, considered as radial growth, in fast-growing poplar plantations.

Preliminary observations showed that 5-year-old *Populus deltoides* clones ('Dvina', 'Neva', and 'Lena') had higher radial growth than *Populus×canadensis* 'I-214', regardless of irrigation regime (irrigated or natural rainfall) and soil type (clay–loam soils and well-drained sandy loam soils) (Facciotto and Zambruno, 2004). The mean annual increment of 'Dvina' was 65% higher than that of hybrid 'I-214' in non-irrigated conditions and clay–loam soils, so planting *P. deltoides* clones is more appropriate in this type of soil as it is less water demanding. The higher radial growth of *P. deltoides* under water-limiting conditions could be consistent with its natural distribution area and life cycle. Some authors reported that natural stands of *P. deltoides* usually grow in semi-arid environments, often along dried-up river beds with recurring short dry periods (Kranjcec *et al.*, 1998;

Kalischuk *et al.*, 2001). However, as only the annual increment of both clones was observed, it was not possible to understand (i) how differences in intra-annual radial growth lead to larger radial growth of *P. deltoides* and (ii) how the different responses to drought stress lead to variation in intra-annual radial growth.

This study aimed to detect clonal differences in intra-annual radial growth by evaluating $\Delta R$ and MDS. The hypothesis was tested that rates of change in $\Delta R$ and MDS reflect drought response, thus supplying information on drought tolerance and water use. To verify this, the effects of different irrigation regimes on radial growth of two highly productive clones, *P. deltoides* 'Dvina' and *Populus×canadensis* 'I-214', were investigated. This paper describes the effect of the irrigation regime on intra-annual radial growth patterns, providing information that could be helpful for poplar breeding programmes.

## Materials and methods

### Study site

The experimental plot was at the CRA-ISP [Istituto di Sperimentazione per la Pioppicoltura (Poplar Research Institute)], in Casale Monferrato (AL), Piedmont, Italy (45°07′52′N, 8°30′17′E, 106 m asl).

The site has a temperate climate with equinoctial rainfall distribution. The long-term (1926–2005) average annual temperature is 12.1 °C with total rainfall of 765 mm and an annual evapotranspiration rate (ET0-FAO) of 879 mm (Viglione, 2004).

### Experimental plantation

Four concrete tanks, 10 m long, 1.5 m wide, and 1.2 m deep, were used for the experiment, set in the soil oriented north–south and physically separated from one another. The base plates of the tanks had a 3% slope and a drainage pipe to drain excess water (Fig. 1a). Each tank was filled with 29 m³ of stirred alluvial sandy–loam soil (70% fine sand, 12% clay, and 18% silt, pH 7.2), with phosphorus (120 kg ha⁻¹ of $P_2O_5$) and potassium (250 kg ha⁻¹ of $K_2O$) added. The soil was left to settle for 3 months before planting the shoots.

Two clones were used: the early-sprouting *Populus×canadensis* Moench 'I-214' and the late-sprouting *P. deltoides* 'Dvina'. Sets
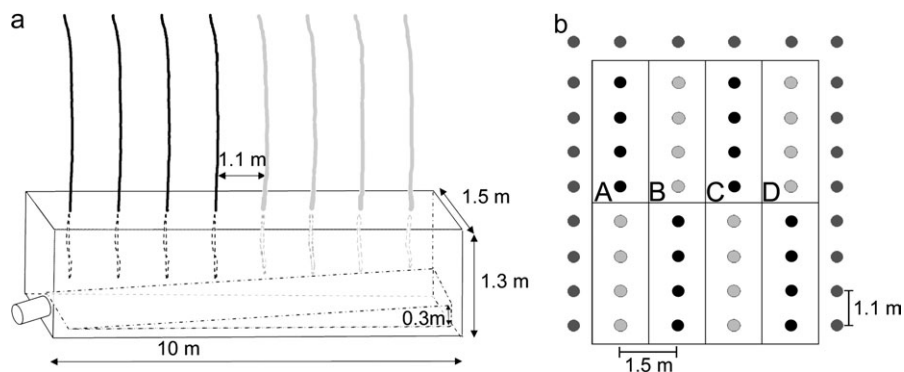


**Fig. 1.** Layout of tanks, plots, and plants. (a) Tanks were divided into two identical sectors, one for each clone, 'Dvina' (in black) and 'I-214' (in light grey). The tanks were oriented north–south, separated by concrete walls, and had a 3% slope at the base to avoid water stagnation. (b) Eight shoots were planted in each tank at 1.50×1.10 m spacing with a control row of plants (1-year-old *Populus nigra* clone, in dark grey) around the perimeters.

(250 cm long, average diameters at breast height of 28.7±0.7 mm for 'I-214' and 29.8±0.7 mm for 'Dvina'), treated according to Facciotto and Zambruno (2004), were taken from second year sprouts of 2-year-old stools in a nursery (5000 trees ha$^{-1}$). In mid-March 2005 the sets were planted in the tanks, at a depth of 70 cm. Four sets per clone were planted in each tank 1.1 m apart (Fig. 1b). To minimize edge effects, a control row of 1-year-old sets was planted along the perimeter of the tanks. The sets were watered every 2 d until April 15 [day of the year (DOY) 105] to promote adventitious root development. Mean plant height was 187±8.7 cm for 'I-214' and 192±10.7 cm for 'Dvina' when the experiment began (DOY 100).

### Irrigation regimes

All tanks received only natural rainfall until DOY 173. At this time, plant growth was similar: height 295±6 cm and 304±10 cm, basal area 360±53 cm$^2$ and 417±30 cm$^2$, total leaf area 1.56±0.15 m$^2$ and 1.62±0.17 m$^2$ for 'I-214'and 'Dvina', respectively. From DOY 174 onwards, two different irrigation regimes were applied: (i) an intensive irrigation regime—Ii—(tanks B and D) in which the soil water content was maintained close to field capacity by flushing the tanks with 70 mm of water every week; and (ii) natural rainfall and late intensive irrigation—Nr—(tanks A and C), in which irrigation, scheduled as in the Ii regime, only started on DOY 224.

