Original article

Compared water deficit response of two *Populus x euramericana* clones, Luisa Avanzo and Dorskamp

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Abstract – The drought response of two *Populus x euramericana* clones cv Dorskamp and Luisa Avanzo was investigated using potted cuttings growing in a greenhouse. The cv Dorskamp clone is known to be more tolerant than Luisa Avanzo under field conditions. Watering was withheld and several parameters followed during the onset of drought. The relation between predawn leaf water potential (Ψ_{wp}) and soil water content differed between clones: (i) Ψ_{wp} tended to decline when soil water content decreased below a threshold of 0.11 v/v and 0.13 v/v for Dorskamp and Luisa Avanzo, respectively; and (ii) Ψ_{wp} of stressed cuttings reached –3.5 MPa and –2.5 MPa for Dorskamp and Luisa Avanzo, respectively. Under well watered conditions, Luisa Avanzo had greater relative rates of increase in number of leaves and leaf growth than in Dorskamp. In response to water stress, the relative rates of increase in number of leaves and leaf growth did not significantly differ between the two clones for similar Ψ_{wp} values. Thus, the growth advantage of Luisa Avanzo observed under control condition appeared counterbalanced by its higher susceptibility to water stress. Our results corroborated field observations on mature trees.

drought / leaf area / *Populus x euramericana* / predawn water potential / soil water content

Résumé – Comparaison de la réponse à un arrêt d'arrosage de deux clones de *Populus x euramericana*, Luisa Avanzo et Dorskamp. La réponse à un stress hydrique de deux clones de *Populus x euramericana* cv Dorskamp et Luisa Avanzo a été étudiée en serre à partir de boutures en pots. Le stress a été initié par arrêt d'arrosage et différents paramètres ont été suivis. La relation entre le potentiel de base foliaire (Ψ_{wp}) et la teneur en eau du sol diffère entre clones : (i) Ψ_{wp} tend à diminuer lorsque la teneur en eau du sol atteint le seuil de 0.11 v/v pour Dorskamp, alors qu'il diminue à partir de 0,13 v/v pour Luisa Avanzo ; (ii) Ψ_{wp} des plants stressés atteint réversiblement –3,5 MPa pour Dorskamp et seulement –2,5 MPa pour Luisa Avanzo. En condition témoin, les taux d'apparition et de croissance des feuilles de Luisa Avanzo sont supérieurs à ceux de Dorskamp. En réponse au stress, ces paramètres ne diffèrent plus entre clones pour des Ψ_{wp} comparables. L'avantage de croissance observé chez Luisa Avanzo en condition témoin n'apparaît plus en réponse au stress ; la plus grande sensibilité de ce clone au stress hydrique corrobore donc les observations réalisées en conditions naturelles.

stress hydrique / surface foliaire / *Populus × euramericana* / potentiel foliaire de base / teneur en eau du sol

1. INTRODUCTION

Fast growing trees, such as poplar, raised in short-rotation intensive cultures represent an alternative use for agricultural land [4, 21, 22]. However, drought periods are responsible for both a decrease in poplar biomass production and an increase in sensitivity to some pathogens [18, 21].

Mechanisms involved in the adaptive response of the whole plant to water stress tend to increase plant water

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use efficiency [15] and to maintain photosynthetic activity [8]. Because of the high degree of variation in drought tolerance among *Populus* genotypes [7, 10, 11, 13, 19, 24], a number of important physiological and morphological traits are receiving attention including: stomatal closure [5, 13, 16], osmotic adjustment [10], leaf area reduction, leaf abscission, and root/shoot ratio increase [7, 14, 16, 24]. Stomatal closure is an essential component of the response to drought, and results in a reduction of water loss by transpiration [5]. Early stomatal closure in response to soil drying can avoid leaf abscission [16] and catastrophic cavitation [5, 13]. Osmotic adjustment, as a result of solute accumulation, can enable plants to survive through, and recover from short dry periods [3, 17]. For several deciduous tree species, a greater capacity for osmotic adjustment seems to be associated with greater dehydration tolerance [1]. However, differences in drought resistance between some poplar clones could not be explained by differences in osmotic adjustment [11]. Leaf area reduction, including leaf abscission and reduced leaf growth, resulting in an increase in root/shoot ratio, are also adaptive response to drought [7, 14, 16, 24]. These responses are adaptive to both short-term intensive drought and long-term site water balance.

While whole plant responses to water stress have been well studied in poplar, cellular mechanisms still remain unclear. Comparison of biochemical and molecular responses between a drought-tolerant and a drought-sensitive clone may help to characterise relevant differences in the pattern of drought response, and then could provide early markers associated with water stress resistance.

In order to study drought tolerance at the cellular level, we have selected two *Populus x euramericana* clones (Luisa Avanzo and Dorskamp) originating from the Aigeiros section. Based on field observations, these two clones differed in drought tolerance, but originated from crossings of the same parental species. The following are limiting cultural conditions for Luisa Avanzo: high clay levels, high density, flooding or lack of water and lack of nutrients. In contrast, the Dorskamp clone is more plastic and less water-demanding [21, 23].

