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Physiological traits of two *Populus × euramericana* clones, Luisa Avanzo and Dorskamp, during a water stress and re-watering cycle

NICOLAS MARRON,¹ DIDIER DELAY,¹ JEAN-MICHEL PETIT,¹ ERWIN DREYER,² GUY KAHLEM,¹ FRANCIS M. DELMOTTE¹ and FRANCK BRIGNOLAS^{1,3}

¹ Laboratoire Biologie des Ligneux et des Grandes Cultures, UPRES EA 1207, UFR—Faculté des Sciences, Université d'Orléans, rue de Chartres, BP 6759, 45067 Orléans Cedex 02, France

² UMR INRA-UHP "Ecologie et Ecophysiologie Forestières," INRA Nancy, 54380 Champenoux, France

³ Author to whom correspondence should be addressed (franck.brignolas@univ-orleans.fr)

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Summary We compared responses to drought and re-watering of greenhouse-grown cuttings of *Populus × euramericana* (Dode) Guinier clones, Luisa Avanzo and Dorskamp. Total leaf area, leaf number, leaf area increment and stomatal conductance were evaluated periodically during a 29-day drought period and for 16 days after re-watering. Soil water content and predawn leaf water potential (Ψ_{wp}) were measured on Days 29 and 45. On the same days, relative water content (RWC), specific leaf area (SLA), nitrogen, chlorophyll, soluble sugars, total phenols, flavanols and antioxidant activity were determined for leaves taken from the bottom to the top of each cutting.

Leaves of Luisa Avanzo cuttings grew more rapidly than leaves of Dorskamp and exhibited higher SLA, but lower concentrations of nitrogen, chlorophyll and soluble sugars and lower antioxidant activity per unit area. On Day 29, after withholding water, both clones had closed their stomata, reduced rates of leaf growth, and lower Ψ_{wp} and RWC; however, the clones differed in their responses to soil water depletion. Compared to Dorskamp, Luisa Avanzo closed its stomata earlier and maintained higher Ψ_{wp} , but lower RWC and leaf sugar concentrations. Antioxidant activity of leaf methanolic extracts decreased in response to water stress only in Luisa Avanzo. Leaf physiology and its modulation by water stress were age dependent in Luisa Avanzo.

Keywords: antioxidant activity, drought, leaf growth, phenolics, predawn leaf water potential, relative leaf water content, soil water content, specific leaf area, stomatal conductance, sugars.

Introduction

Fast-growing trees, such as poplar (*Populus* L.), raised in short-rotation intensive cultures, represent an alternative use for agricultural land (Bisoffi and Gullberg 1996). However, drought episodes may severely reduce poplar productivity and increase sensitivity to various pathogens (Soulères 1992, Pinon and Valadon 1997). Whole-plant responses to water stress

range from stomatal closure to increased root/shoot ratio, leaf area reduction and osmotic adjustment. Such mechanisms either increase water availability (Levitt 1980) or reduce water loss, thereby increasing plant water-use efficiency but decreasing productivity. Poplar clones display high variability in drought tolerance (Gebre et al. 1994, 1998, Harvey and van den Driessche 1997, Robison and Raffa 1998, Tschaplinski et al. 1998). Several physiological and morphological traits of poplar clones, including stomatal sensitivity to water stress (Liu and Dickmann 1992, Blake et al. 1996, Harvey and van den Driessche 1997), potential for osmotic adjustment (Gebre et al. 1994), sensitivity of leaf expansion, extent of leaf abscission and root/shoot ratio increase (Liu and Dickmann 1992, Chen et al. 1997, Ibrahim et al. 1997, Tschaplinski et al. 1998) vary widely among clones and this variation is receiving increased attention in breeding programs.

Early stomatal closure in response to soil drying may protect trees from catastrophic xylem cavitation (Blake et al. 1996, Harvey and van den Driessche 1997) and subsequent leaf abscission (Liu and Dickmann 1992). Osmotic adjustment, using soluble sugars, has also been observed among poplar clones subjected to drought, and contributes to both plant survival and growth maintenance (Meyer and Boyer 1981, Tschaplinski and Blake 1989, Tan et al. 1992, Gebre et al. 1994). In several deciduous tree species, a large capacity for osmotic adjustment has been associated with high dehydration tolerance (Augé et al. 1998). However, differences in drought tolerance among poplar clones cannot be solely explained by differences in osmotic adjustment (Gebre et al. 1998), because thickening of cell walls, by lignification and polysaccharide deposits, also contributes to the maintenance of leaf shape (Niinemets 2001). Leaf area reduction, caused by leaf abscission and reduced leaf growth, is also an adaptive response to drought that results in an increase in root/shoot ratio (Liu and Dickmann 1992, Chen et al. 1997, Ibrahim et al. 1997, Tschaplinski et al. 1998).

When carbon dioxide assimilation is limited by water deficits, the rate of active oxygen formation may increase in chlor-

oplasts (Smirnov 1993, Foyer and Harbinson 1994, Foyer et al. 1997). A drought-tolerant genotype should, therefore, be able to increase water-use efficiency and prevent oxidative injury during drought. Plant cells contain an integrated system of enzymatic and non-enzymatic antioxidants that remove reactive oxygen species (Alscher et al. 1997). Non-enzymatic systems include many classes of metabolites that can play an antioxidant role in vivo (Larson 1988, Smirnov 1993, Foyer and Harbinson 1994). Plant polyphenols can function as free radical scavengers (Larson 1988, Tamagnone et al. 1998). Previous studies have shown that poplars exhibiting resistance to ozone-induced injury are characterized by an increase in polyphenols, such as chlorogenic acid in chloroplasts, which are known for their antioxidant properties (Biagioni et al. 1997). Although the evidence for a direct contribution of plant polyphenols to the protection of mesophyll cells against oxidation resulting from severe water stress is equivocal, some of these compounds are lignin precursors and can participate in cell wall strengthening during drought (Niinemets 2001).