The timing of the start of irrigation was based on threshold values of soil water content (<10%) and pre-dawn leaf water potential (Ψpd; less than –0.8 MPa). At these values, plants growing in the Nr regime (named 'Nr plants' in the text) showed a 90% greater reduction in CO$_2$ assimilation and leaf stomatal conductance than those growing in the Ii regime ('Ii plants' in the text). In both regimes, irrigation continued until DOY 274.

### Data collection

Stem radius variations were monitored during 2005 using automatic point dendrometers (Label *et al.*, 2000). These instruments measure the linear displacement of a sensing rod pressed against the bark. The operating principle is based on the use of a linear variable transducer (AB Electronics Ltd, Romford, Essex, UK) that responds to stem radial variation with an average sensitivity of 2±0.01 mm V$^{-1}$ (linearity of ±2%, linear expansion coefficient of 16 μm m$^{-1}$ °C$^{-1}$). As the stem expands and contracts, the rod transmits the signal to the transducer. The variable potential is digitized by an analogue-to-digital converter (PCI-1710 pg, Advanthech, Taiwan) connected to a PC-based data recording system. A total of 16 trees (two trees per clone per tank) were monitored with dendrometers installed at breast height and shielded from direct sunlight and weather damage by aluminium foil. Raw data were recorded every 15 min, and hourly averages were calculated.

Climate parameters [air temperature (°C), relative humidity (%), rainfall (mm), and global radiation (Wm$^{-2}$)] were measured at a 15-m-tall weather station close to the tanks. These were recorded every 15 min and the data stored in a SILIDATA AD2 Silimet® datalogger. Volumetric soil moisture content (θv) and soil temperature were measured by ThetaProbe soil moisture sensors (type ML1, Delta-T® Cambridge, UK) installed at 20 cm and 40 cm depths in each tank (midway between two sectors). Soil moisture content was monitored every 15 min and the data stored in a DL6 Delta-T® datalogger (Cambridge, UK). The mean soil water content (SWC, %) for each tank was calculated from the measurements taken at the two depths. From DOY 123 to DOY 222, SWC was also measured by a gravimetric method. A calibrated volume of homogeneous soil (609.92 cm$^{-3}$) was collected at 15 cm and 35 cm depths from three sectors of each tank: at 3, 5.5, and 8.5 m from the end of the tank, i.e. near clone 'I-214', in the centre of

both clones, and near 'Dvina'. The samples were immediately weighed and then kept at 120 °C for 48 h before measuring the dry weight. Soil water depletion in each sector was calculated from these data by dividing each value by the field capacity (30%). Analysis of variance (ANOVA) was performed to compare the soil water depletion between sectors (3, 5.5, and 8.5 m) (GLM procedure, SAS system).

Ψpd (MPa) was measured several times from DOY 160 to DOY 270 with a pressure chamber (PMS Instruments Co., Corvallis, OR, USA). For each clone and tank, Ψpd measurements were taken on 2–3 fully expanded leaves collected from randomly selected shoots of trees adjacent to a soil moisture sensor. Leaf gas exchange measurements (light-saturated photosynthesis and stomatal conductance) were performed using a portable open system gas analyser (CIRAS, PP-system, Hitchin, UK) on intact attached leaves. Fully expanded leaves (ninth from the apex) were sampled on two trees per sector. Maximum photosynthetic rate ($A_{max}$, μmol m$^{-2}$ s$^{-1}$) and stomatal conductance ($g_s$, mol m$^{-2}$ s$^{-1}$) were measured on sunny days (DOY 174, 222, and 240) from 10.00 h to 13.00 h, under saturating PPDF conditions of about 1400 mmol m$^{-2}$ s$^{-1}$.

### Extraction of ΔR and MDS

The daily $\Delta R$ was calculated by dividing the stem radius diurnal cycle into three distinct phases (Fig. 2) (Downes *et al.*, 1999; Deslauriers *et al.*, 2003) and identifying the (i) contraction phase, i.e. the period between morning maximum and afternoon minimum; (ii) expansion phase, i.e. the period from the minimum to the following morning maximum; and (iii) stem radius increment phase, i.e. part of the expansion phase from the time when the stem radius exceeds the morning maximum until the subsequent maximum. The difference between maximum expansion and the beginning of the third phase represents the positive $\Delta R$ (μm). When the maximum value of the previous cycle was not reached, negative $\Delta R$ (μm) was calculated and no stem radius increment phase defined (Fig. 1). The MDS (μm) was calculated as the difference between the afternoon minimum and morning maximum. The duration (h) of each cycle and phase was also calculated. The rate of MDS or $\Delta R$ (μm h$^{-1}$) was calculated by dividing the values by the duration of stem contraction or expansion, respectively.

### Statistical analysis

The growing season was divided into 17 periods of 10 d (from DOY 105 to 274) in order to assess the effect of different irrigation regimes on MDS and $\Delta R$. A 10 d scale was chosen to eliminate high frequency variations in the daily time series (Deslauriers *et al.*, 2007b). Period timing started from the maximum water stress conditions recorded in Nr plants on DOY 224, then going back and forth (in time) with equal periods until the entire growing season was covered.