The first step in developing model clones for biochemical and molecular studies is the controlled validation of the above mentioned cultural observations. The purpose of this study was to test the drought behaviour of both clones by measuring several parameters on control and drought-stressed cuttings grown under greenhouse conditions. The measured parameters included: soil water content, predawn leaf water potential [7, 24], number of leaves [7, 14], and leaf area [2, 6, 12]. For the latter, a basic, but not-destructive, allometric relationship had to be developed between leaf dimensions (length, width) and leaf area [6, 9, 25].

2. MATERIALS AND METHODS

Two-month-old 20-cm woody stem cuttings, from 1-year-old cut-back stems of two *Populus x euramericana* (Dode) Guinier (*P. deltoides* (Bartr.) Marsh. *x P. nigra* L.) cv "Dorskamp" [male] and "Luisa Avanzo" [female] clones, were used in all experiments.

2.1. Establishment and validation of basic allometric relationships between leaf dimensions and leaf area

In October 1997, 40 leaves of Dorskamp and 32 leaves of Luisa Avanzo were collected from 4 woody-stem cuttings of each clone. Leaf length (from lamina tip to petiole insertion along the main vein), maximum width (perpendicularly to the main vein) and leaf area were measured. Leaf area was determined using an automatic image analyzer (Biocom Imagenia 5000 2.0).

To establish the basic relations between leaf dimensions and leaf area for each clone, two models were tested: a linear model ($A_m = aX + b$) and a logarithmic one ($\log A_m = a \log X + b$) i.e. ($A_m = 10^{a \log X + b}$), where A_m is the measured leaf area and X the leaf length or leaf width. These formulae were validated at both leaf and cutting levels by measuring leaf area (A_m) and cutting leaf area ($C_m = \Sigma A_m$) on 5 other heterogeneous cuttings of each clone (in a range of one to two-months-old cuttings, with 5 to 16 leaves each). Overall leaves of cuttings, 52 for Dorskamp and 58 for Luisa Avanzo, were measured. The width of each leaf was manually measured and the selected relationships were applied for each clone to estimate leaf area (A_e) and total leaf area per cutting ($C_e = \Sigma A_e$). The correlation between calculated (A_m, C_m) and estimated (A_e, C_e) parameters were checked by linear regression (Pearson's coefficient), and tested with the Student's distribution ($p \leq 0.05$) using SPSS software.

2.2. Stress experiment

In February 1998, 130 twenty cm woody stem cuttings of each clone were planted into 300 ml plastic pots until rooting occurred. One month later, each cutting was repotted in 1 l pots containing sand/peat moss/clay (50/45/5, v/v/v, pH 7) together with dolomite (3 kg/m^3), and chemical fertilisers N-P-K (15-9-15) to a total concentration of 2 kg/m^3. All cuttings were regularly

watered to field capacity until April, the start of the experiment.

Drought stress experiment was conducted in a greenhouse exposed to natural daylight. On April 6, 1998 (d 96 of the year) water stress was induced by withholding water from 100 cuttings of each clone. The remaining cuttings were regularly watered to field capacity and constituted the controls. For each clone, 5 drought stressed cuttings and 3 or 5 controls were collected weekly for estimating the following parameters:

1- Predawn leaf water potential (Ψ_{wp}) was measured with a Scholander pressure chamber on a fully expanded leaf [20]. When Ψ_{wp} of stressed cuttings became significantly lower than that of controls, 5 other stressed cuttings per clone, chosen at random, were rewatered, and their Ψ_{wp} was estimated c.a. one week later. The experiment was stopped when Ψ_{wp} of rewatered cuttings did not return to a control value.

2- Soil water content was measured from a calibrated volume of homogeneous soil (345 cm^3). This soil was weighted and placed at 100 °C for 24 hours before measure of dry weight. Results were expressed in grams of water per cm^3 soil (= v / v).

From April 6, 1998, the number of leaves and each leaf's width were measured fortnightly for each remaining cutting. Results were expressed in two ways: (i) at each date of measure, the total number of leaves and the total foliar area of each cutting were expressed as the percent of their initial value measured at day 96; (ii) for each level of water stress (Ψ_{wp} values), the relative rates of increase in number of leaves (day $^{-1}$) and leaf growth (day $^{-1}$) of each cutting were calculated using the following equation: $[(X_2 - X_1) / (t_2 - t_1)]$, where X_1 and X_2 are the number of leaves or leaf area at times t_1 and t_2, respectively. All leaves were taken into account in these calculations over the experiment, including the fallen leaves. These parameters were also estimated for weekly collected cuttings. Means (± standard error) were compared by variance analysis (SPSS software), and were considered significantly different when $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Relation between leaf dimensions and leaf area for each clone