We attempted to associate variations in leaf polyphenols and antioxidant activity of leaf methanolic extracts with variations in water relations parameters during a soil drying and re-watering cycle in two *Populus × euramericana* (Dode) Guinier cultivars, Luisa Avanzo and Dorskamp, that differ in drought tolerance, based on field observations (Brignolas et al. 2000). High clay content, high soil density, flooding or lack of water and lack of nutrients limit growth of Luisa Avanzo, whereas Dorskamp is more plastic and less water-demanding (Terrasson 1988, Soulères 1992). Subjected to drought of similar severity, growth reductions in Luisa Avanzo cuttings are larger than those in Dorskamp cuttings and irreversible damage occurs at a lower predawn leaf water potential in Dorskamp than in Luisa Avanzo (Brignolas et al. 2000). Specific objectives were to characterize differences in the patterns of drought response between the clones, and to identify early traits associated with water stress tolerance. Accordingly, we measured several plant and leaf parameters on well-watered, drought treated and re-watered cuttings grown in a greenhouse. Measurements on both growing and mature leaves are reported.

Materials and methods

Plant material and growth

Two-month-old, 20-cm woody stem cuttings obtained from 1-year-old stems of *Populus × euramericana* (Dode) Guinier (*P. deltoides* (Bartr.) Marsh. × *P. nigra* L.) cv Dorskamp (male) and cv Luisa Avanzo (female), were used in all experiments. During February 2000, 20 1-month-old rooted cuttings of each clone were repotted in 1-l pots containing a mixture of sand, clay, brown peat, composted cluster pine bark, wood fiber, blond peat and moss (10:10:10:20:20:30, v/v, pH 6.5) and including N,P,K,Mg (15:9:17:4) at a total concentration of 1.7 kg m⁻³. All cuttings were placed in a greenhouse heated to 20 °C and exposed to natural daylight. Cuttings were watered every third day until the start of the experiment.

From April 2000, leaves of each cutting were numbered from the bottom (Rank 1) to the top (Rank *n*) of the stem. Every second day, individual leaf area was calculated as $\log A_m = a \log x$, where A_m is leaf area, x is measured leaf width, and a is a coefficient equal to 1.73 and 1.81 for Dorskamp and Luisa Avanzo, respectively (Brignolas et al. 2000). Growth parameters were expressed as total cutting leaf area (calculated by summing individual leaf areas), leaf number increase (day⁻¹) and leaf growth (cm² day⁻¹).

Drought treatment

Beginning on April 10, 2000 (Day 0), water stress was induced by withholding water from 10 cuttings per clone. The 10 remaining cuttings were well watered and used as controls. On Day 0 and twice weekly thereafter, stomatal conductance of two control and two water-stressed cuttings of each clone was estimated on both upper and lower sides of a mature leaf (Rank 3) with a steady-state diffusion porometer (PMR-2, PP Systems, Hertfordshire, U.K.).

On May 9, 2000 (Day 29), six drought treated and six control cuttings of each clone were used to estimate predawn leaf water potential (Ψ_{wp}), relative water content (RWC) and specific leaf area (SLA) and for chemical analyses. Volumetric soil water content (SWC) was measured in a calibrated volume of homogeneous substrate (150 cm³). The substrate was weighed, incubated at 100 °C for 24 h and reweighed. Results were expressed in g water cm⁻³ soil. Predawn leaf water potential was measured with a pressure chamber on a mature leaf (Rank 6). Three cuttings were used to determine RWC and SLA. All leaves were weighed immediately after collection to determine fresh weight (FW). The cut end of each leaf was placed in distilled water, and kept in dim light at 4 °C for 24 h before turgid weight (TW) was recorded. Dry weight (DW) was measured after leaves were dried for 24 h at 75 °C.

Leaves of the three remaining cuttings were used for chemical analyses. Mature leaves were collected in pairs from the bottom (Group 1, old adult leaves) to the top (Group 8, young adult leaves) of each cutting. Expanding leaves (first leaves of about 2 cm length) were pooled and corresponded to Group 9. Samples were frozen in liquid nitrogen, freeze dried, ground to powder and kept under vacuum on anhydrous silica gel until analyzed.

For each clone, the four remaining drought-treated cuttings were re-watered and maintained at field capacity until May 25, 2000 (Day 45). On this date, Ψ_{wp} was estimated on four controls and four re-watered cuttings. We also determined SWC of the rooting medium for these cuttings. Measurements of RWC were made on one cutting per clone per treatment. Leaves of the remaining cuttings were collected for chemical analyses as described previously.

Extraction and assay of chlorophyll and nitrogen

Ten mg of freeze-dried leaf powder was suspended in 500 μ l of acetone:distilled water (8:2, v/v). The mixture was subjected to an ultrasonic bath for 30 min at 4 °C and then centrifuged at 20,000 g for 10 min. The supernatant (300 μ l) was

collected and the residue re-extracted once under the same conditions, and the recovered supernatants ($2 \times 300 \mu\text{l}$) pooled. Chlorophyll was determined spectrophotometrically at 652 nm (Bruinsma 1961) and chlorophyll concentration was calculated according to the McKinney equation and expressed in $\mu\text{mol m}^{-2}$ of leaf area. Total nitrogen in 1.5 mg of powder was measured with an elemental analyzer (2500 NCS, ThermoQuest, Finnigan CE Instruments, Waltham, MA) and expressed in g m^{-2} of leaf area.