The effect of the irrigation regime (treatment) and time (period) on MDS and $\Delta R$ was tested by a model fitting approach and using multivariate and univariate methods (Potvin *et al.*, 1990). To verify if treatment or clone had a significant effect on stem growth response, comparisons of fitted curves were performed on the sum of $\Delta R$ averaged by period. Logistic functions were fitted by non-linear regressions (NLIN procedure, SAS system) to the four combinations of clone and irrigation regime and compared as described in Potvin *et al.* (1990). The logistic equation fitted between the sum $\Delta R$ (sum) and time (*t*, computed DOY) was:

$$\text{sum} = A + e^{\beta - \kappa t}$$

where *A*, β, and κ were the parameters of the function representing growth asymptote, time-axis placement, and rate of change of the curve, respectively.
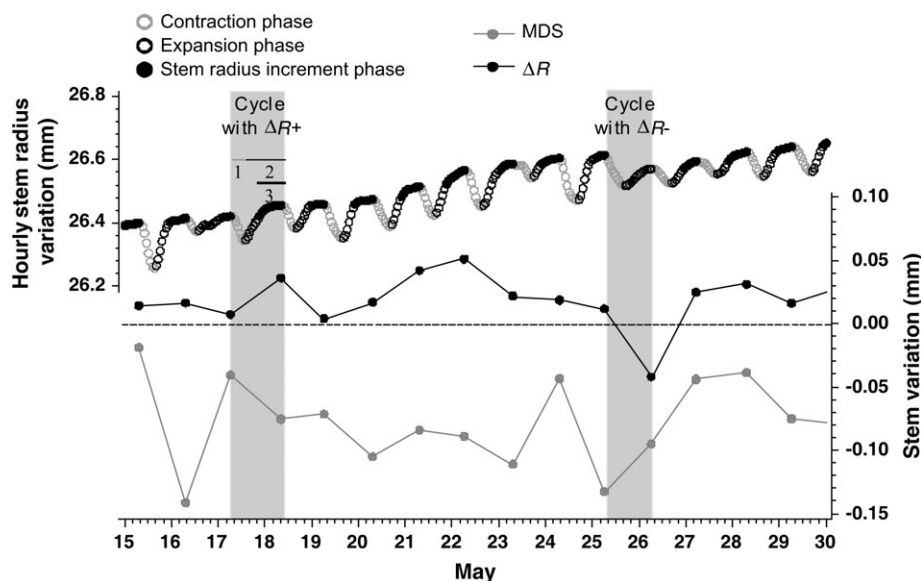
**Fig. 2.**  The stem cycle divided into three phases of contraction, expansion, and stem radius increase for the clone 'Dvina' (tree 12). A $\Delta R+$ (mm, black line) occurred when the stem radius exceeded the morning maximum until the subsequent maximum, while a $\Delta R-$ occurred when the previous cycle maximum was not reached. MDS (mm, grey line) was calculated as the difference between the afternoon minimum and morning maximum.

Treatments were compared using univariate ANOVA performed on MDS and $\Delta R$ for each assessment time (GLM procedure, SAS system). The effect of treatment [Ii and Nr (df = 1)] was tested based on a nested model, with tank [tanks A–D (df = 2)] as the experimental unit, and tree (df = 4) the error term for testing the treatment effect (Quinn and Keough, 2002). Differences between treatments were found by using the LS-means with the Scheffe test. The model was validated by testing for evidence of non-normality in residual distribution and examining the plots of residuals against predicted values (Quinn and Keough, 2002).

Log-linear models (CATMOD procedure, SAS system) were used to test the hypothesis that the frequency distribution of positive and negative $\Delta R$ was similar between periods and treatments. In order to compare periods and treatments, three 10 d periods were grouped in three homogeneous phases on the basis of changes in leaf $\Psi$pd in response to different soil water availability recorded in the Nr regime: (i) the pre-water stress period (from DOY 165 to 194) corresponding to an SWC of 14–22% and leaf $\Psi$pd below $-0.3$ MPa; (ii) the water stress period (from DOY 195 to 224) in which the SWC dropped from 14% to 8% and leaf $\Psi$pd decreased to $-0.8$ MPa; and (iii) the recovery period (from DOY 225 to 254) in which SWC ranged between 20% and 26% and $\Psi$pd was above $-0.2$ MPa, after resumption of irrigation. The differences between the three periods were examined using contrasts. The model was validated using the goodness of fit statistic ($\chi^2$) of the likelihood ratio (McCullagh and Nelder, 1989).

## Results

### Plant and soil water status under non-limiting water conditions

Field capacity was estimated at 28% and 30% during 2005 (Fig. 3B). These values were derived from SWC recorded after two rainy periods, one at the start of the growing season (DOY 104–113, 64.2 mm in seven events) and the other after bud set (DOY 247–253,

69.5 mm in six events, Fig. 3A). From DOY 104 until starting the irrigation regimes (DOY 175), SWC gradually decreased in all the tanks even if the soil water reserves partially recovered after abundant rainfall (>10 mm). With this water availability, $\Psi$pd remained above $-0.1$ MPa (Fig. 4A), and $g_s$ and $A_{max}$ were similar in the two clones (DOY 174, Table 1).

After irrigation began, SWC increased by 13% and field capacity was reached on DOY 182, 7 d later (Fig. 3B, C). After DOY 182, the SWC remained above 26% until the end of the experiment. Soil water depletion was similar in the three sectors of the tanks ($P=0.97$), with no significant differences between sectors on any measurement day ($P >0.05$). During irrigation, $\Psi$pd was maintained at around $-0.15$ MPa in both clones (Fig. 4A).