Extraction of soluble sugars and phenolic compounds

Thirty mg of freeze-dried powder was suspended in 1.5 ml of methanol:distilled water (8:2, v/v) (Scalbert 1992) and subjected to an ultrasound bath for 30 min at 4 °C. After centrifugation at 20,000 g for 10 min, 900 μl of the supernatant was collected. The pellet was re-extracted under the same conditions and 900 μl of the supernatant was removed and pooled with the first supernatant. The resulting extract was filtered through a Sep-Pak C18 cartridge (Waters, Milford, MA) to remove photosynthetic pigments.

Soluble sugars assay

Soluble sugars in each extract were determined by a resorcinol sulfuric acid micromethod (Monsigny et al. 1988). Each 20- μl aliquot of extract was mixed with 20 μl of 6 g l^{-1} aqueous resorcinol (Merck-Eurolab) and 100 μl of 75% aqueous sulfuric acid. The mixture was homogenized and heated to 90 °C in an oven for 30 min and subsequently kept in the dark at room temperature for 30 min. The absorbance at 450 nm was recorded with a microtiter plate reader (Titertek Multiscan Plus, Molecular Devices, Wokingham, U.K.). Results were expressed in mg equivalents of mannose per m^2 of leaf area, based on a mannose standard range.

Phenolic compounds assay

Absorption spectra of phenolic extracts from expanding and mature leaves of each clone were recorded from 250 to 400 nm. To estimate the amount of phenolics in the extracts we used Folin-Ciocalteu reagent (Carlo Erba Reagenti, Rodano, Italy) (Scalbert et al. 1989). First, methanolic extracts were diluted 1:50 with water. Folin-Ciocalteu reagent diluted 10 times with distilled water (2.5 ml) and 0.7 M Na_2CO_3 (2 ml) were added to the samples (500 μl). Tubes were incubated for 5 min at 50 °C and then cooled to room temperature. Absorbance was measured at 765 nm (Uvikon 930, Kontron Instruments, Zurich, Switzerland). Results were expressed in catechin equivalents per m^2 of leaf area, based on a (+)-catechin (Sigma) standard range.

Flavanols assay

Extracts were diluted to 1:100–1:400 and 20 μl of DMACA reagent (50 mg of 4-(dimethylamino)cinnamaldehyde (Aldrich) dissolved in 5 ml of 1.5 M methanolic sulfuric acid) was added to the diluted extracts (1 ml). The mixtures were then incubated for 1 h at room temperature. Absorbance was measured at 637 nm. Results were expressed in mg of catechin

equivalents per m^2 of leaf area, based on a (+)-catechin standard range (Treutter et al. 1994).

Antioxidant activity estimation of methanolic leaf extracts

Antioxidant activity of methanolic leaf extracts was estimated by the linoleic acid- β -carotene oxidation method (Miller 1970). A linoleic acid- β -carotene emulsion was prepared by mixing 10 mg of linoleic acid (Sigma) with 750 μl of 0.2 mg ml^{-1} chloroformic β -carotene solution and 100 mg of Tween 40 (polyoxyethylenesorbitan monopalmitate) in a stoppered tube. Chloroform was evaporated under nitrogen flow for 10 min. The resulting mixture was adjusted to 25 ml with distilled water and vigorously shaken for 10 s. Ten- μl aliquots of extract were adjusted to 250 μl with methanol:distilled water (8:2, v/v) and 2.5 ml of linoleic acid- β -carotene emulsion was added and the mixture was heated to 50 °C. The control consisted of 250 μl of methanol:distilled water (8:2, v/v) and 2.5 ml of linoleic acid- β -carotene emulsion. Absorbance was measured at 470 nm every 15 min for 90 min. Results were computed as the ratio of β -carotene protection of the extract to the methanol:distilled water (8:2, v/v) control, and presented as the leaf area providing protection of 50% of the β -carotene (Leaf Area 50).

Statistical analyses

Results were evaluated by linear regression analysis and analysis of variance using the SPSS statistical software package (SPSS, Chicago, IL). Means are expressed with their standard error (\pm SE). Where regression analysis indicated an effect of leaf rank, this factor was included in the statistical model as a covariate. All statistical tests were considered significant at $P \leq 0.05$.

Results

Plant growth

On Day 0 (April 10, 2000), Dorskamp cuttings had a larger total leaf area than Luisa Avanzo (Figure 1); however, leaf number increment and leaf expansion rate were twice as high in Luisa Avanzo as in Dorskamp. Independently of the applied treatment, increases in leaf number and leaf area of all cuttings were reduced during the first 10 days of the experiment by about 50% in Luisa Avanzo and 100% in Dorskamp. From Day 10 until the end of the experiment, leaf growth gradually recovered to initial rates in control cuttings of Luisa Avanzo, whereas there was only a slight resumption of leaf growth in control cuttings of Dorskamp.

From Day 22 on, drought-treated cuttings of Luisa Avanzo exhibited a significant decrease in leaf number increment and leaf area expansion compared with control cuttings (Figure 1). On Day 29, leaf number increment and leaf area expansion of drought-treated cuttings of Luisa Avanzo were six and two times lower, respectively, than in control cuttings. Two days after re-watering (Day 31), leaf number increment and leaf area expansion had increased to control values.