### Plant and soil water status under limiting water conditions

SWC in non-irrigated tanks was between 15% and 17% on DOY 175 (Fig. 3B). From DOY 175 to 224, SWC remained lower in non-irrigated tanks than in irrigated ones, and leaf $\Psi$pd declined when SWC was <12%. Even so, plant and soil water status were influenced by rainfall events. During a rainy period (DOY 182–188, 45 mm in five events, Fig. 3A), SWC rapidly increased from 11% to 18% and leaf $\Psi$pd remained above $-0.35$ MPa for 10 d. Thereafter, from DOY 189 to 224, with persistent high temperatures and no rainfall, SWC gradually decreased below 12% and the average leaf $\Psi$pd declined below $-0.7$ MPa in both clones (Figs 3A, 4A). In 'Dvina', at maximum water stress (DOY 222), $g_s$ in Nr plants was reduced to 0.02 mol m$^{-2}$ s$^{-1}$ compared with 0.35 mol m$^{-2}$ s$^{-1}$ in
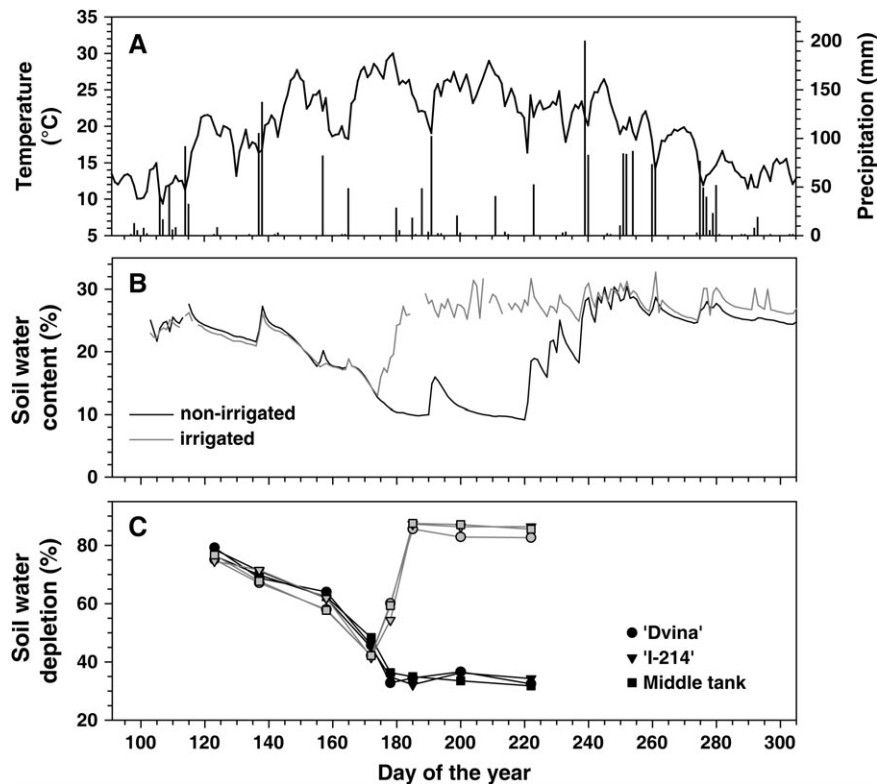
**Fig. 3.** Seasonal patterns of microclimatic conditions and soil response for 'Dvina' and 'I-214' clones from April to October 2005 (DOY 90–300). (A) Air temperature (°C) and precipitation (mm). (B) Soil water content (%) of the intensive irrigation (grey) and natural rainfall (black) treatments. (C) Soil water depletion (%) in three different sectors of the tanks, 'Dvina' (dots), 'I-214' (triangles), and tank centre (squares), for both intensive irrigation (grey lines) and natural rainfall (black lines) treatments.

Ii plants, while $A_{max}$ was reduced by 85% (Table 1). Neither $g_s$ nor $A_{max}$ was measured in 'I-214', as >70% of total mature leaf area had been shed. In 'Dvina', leaf shedding was estimated as only 25% of the total leaf area.

When the intensive irrigation was resumed on DOY 224, lower leaf $\Psi$pd was measured in 'I-214' (–0.88 MPa) than in 'Dvina' (–0.75 MPa). Twelve days later, the SWC of non-irrigated tanks reached field capacity and leaf $\Psi$pd was similar in the two irrigation regimes (Figs 3B, 4A). At DOY 240, the values of $g_s$ and $A_{max}$ were similar between clones and irrigation regimes (Table 1). During the experiment, soil water depletion and re-watering occurred in a similar way, as no significant differences were found either between the three sectors of the tank ($P=0.99$) or on each measurement day ($P > 0.05$) (Fig. 3B).

### Relating MDS and $\Delta$R to irrigation regimes

In order to assess the effect of different irrigation regimes on the rate of MDS and $\Delta R$, univariate ANOVAs were performed for each 10 d period (Fig. 4). From the start of the growing season to DOY 174, no difference was observed in the rate of $\Delta R$ (Fig. 4B, C) and MDS (Fig. 4D, E) in either clone. During the development of long shoots with mature leaves (DOY 115–135), MDS changed from nearly zero to –40 $\mu$m h$^{-1}$ and –20 $\mu$m h$^{-1}$ in 'I-214' and 'Dvina', respectively. The $\Delta R$ rates oscillated around zero in the same period, indicating no radial stem growth. After DOY 135, the MDS rate again decreased to –5 $\mu$m h$^{-1}$, while $\Delta R$ rates progressively increased and reached a mean of 5 $\mu$m h$^{-1}$ for clone 'I-214' and 9 $\mu$m h$^{-1}$ for 'Dvina'. Although some Nr plants seemed to grow at a faster rate (Fig. 4B, C), no significant difference in $\Delta R$ rates was found between irrigation regimes for most of the 10 d periods prior to DOY 174, with two exceptions: from DOY 145 to 154 for 'I-214' and from 165 to 174 for 'Dvina' higher growth rates were measured in Nr plants (Fig. 4B, C).

From DOY 175 to 224, higher negative rates of MDS were observed in Nr plants, with means varying around –20 $\mu$m h$^{-1}$, compared with –7 $\mu$m h$^{-1}$ for the Ii plants, with similar variations between clones (Fig. 4D, E). By contrast, the $\Delta R$ rates in Nr plants significantly decreased only after DOY 194 for clone 'I-214' and DOY 204 for 'Dvina', but with high variability between trees, indicated by the 5–95% confidence limits (Fig. 4B, C). However, the $\Delta R$ rates decreased when SWC was lower than 10% and leaf $\Psi$pd below –0.4 MPa (Fig. 4A). Under these conditions, the $\Delta R$ rates decreased more rapidly for

'I-214' than for 'Dvina'. Negative $\Delta R$ values were recorded for 'I-214' on DOY 205 and for 'Dvina' on DOY 215.