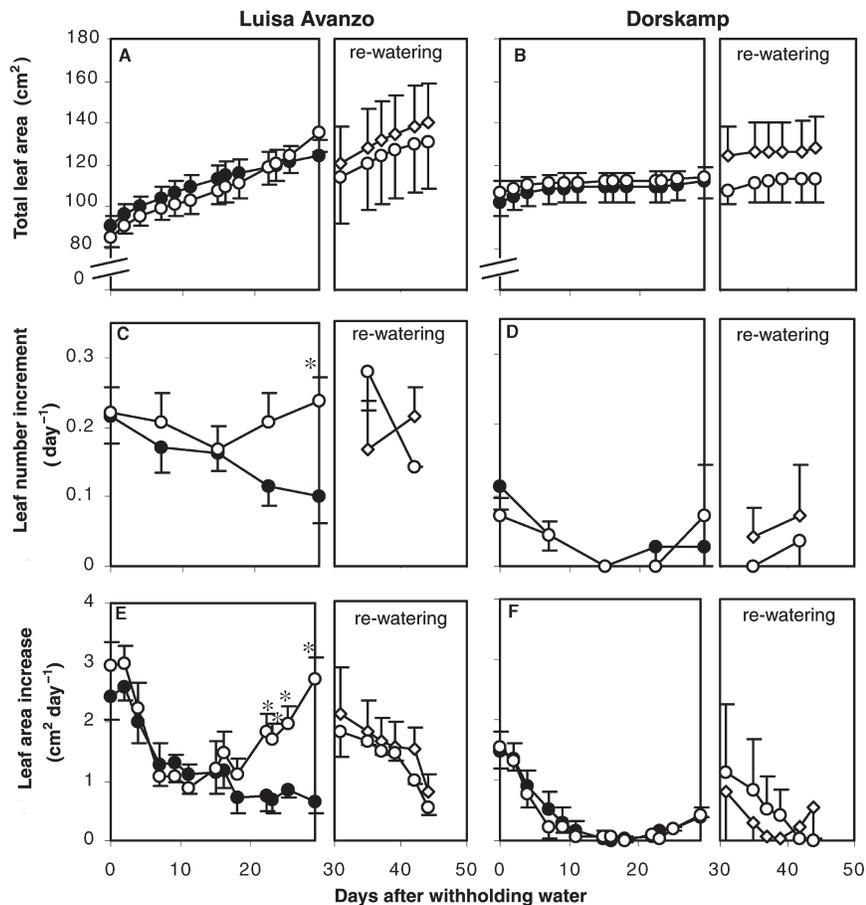


Figure 1. Time course of changes in total leaf area (A and B), leaf number increment (C and D) and leaf area increase (E and F) in control (○), water-stressed (●) and re-watered cuttings (◇) of Luisa Avanzo and Dorskamp. Means (\pm SE) were compared between 10 control cuttings and 10 water-stressed cuttings before re-watering and between four control cuttings and four re-watered cuttings after re-watering. Significant differences ($P \leq 0.05$) between control and water-stress conditions and between control and re-watering conditions are indicated by asterisks (*).

Water relations

Both clones have amphistomatous leaves, but stomatal conductance of the lower leaf side was twice that of the upper leaf side. At Day 0, stomatal conductance of the lower side ranged from 150 to 250 $\text{mmol m}^{-2} \text{s}^{-1}$ and did not vary between clones. Drought caused a concomitant decrease in stomatal conductance on both sides, which was significant 1 week earlier for Luisa Avanzo than for Dorskamp (Figure 2). Three days after re-watering (Day 32), stomatal conductance of both clones returned to control values.

At Days 29 and 45, RWC (Figure 3) and Ψ_{wp} of control cuttings from both clones remained above 94% and -0.8 MPa, respectively, and SWC remained above $0.27 \pm 0.03 \text{ g cm}^{-3}$. Water-stressed cuttings displayed significantly lower RWC and Ψ_{wp} than control cuttings, and SWC was also lower in the drought treatment than in the control treatment. Leaf RWC was lower in Luisa Avanzo ($< 90\%$) than in Dorskamp ($> 90\%$, Figure 3). The drought-induced decrease in RWC was observed in all leaves of Luisa Avanzo, but mainly in old mature leaves of Dorskamp. Values of Ψ_{wp} were lower in Dorskamp (-1.6 MPa) than in Luisa Avanzo (-1.3 MPa). Between-clone variations in RWC and Ψ_{wp} could not be explained by differences in SWC, which was similar for both clones (0.11 g cm^{-3}) in the drought treatment. Sixteen days after re-watering

(Day 45), cuttings of both clones exhibited similar RWC and Ψ_{wp} to the control cuttings, and SWC also recovered to control values.

Specific leaf area

A relationship between SLA and leaf rank on the stem was established for each clone and each treatment (Figure 3 and Table 1). Expanding leaves differed significantly from mature leaves in several traits and were not taken into account in the SLA–rank relationship or in the rank–trait correlations (Table 2). In Dorskamp, SLA was unaffected by either leaf rank or treatment. Expanding leaves of Luisa Avanzo had a lower SLA than mature leaves and did not vary in response to either water stress or re-watering. The SLA of mature leaves was significantly higher in Luisa Avanzo than in Dorskamp, and the SLA of mature leaves of Luisa Avanzo was dependent on both rank on the stem and treatment. In Luisa Avanzo, SLA increased with leaf age in water-stressed cuttings, reflecting product remobilization from mature leaves. After re-watering, SLA–rank variations were similar to those observed for control cuttings but with lower values, perhaps indicating product accumulation or thickening of cell walls. Because of the observed differences in SLA, results were expressed per unit leaf area in order to compare clones and treatments on a similar functional basis.