A clear change in MDS of clone 'I-214' was recorded in the Nr plants on DOY 205, which reached an equal value to Ii plants on DOY 215, 10 d before irrigation was resumed. The MDS of 'Dvina' Nr plants changed rapidly and reached values similar to the Ii plants only after irrigation began on DOY 224 (Fig. 4D, E). The $\Delta R$ increased rapidly in 'Dvina' and, after the first 10 d period, the values were similar to the Ii plants (7 $\mu$m h$^{-1}$), whilst it remained unchanged after resumption of
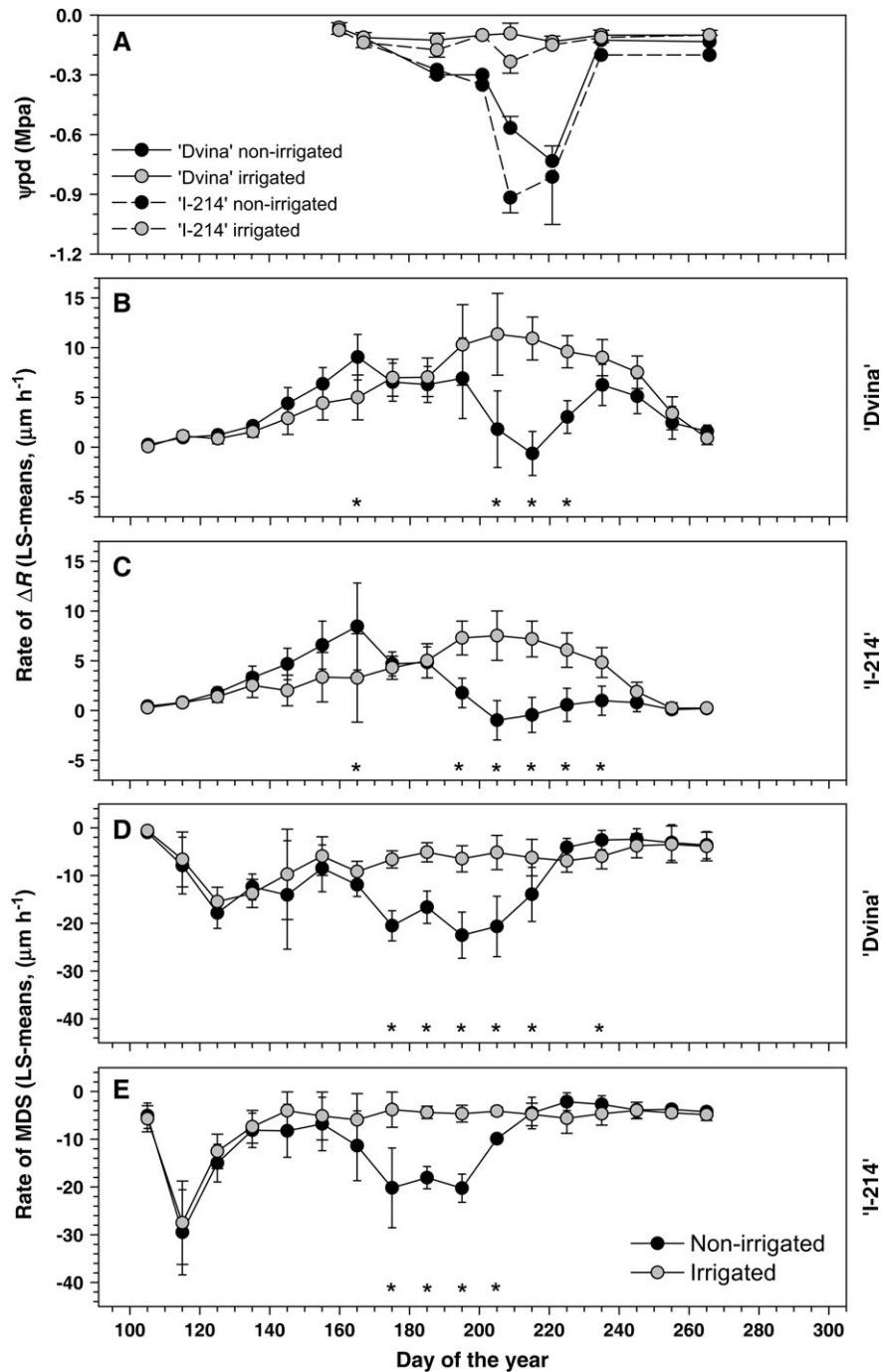


**Fig. 4.** (A) Pre-dawn leaf water potential ($\Psi_{pd}$, MPa) for clones 'Dvina' (solid line) and 'I-214' (dashed line) of intensive irrigation (grey) and natural rainfall (black). Comparisons of the rates of $\Delta R$ (B and C) and MDS (D and E) between Ii individuals and Nr individuals for 'Dvina' and 'I-214'. LS-means and their 95% interval are shown for the seventeen 10 d periods. Asterisks indicate significant differences between irrigation regimes.

**Table 1.** *Stomatal conductance* ($g_s$, *mol* $m^{-2}$ $s^{-1}$) *and light-saturated photosynthesis* ($A_{max}$, *μmol* $m^{-2}$ $s^{-1}$) *measured for intensive irrigation (Ii) and natural rainfall (Nr) treatments*

In both clones, one measurement was taken during pre-water deficit [day of the year (DOY) 174], water deficit (DOY 222), and recovery (DOY 240) periods. Values are reported as mean ±SD. nd, not detectable. Over 70% of mature leaves were shed under prolonged water deficit conditions and the remaining 30% were yellowing or showed wide necrotic areas.

| DOY | Clone | $g_s$ | | $A_{max}$ | |
| --- | --- | --- | --- | --- | --- |
| | | Nr | Ii | Nr | Ii |
| 174 | I-214 | 0.34±0.02 | 0.36±0.01 | 15.2±1.0 | 14.4±0.6 |
| | Dvina | 0.36±0.02 | 0.37±0.02 | 13.8±1.7 | 14.5±0.9 |
| 222 | I-214 | nd | 0.36±0.01 | nd | 13.4±1.0 |
| | Dvina | 0.02±0.001 | 0.35±0.02 | 2.1±0.2 | 13.4±1.1 |
| 240 | I-214 | 0.27±0.02 | 0.29±0.01 | 12.8±1.3 | 11.3±0.8 |
| | Dvina | 0.29±0.01 | 0.30±0.02 | 12.9±0.8 | 13.2±0.7 |

irrigation in clone 'I-214' and no further $\Delta R$ increase was detected.

Frequency distribution analyses of the positive and negative $\Delta R$ between treatments and periods were performed for each clone (Fig. 5, Table 2). These analyses had the advantage of removing differences in growth rates between tanks and combining the effect of time. The occurrence of $\Delta R+$ and $\Delta R-$ (Fig. 5) changed significantly between periods for both clones (Table 2, $P <0.001$), as did the interaction between periods and treatments ($P <0.001$). In the pre-water stress period, there was almost no $\Delta R-$ in either treatment (Fig. 5). Then, during the water stress period, between 27% and 52% of $\Delta R-$ was observed in Nr plants for 'I-214' and 'Dvina', respectively, whilst the proportion of $\Delta R-$ remained below 1% for Ii plants. After irrigation was resumed on DOY 224 (recovery period), 26% of $\Delta R-$ was still observed for 'I-214' whilst no difference was detected between irrigation regimes for 'Dvina' (Fig. 5). Contrasts analysis was used to test whether one specific period differed from another. For 'Dvina', the tests for differences between pre-water stress, water stress, and recovery were all highly significant, except for pre-water stress versus recovery ($P=0.93$), as the frequencies of $\Delta R+$ and $\Delta R-$ were very similar (Table 2, Fig. 5). Significant differences were found ($P <0.0001$, Table 2) for 'I-214' because of a higher proportion of $\Delta R-$ detected under water stress conditions. However, no difference was found between water stress and recovery ($P=0.82$).

The cumulative sums of $\Delta R$ showed a sharp increase starting around DOY 140, followed by a plateau indicating the end of radial growth and resulting in a typical S-shaped curve (Fig. 6). Significance testing indicated the existence of a group effect on the growth response curve (Table 3). Two successive pairwise comparisons revealed a significant difference between clones and irrigation

regimes. 'Dvina' had a different growth response with higher growth rates and total increments, independently of irrigation regime. For both clones, the growth response of Nr plants differed from that of the Ii plants (Table 3, Fig. 6), with a lower radial increment.

## Discussion

Significant differences were found in intra-annual stem growth patterns of *P. deltoides* and *Populus×canadensis* by splitting the diurnal cycle into separate phases and resolving the $\Delta R$ in terms of rate and MDS. The clone 'Dvina' displayed larger radial growth than clone 'I-214', independently of soil water availability. The results also showed that the two clones had different responses to soil water depletion. Under limited water availability, 'I-214' exhibited a significant reduction in stem growth, with high leaf senescence and abscission. The strategy of 'Dvina' differed, with partial plant dormancy, i.e. stem growth stopped in response to the dry period and leaf $\Psi$pd was maintained as high as possible. Minor osmotic adjustment normally occurs in *P. deltoides* clones under water deficit conditions (Tschaplinsky and Tuskan, 1994; Gebre *et al.*, 1998), and this could suggest that they use dehydration postponement, such as stomatal control and adjustment of tissue elastic properties, as their main drought tolerance mechanism (Kramer, 1983; White *et al.*, 1994). In *Eucalyptus globulus*, a low elastic modulus (ε), i.e. higher cell wall elasticity, facilitated longer maintenance of turgor pressure and higher growth rates than *E. nitens* under water stress conditions (White *et al.*, 1996). In *P. deltoides*, a difference in cell wall elasticity could maintain turgor pressure over a wider range of relative water content and allow higher growth rates to be maintained under prolonged water stress.

### Plant water status and $\Delta$R in non-limiting soil water conditions

In non-limiting water availability conditions, different increments of $\Delta R$ were observed between clones at the end of the growing season, with a seasonal radius increment of 'Dvina' about twice that of 'I-214'. Under optimal irrigated conditions, high clonal variability exists within *Populus* species and hybrids (Marron *et al.*, 2005; Monclus *et al.*, 2005). Analysis on a 10 d time scale indicated that 'Dvina' had a longer period (~20 d) with $\Delta R$ higher than 0. Even so, the higher radial increment of 'Dvina' was mainly the result of higher $\Delta R$ rates during the period mid-June to early-September (DOY 190–250). The higher cumulative $\Delta R$ of 'Dvina' was due to a cumulative effect of both higher daily stem growth and a longer period with positive $\Delta R$. In faster growing *Liriodendron tulipifera* L., trees were more affected by rainfall and temperature and optimized their stem radial
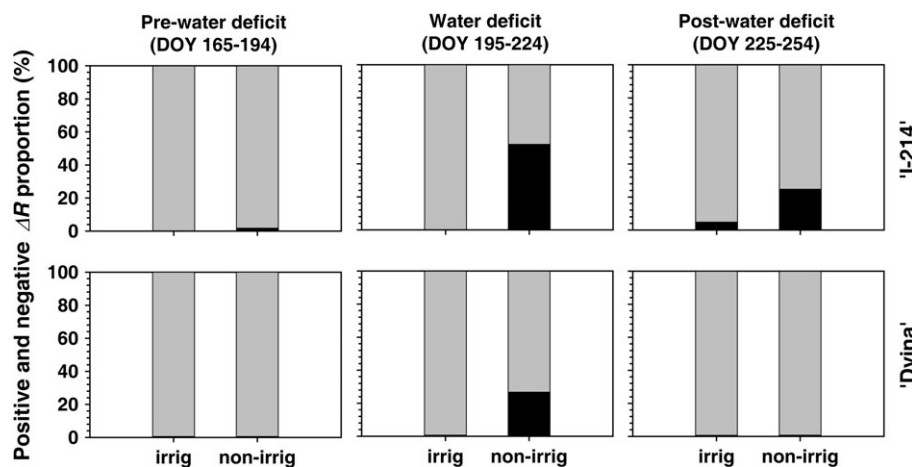
**Fig. 5.** Frequency distribution (%) of ΔR+ (grey) and ΔR– (black) for intensive irrigation (Ii) and natural rainfall (Nr) during three different periods for clones 'Dvina' and 'I-214'. Differences between irrigation regimes and periods are reported in Table 2.