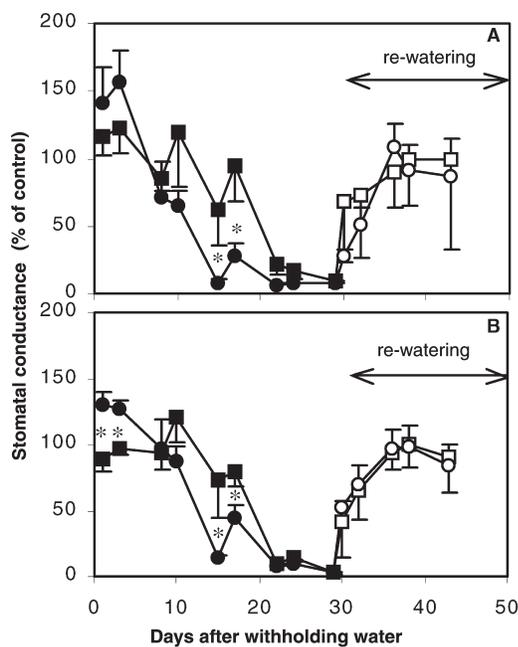


Figure 2. Time course of changes in stomatal conductance estimated on upper (A) and lower (B) leaf surfaces and expressed as percent of control for water-stressed (●, ■) and re-watered cuttings (○, □) of Luisa Avanzo (●, ○) and Dorskamp (■, □). Means (\pm SE) were calculated from two cuttings per treatment. Significant differences ($P \leq 0.05$) between clones are represented by asterisks (*).

Nitrogen and chlorophyll

When nitrogen data were expressed per unit weight (N_M), no significant differences were observed between clones or between control and water-stressed cuttings (data not shown). Nevertheless, re-watering caused a significant decrease in N_M , mainly in the oldest leaves of both clones. Values varied between 7 and 15 mg g⁻¹ and were lower than those commonly measured in other studies (25 mg g⁻¹), indicating that N could be a limiting factor for growth (Niinemets 1997, Peterson et al. 1999). When data were expressed per unit leaf area (N_A) (Figure 4), leaves of control and water-stressed cuttings exhibited significantly higher N_A in Dorskamp than in Luisa Avanzo. In Luisa Avanzo, the drought treatment caused a significant decrease in N_A of the oldest leaves, reflecting SLA variations. Compared with leaves of control cuttings, re-watering resulted in a significant decrease in N_A in mature leaves of Dorskamp, whereas N_A in leaves of re-watered cuttings of Luisa Avanzo was similar to that in control leaves as a result of variations in SLA.

Chlorophyll per unit leaf area (Chl_A) increased significantly with leaf aging in control cuttings of Luisa Avanzo, but not in control cuttings of Dorskamp (Figure 4 and Table 2). However, Chl_A was significantly higher in Dorskamp than in Luisa Avanzo, especially in younger leaves. For both clones, the oldest leaves of water-stressed cuttings exhibited a large decrease in Chl_A ; however, it was linked to SLA variations between control and water-stressed cuttings for Luisa Avanzo only. After re-watering, Chl_A in Luisa Avanzo recovered to

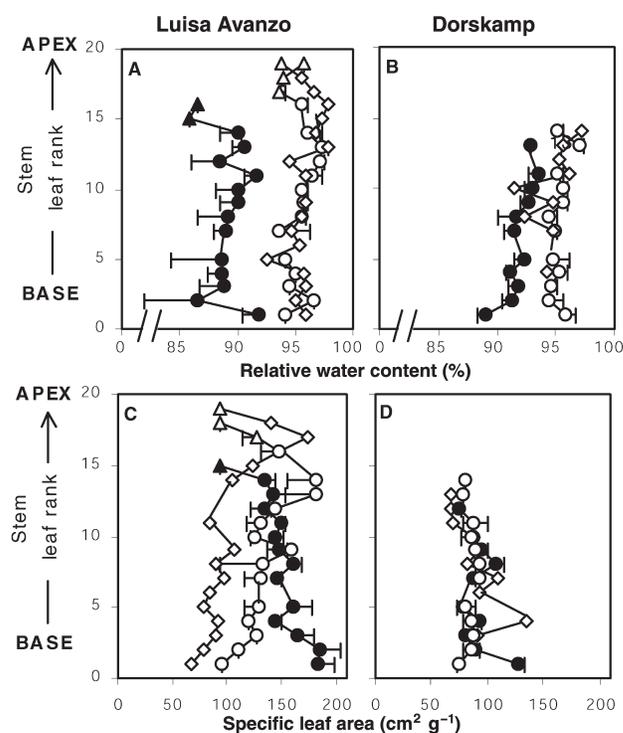


Figure 3. Effects of leaf position on leaf relative water content (A and B) and specific leaf area (C and D) in control (○, □) and re-watered (◇, ▣) cuttings of Luisa Avanzo and Dorskamp. Leaves are numbered from the bottom to the top of the stem, and control and water-stressed expanding leaves are symbolized by open and closed triangles (Δ , \blacktriangle , respectively). Means (\pm SE) were calculated from three control cuttings and three water-stressed cuttings and one re-watered cutting.

control values, whereas no recovery was observed in Dorskamp.

Soluble sugars

Among clones and leaves, expanding leaves of Luisa Avanzo exhibited the highest concentration of soluble sugars and the

Table 1. Regression analysis of specific leaf area (SLA) (cm² g⁻¹) and the leaf position on the stem (rank). The regression equation is: $S/DW = a \times \text{rank} + b$ when the ratio is linked to the leaf position, in other cases, the relationship becomes: $S/DW = A$; where S = leaf area, DW = dry weight and A = constant.

Clone	Treatment	Regression parameters		
		a	b	A
Luisa Avanzo	Control	4.006	104.661	
	Drought treated	-3.108	177.251	
	Re-watered	4.501	63.394	
Dorskamp	Control			83.405
	Drought treated			77.146
	Re-watered	-4.824	134.568	

Table 2. Correlations between measured parameters and leaf position on stem. Significant correlations are indicated by asterisks: * = $P \leq 0.05$, ** = $P \leq 0.01$ and *** = $P \leq 0.001$. Expanding leaves were excluded from this analysis. Negative correlations indicate an increase with leaf age.

Parameter	Luisa Avanzo			Dorskamp		
	Control	Drought treated	Re-watered	Control	Drought treated	Re-watered
RWC	0.569 *	0.324	0.434	0.322	0.855 ***	0.745 **
SLA	0.796 ***	-0.586 ***	0.863 ***	0.067	-0.422	-0.762 **
Nitrogen	-0.929 **	0.829 *	-0.333	0.486	0.943 **	1 ***
Chlorophyll	-1 ***	0.029	-0.881 **	0.086	0.486	0.829 *
Sugars	0.179	-0.774	0.643	0.069	-0.886 *	0.685
Total phenols	-0.714	-0.257	0.381	-0.5	-0.6	0.6
Flavanols	-0.964 ***	-1 ***	-0.952 **	-0.886 *	-1 ***	-0.714
Leaf Area 50	0.857 *	0.771	0.964 ***	1 ***	0.886 *	-0.543

concentration was unaffected by drought treatment or re-watering (Figure 4). Among clones and ranks, mature leaves had similar concentrations of soluble sugars; however, mature leaves of both clones showed a significant increase in soluble sugars per unit weight (S_M) in response to water stress (data not shown). When the data were expressed on an area basis (S_A), this increase was evident in all leaves of Dorskamp, whereas it was evident only in the youngest mature leaves of Luisa Avanzo (Figure 4). The drought-induced increase in S_M in Luisa Avanzo was counterbalanced by variations in SLA, whereas it was independent of SLA in Dorskamp. After re-watering, soluble sugar concentrations returned to control values in both clones.

Total phenolics, flavanols and antioxidant activity

Spectra of extracts from mature and expanding leaves displayed a maximum absorption between 270 and 290 nm, and between 290 and 330 nm, respectively (data not shown). Total phenolics were always higher in expanding leaves than in mature leaves. Under well-watered conditions, mature leaves of both clones had similar amounts of total phenol and flavanols (Figure 5). However, under drought conditions, mature leaves of Dorskamp cuttings had more total phenol and flavanols than mature leaves of Luisa Avanzo cuttings. In both clones, re-watering mainly affected the oldest mature leaves, but the amounts of phenolics and flavanols decreased in Dorskamp, whereas they increased in Luisa Avanzo. With the exception of re-watered cuttings of Dorskamp, amounts of leaf flavanols increased with leaf age in both clones (Figure 5 and Table 2).

Antioxidant activity (AA) of control and water-stressed cuttings was higher for Dorskamp than for Luisa Avanzo (Figure 5). With the exception of re-watered cuttings of Dorskamp, AA of mature leaves increased with leaf age (Table 2). In control Luisa Avanzo cuttings, expanding leaves and the oldest mature leaves had similar AA values. Drought had no effect on leaf AA of Dorskamp, whereas drought caused a decrease in AA of the youngest mature leaves of Luisa Avanzo. After re-watering, a decrease in AA was observed in mature leaves of Dorskamp only.

Discussion

Leaf characterization of Dorskamp and Luisa Avanzo under well-watered conditions

Although Dorskamp cuttings initially had a larger leaf area than Luisa Avanzo cuttings, Luisa Avanzo cuttings exhibited higher increments in leaf number and leaf area growth during the study. This is in agreement with the trend to rank Luisa Avanzo as a faster growing clone than Dorskamp (Terrasson 1988, Brignolas et al. 2000). Values of N_A and Chl_A were significantly higher for Dorskamp than for Luisa Avanzo. These findings may reflect the balance between foliage production and cost of foliage in terms of nitrogen (Niinemets 1997). All of our cuttings exhibited variations in leaf growth that were unrelated to the drought treatment, but were attributed to fluctuations in solar irradiance and mineral nutrient supply.

Clones differed in leaf structure as indicated by the low SLA of mature leaves of Dorskamp. Natural variations in both leaf thickness and density account for variations in SLA across a range of species (Abrams et al. 1994, Garnier and Laurent 1994, Niinemets 2001). Under well-watered conditions, Luisa Avanzo grows faster than Dorskamp, and leaf area is greater although leaves are thinner. Similar relationships between growth parameters and SLA have been observed between fast- and slow-growing species of herbs (Poorter and Remkes 1990, Van der Werf et al. 1993, Poorter and Evans 1998) and trees (Reich et al. 1991, 1995, Walters et al. 1993). The decrease in SLA with leaf age in Luisa Avanzo cuttings was caused by compound accumulation or thickening of cell walls, or both.

For both clones, soluble sugar concentrations were higher in expanding leaves than in mature leaves, perhaps reflecting a requirement for high cell osmolarity in the expanding leaves. Accumulation of osmotically active solutes results in increased influx (or reduced efflux) of water into cells thereby maintaining the turgor necessary for cell expansion (Hare et al. 1998).