**Table 2.** *Comparison of ΔR+ and ΔR– frequency between treatment (intensive irrigation and natural rainfall), period (pre-water deficit, water deficit, and recovery), and their interaction*

Contrast tests were calculated to illustrate the differences between periods for both 'Dvina' and 'I-214'. ns, not significant.

| Source | df | Dvina | | I-214 | |
|---|---|---|---|---|---|
| | | $\chi^2$ | *P*-value | $\chi^2$ | *P*-value |
| Intercept | 1 | 111.6 | <0.0001 | 111.6 | <0.0001 |
| Period | 2 | 17.1 | <0.001 | 42.4 | <0.0001 |
| Period×treatment | 2 | 16.9 | <0.001 | 40.6 | <0.0001 |
| Likelihood ratio contrast | 1 | 2.7 | 0.0978 | 35.0 | <0.0001 |
| Pre-water deficit versus water deficit | 1 | 7.6 | <0.01 | 41.8 | <0.0001 |
| Pre-water deficit versus recovery | 1 | 0.01 | ns | 34.6 | <0.0001 |
| Water deficit versus recovery | 1 | 6.9 | <0.01 | 0.05 | ns |
| Water deficit versus others | 1 | 17.1 | <0.0001 | 29.2 | <0.0001 |

growth during periods with higher water availability and higher temperature (McLaughlin *et al.*, 2003).

### Plant water status and MSD in limiting water conditions

Long-lasting soil water deficit induced a significant reduction in leaf water potential. In Nr plants of both clones, the first detectable response to change in the soil water content was a significant increase in the MDS rate, which tripled after SWC decreased below 14%. The increase in MDS occurred in the absence of differences in leaf Ψpd and before a detectable decrease in ΔR. This supports the hypothesis that stem contraction provides information on tree water relations (Zweifel *et al.*, 2000) and that the increase in MDS is the first detectable morpho-physiological signal of changes in the water status of the whole plant (Goldhamer *et al.*, 1999; Naor and Cohen, 2003; Remorini and Massai, 2003; Conejero *et al.*, 2007). Two combined causes could have led to the sudden increase in MDS. In water deficit conditions, longer contraction phases resulted in a balanced cycle of about 12 h/12 h of stem contraction/expansion (Deslauriers *et al.*, 2007a). In slowly developing water shortage (e.g. days or weeks), the hydraulic resistance of the whole path (roots–leaves) increases, leading to a slower recovery rate of the plant water balance. Therefore, even if stomatal closure occurs earlier in the morning as the water stress persists, the increase in stem contraction duration combined with a poorer re-hydration could lead to higher MDS. In the present study, the plants were probably able to sustain such high MDS for almost 40 d because of a few rainfall events during the induced drought period, allowing some recovery of the soil and stem water reserves.

Asynchronous changes between plant water status and leaf Ψpd have already been reported in poplar (Tardieu and Simonneau, 1998) and are probably related to high endogenous leaf control mechanisms (solute accumulation and abscisic acid concentration), which maintain stable leaf water status over a wide range of evaporative demand or soil water supplies (Jones, 2004). It can therefore be hypothesized that in the early drought period, while leaf Ψpd was maintained as a strategy to avoid tissue dehydration, a decline in tree water status was already taking place in the stem, as indicated by higher MDS. In Nr plants of both clones, leaf Ψpd was influenced by rainfall events of >10 mm even if no significant changes were observed in MDS and ΔR rates at a 10 d time scale. However, analyses on a daily scale (data not shown) highlight a strong but transient recovery of the MDS rate (from –20 μm h$^{-1}$ to –10 μm h$^{-1}$) after abundant rainfall.
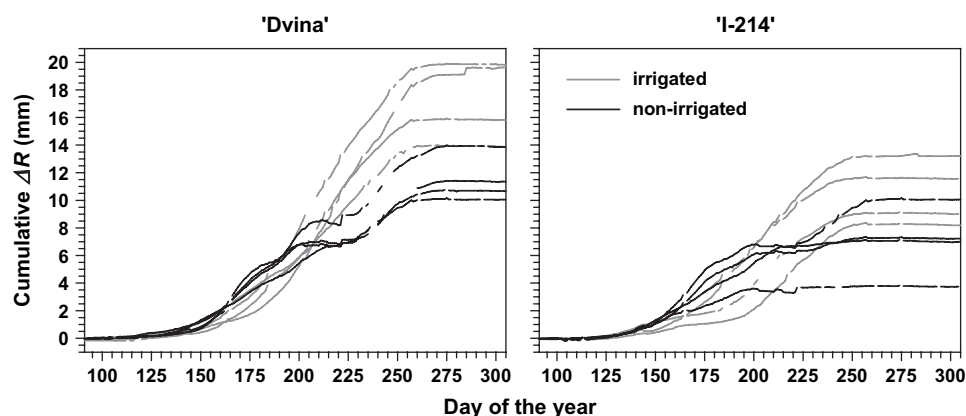
**Fig. 6.** Cumulative $\Delta R$ (mm) for intensive irrigation (black line) and natural rainfall (grey line) treatments in 'Dvina' and 'I-214'. Differences between groups of curves are reported in Table 3.