Expanding and mature leaves differed in phenolic composition (data from Luisa Avanzo). These differences were significant as soon as leaf growth was completed. Large amounts of phenolics and a maximum UV spectrum ranging from 290 to 330 nm characterized expanding leaves, whereas matures

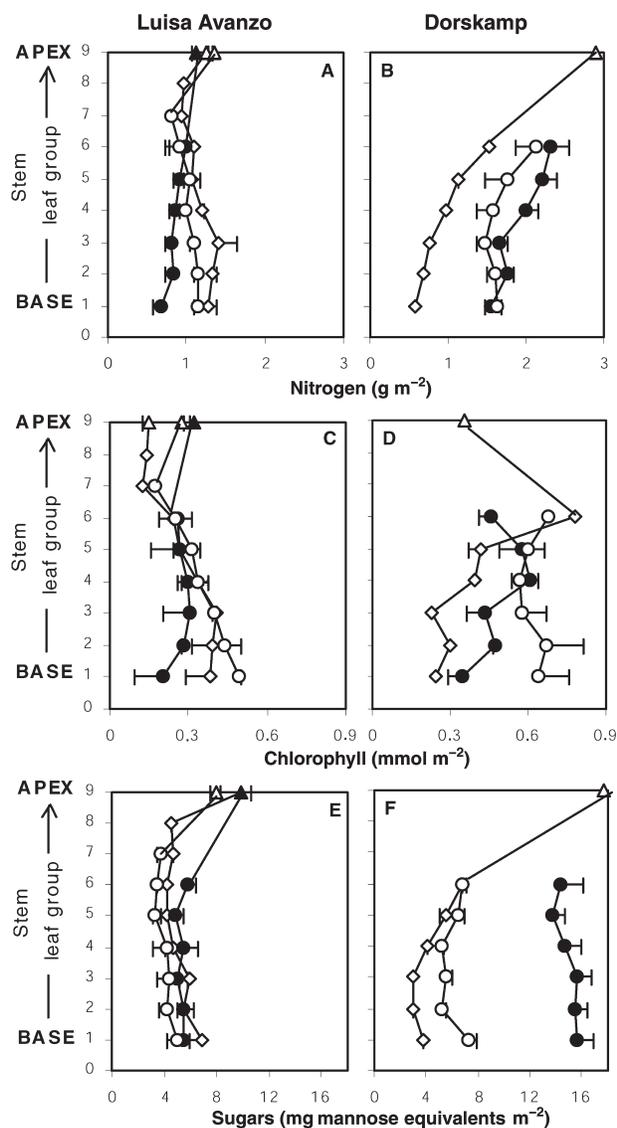


Figure 4. Effects of leaf position on leaf nitrogen (A and B), chlorophyll (C and D) and soluble sugars (E and F) in control (○), water-stressed (●) and re-watered (◇) cuttings of Luisa Avanzo and Dorskamp. Leaves are numbered from the bottom to the top of the stem, and expanding leaves are symbolized by triangles. Means (\pm SE) were calculated from three control cuttings and three water-stressed cuttings or two re-watered cuttings.

leaves had lower amounts of phenolics and a maximum UV spectrum ranging from 270 to 290 nm.

Antioxidant activity per unit area was significantly higher in both expanding and older mature leaves than in young mature leaves, reflecting a possible link between antioxidant activity and the presence of phenolics in the extracts. Previous studies have demonstrated a significant increase in production of reactive oxygen species (ROS) in mature and senescent leaves of tobacco plants (*Nicotiana tabacum* L.) (Thompson et al. 1987, Pastori and del Rio 1997). Cell death may result from the direct effect of elevated ROS, and the increase in phenolic

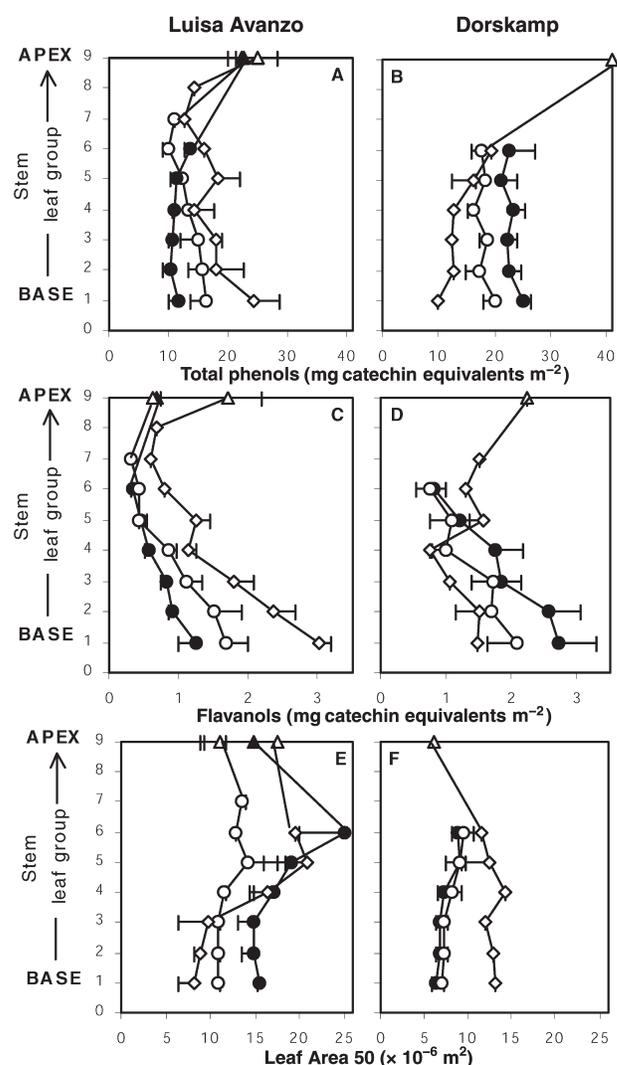


Figure 5. Effects of leaf position on leaf total phenols (A and B), flavanols (C and D) and Leaf Area 50 (E and F) in control (○), drought-treated (●) and re-watered (◇) cuttings of Luisa Avanzo and Dorskamp. Leaves are numbered from the bottom to the top of the stem, and expanding leaves are symbolized by triangles. Means (\pm SE) were calculated from three control cuttings and three water-stressed cuttings or two re-watered cuttings. Leaf Area 50 represents the leaf area providing 50% protection of β -carotene under standard assay conditions.

concentration may be associated with the regulation of cell death during leaf senescence (Korsmeyer 1995, Sharma and Vaid 1997, Heath 1998, Tamagnone et al. 1998).

Leaves of Luisa Avanzo had a higher SLA than those of Dorskamp, which contained more nitrogen, chlorophyll, soluble sugars and antioxidant activity per unit area. The structure and physiology of Luisa Avanzo leaves were dependent on developmental stage: compared with mature leaves, expanding leaves had a lower SLA, higher sugar and phenolic concentrations, higher antioxidant activity and lower chlorophyll and flavanol concentrations. Young mature leaves also differed

from the oldest leaves in their higher SLA, lower chlorophyll and flavanol concentrations and lower antioxidant activity.

Leaf traits during water stress and after re-watering in Dorskamp and Luisa Avanzo

Growth of Luisa Avanzo leaves was decreased by drought but recovered to control values after re-watering. The SLA of the oldest mature leaves increased in response to drought compared with SLA of control and re-watered cuttings, suggesting remobilization of compounds from the oldest mature leaves during drought.

Although N_M was unaffected by water stress, a decrease was observed after re-watering, mostly in the oldest mature leaves of both clones. Variations in N_A resulting from various treatments mainly reflected variations in SLA. A decrease in chlorophyll concentration, commonly observed in response to environmental variation (Upreti et al. 1997, Skärby et al. 1998, Medlyn et al. 1999), was detected after drought and re-watering in the oldest leaves of both clones.

In response to soil drying, stomatal conductance declined 1 week earlier in Luisa Avanzo than in Dorskamp. The timing of this response was unrelated to differences in rates of soil water depletion, because both clones displayed similar leaf areas and therefore transpiration rates. Furthermore, SWC was similar for both clones. The earlier decline in stomatal conductance enabled Luisa Avanzo to maintain a higher soil water potential for longer, as shown by the lower Ψ_{wp} for Dorskamp than for Luisa Avanzo on Day 29 (−1.6 versus −1.3 MPa). Dorskamp cuttings showed large changes in water potential for a given change in leaf RWC, indicating that leaves of Dorskamp have a high bulk elastic modulus (Niinemets 2001). The ability to maintain a high RWC, especially when water potential decreases, is considered indicative of drought tolerance (Matin et al. 1989, Irigoyen et al. 1992, Kimani et al. 1994). The RWC of Luisa Avanzo expanding leaves was affected by drought-treatment and could account for the observed drought-induced reduction in leaf growth. In both clones, re-watering of drought-treated cuttings caused rapid reopening of stomata, and RWC and Ψ_{wp} recovered to control values within 2 weeks.

Dehydration tolerance is often associated with osmotic adjustment (Morgan 1984, Sinclair and Ludlow 1986, Ludlow 1989, Gebre et al. 1998). It has been suggested that the drought-induced increase in soluble sugar concentration decreases the solute potential thereby helping cells maintain turgor in an environment with low water potential (Venkateswarlu et al. 1989, Irigoyen et al. 1992). Drought significantly increased soluble sugars per unit weight of both clones, but the increase was less for Luisa Avanzo than for Dorskamp on an area basis. Such clonal differences suggest that Dorskamp is more drought tolerant than Luisa Avanzo (cf. Gebre et al. 1998). In both clones, sugar concentrations returned to control values when the cuttings were re-watered, confirming the role of soluble sugars as a contributor to osmotic adjustment.

Antioxidant activity of mature leaves of Luisa Avanzo was decreased by water stress and the decrease was amplified in

the youngest mature leaves, which contained a lower concentration of phenolic compounds. This finding suggests that phenolic compounds are partially responsible for the variations in antioxidant activity among treatments. Moreover, the low antioxidant activity and low concentrations of phenolic compounds in the youngest mature leaves reflects their high susceptibility to oxidation. For Dorskamp, drought had no effect on antioxidant activity; however, we note that Dorskamp leaves maintained higher RWC in the drought treatment than Luisa Avanzo leaves.

In summary, in both clones, soil water depletion resulted in decreases in stomatal conductance, Ψ_{wp} , leaf growth and leaf RWC. In both clones, re-watering after drought treatment caused a rapid reopening of stomata and a return of Ψ_{wp} , leaf growth parameters and RWC to control values. However, the drought-induced effects differed in time and amplitude between clones. Although stomatal conductance in Luisa Avanzo declined 1 week earlier than in Dorskamp resulting in better Ψ_{wp} maintenance, Luisa Avanzo suffered a greater decrease in leaf RWC than Dorskamp. These observations suggest that Dorskamp has a higher bulk elastic modulus than Luisa Avanzo. Drought increased SLA only in mature leaves of Luisa Avanzo cuttings, indicating product remobilization. Compared with Luisa Avanzo leaves, Dorskamp leaves had higher antioxidant activity and a greater ability to accumulate sugars in response to drought. The observed drought-induced differences between clones could account for the greater tolerance to water stress exhibited by field-grown trees and cuttings of Dorskamp (Soulères 1992, Brignolas et al. 2000).

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