**Table 3.** *Comparisons between logistic growth response curves, fitted to the cumulative sum of stem radius variation (μm) averaged by period*

$F$-values were calculated among all groups, between clones (groups 1–2 versus 3–4), and between irrigation regimes (groups 1–3 versus 2–4). Groups represent (1) Nr plants 'Dvina', (2) Ii plants 'Dvina', (3) Nr plants 'I-214', and (4) Ii- plants 'I-214'.

| | 'Dvina' | | 'I-214' | | Source of variation | | |
|---|---|---|---|---|---|---|---|
| | Non-irrigated | Irrigated | Non-irrigated | Irrigated | Among groups | Between clones | Between treatments |
| $A$ | 11287.6 | 17906.8 | 6056.1 | 10966.7 | | | |
| $\beta$ | 8.18 | 11.23 | 14.24 | 10.87 | | | |
| $\kappa$ ($10^{-2}$) | 4.07 | 5.19 | 8.34 | 5.27 | | | |
| $F$-value | 547.8 | 795.0 | 172.9 | 244.2 | 53.2 | 34.9 | 37.0 |
| df ($\upsilon_1$, $\upsilon_2$) | 3, 65 | 3, 65 | 3, 65 | 3, 65 | 12, 256 | 4, 264 | 4, 264 |
| $P$ | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.001 | <0.001 | <0.001 |

*Plant water status and $\Delta$R in limiting water conditions*

Compared with MDS, there was a delay of 20 d before $\Delta R$ was near zero. In 'I-214', $\Delta R$ was already close to zero on DOY 195 when leaf $\Psi$pd was still higher than −0.4 MPa. In 'Dvina', $\Delta R$ was approximately zero on DOY 210 at a leaf $\Psi$pd of –0.5 MPa, suggesting a better water use (carbon uptake versus water loss). Cell enlargement is known to be directly inhibited by a decrease in hydrostatic pressure of apoplastic water during the early stage of water deficit (Savidge, 2000; Abe *et al*., 2003; Steppe *et al*., 2006). In drought-stressed oak, Saveyn *et al*. (2007) observed that from the moment $\Delta R$ was near zero, a depression in stem $CO_2$ efflux rate occurred linked to a reduction in metabolic activity (growth and maintenance processes) caused by the lowered daytime stem water status. Under the present field conditions, it was not possible to separate the direct (water) and indirect (carbon limitation) effects causing reduced radial growth as in Daudet *et al*. (2005). However, when growth stopped, both carbon and water availability probably limited radial growth. At maximum stress (DOY 222), $g_s$ and $A_{max}$ were strongly reduced, corresponding to a negative $\Delta R$. In slowly developing water stress, a reduction in the biochemical capacity for carbon assimilation and utilization may occur along with restrictions in gaseous diffusion (Chaves *et al*., 2002; Flexas *et al*., 2002), inevitably leading to a decrease and halting of radial growth.

The impact of drought on biomass production is mostly genotype dependent (Monclus *et al*., 2006). The $\Delta R$ rate decreased more slowly in 'Dvina' than in 'I-214' and, as a consequence, 'Dvina' maintained an $\Delta R+$ equivalent to the irrigated individuals for longer. The maintenance of high growth rates in the trunk and reduced leaf senescence under stress conditions are suitable parameters to assess drought tolerance (Tschaplinsky *et al*., 1998). The extent of senescence reflects the severity of drought stress and, together with xylem cavitation, reduces transpiration demand, improving the water balance of the tree (Sperry and Pockman, 1993). Lower leaf $\Psi$pd and greater leaf senescence were detected in clone 'I-214' at the end of the Nr irrigation regime, so its higher degree of leaf abscission of about 70% could suggest an avoidance strategy in order to maintain a favourable water balance.

After resumption of irrigation, 'Dvina' gradually recovered high $\Delta R+$ whilst 'I-214' was unable to restore stem growth even if parameters such as leaf $\Psi$pd (Fig. 4), stomatal conductance, photosynthesis, and shoot growth

did not differ from those recorded in the irrigated individuals. However, the behaviour of 'I-214' could be a consequence of the higher leaf abscission during the drought period and the production of newly expanded leaves shortly after. Shoot apices and leaves have a higher ranking priority than cambium as a sink for carbon (Minchin and Lacointe, 2004). After the onset of irrigation, nutritional reserves and other growth regulators (auxin) may thus be shifted preferentially towards shoot elongation and new leaf production at the expense of stem and root, as reported in *L. tulipifera* (McLaughlin *et al.*, 2003). Compared with 'Dvina' and Ii plants of 'I-214', this strategy removed about 25 d of possible stem radial growth, which clearly decreases wood production. According to Monclus *et al.* (2006), the most productive poplar genotypes were also the ones more sensitive to drought. However, the present results did not confirm this hypothesis, as the more productive clone 'Dvina' was more tolerant.

The findings of this study lead to the conclusion that the *P. deltoides* clone 'Dvina' has a greater ability to maintain stem growth for a given SWC as it was more productive than 'I-214' under conditions of limited water availability. However, its high capacity to restore stem growth after drought might influence the wood quality, as annual rings with different properties may be produced under recurrent drought conditions (Wimmer *et al.*, 2002). By contrast, clone 'I-214' could produce smaller but uniform ring widths under limited water availability. Trials are currently in progress to investigate the relationship between stem growth pattern and wood properties of annual rings in these clones in order to include this information in breeding programmes.

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