Leaf sizes in the Dorskamp cuttings ranged from 19 to 63 mm wide, 27 to 67 mm long, and from 3.5 to 26 cm^2 in area. The respective dimensions of Luisa Avanzo leaves were 23 – 62 mm, 32 – 58 mm, and 4.5 – 24 cm^2. The logarithmic and linear equations were developed between leaf area (A_m) and either leaf length or width; both equations were highly significant ($P \leq 0.01$). Best fits were obtained with the width parameter (w) particularly for Dorskamp (table I). The linear model had a negative intercept which was significantly different from zero. Because such intercept values could lead to negative leaf areas for small leaves, the logarithmic model was chosen for estimating leaf area at both the leaf and cutting levels. This model was then validated on other cuttings by linear regression at leaf ($A_e = a A_m + b$) and cutting levels ($C_e = a C_m + b$). According to the significant results (table II), the equations retained for further estimation of cutting leaf area were: $A_e = 10^{1.81 \log w}$ and $A_e = 10^{1.73 \log w}$ for Luisa Avanzo and Dorskamp, respectively.

3.2. Stress experiment

Woody stem cuttings of both clones (n = 130 cuttings by clone) were prepared exactly at the same time; however, at the beginning of the experiment (April 6, 1998 = d 96), Luisa Avanzo cuttings exhibited a smaller number of leaves and total leaf area than Dorskamp (8.5 ± 0.3 and 67.9 cm^2 ± 2.6 *vs.* 11.5 ± 0.3 and 84.4 cm^2 ± 3.1,

Table I. Establishment of a mathematical function between measured leaf area and measured leaf width or measured leaf length. Two models were tested: model 1, linear function ($A_m = a X + b$); model 2, logarithmic function (log $A_m = a \log X + b$). A_m = measured leaf area; X = measured leaf width (w), or measured leaf length (l); r = Pearson's coefficient; (**) = Significant correlation at $P \leq 0.01$. n = number of data points used for linear regression.

		model 1 regression parameters			model 2 regression parameters		
		a	b	r	a	b	r
Dorskamp	$f(w)$	4.97	−8	0.96(**)	1.73	0	0.97(**)
(n = 40)	$f(l)$	4.21	−7.6	0.91(**)	0.833	0.04	0.91(**)
Luisa Avanzo	$f(w)$	5.05	−7.5	0.97(**)	1.81	0	0.97(**)
(n = 32)	$f(l)$	6.15	−5.4	0.96(**)	2.36	0	0.96(**)

Table II. Linear regression parameters between measured areas using an image analyser, and calculated areas using the logarithmic model at both leaf and cutting levels ($A_e = a\,A_m + b$ and $C_e = a\,C_m + b$). Regression parameters: slope (s), intercept (i), Pearson's coefficient (r). Significant correlation are indicated by (*) ($P \leq 0.05$); C_e = estimated leaf area per cutting: i.e. sum of individual estimated leaf area of all the cutting leaves ($C_e = \Sigma A_e$); C_m = measured leaf area per cutting; A_e = estimate leaf area; A_m = measured leaf area and n = number of data points used for linear regression.
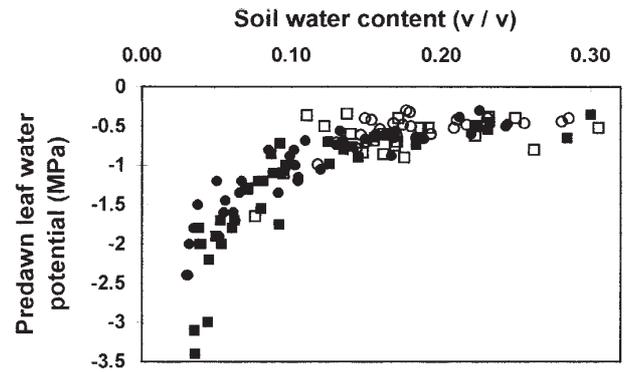
| | | Logarithmic model regression parameters | | |
		s	i	r
Dorskamp	per leaf	0.913	0	0.97*
($n = 52$)	per cutting	0.986	0	0.96*
Luisa Avanzo	per leaf	1.04	0	0.98*
($n = 58$)	per cutting	1.09	0	0.95*

Figure 1. Predawn leaf water potentials (Ψ_{wp}) and soil water contents in control ($\bigcirc$/$\square$) and stressed cuttings ($\bullet$/$\blacksquare$) of clones Dorskamp ($\square$/$\blacksquare$) and Luisa Avanzo ($\bigcirc$/$\bullet$).

respectively). The 24.3% difference in leaf area likely affected the rate of water use and, therefore, drought development.

3.2.1. Soil water content and predawn leaf water potential

Soil water content and predawn leaf water potential (Ψ_{wp}) of control cuttings, which were watered weekly to field capacity, remained above 0.14 v/v and –1 MPa, respectively (*figure 1*). These parameters did not significantly differ between both control clones throughout the experiment.

Soil water content of stressed cuttings, which gradually decreased during the time course of the experiment, became significantly lower than in controls, 14 and 21 days after withholding irrigation for Dorskamp and Luisa Avanzo, respectively. From day 14 to day 28, the decrease in soil water content was faster and greater for Dorskamp than for Luisa Avanzo (data not shown). Hence, during this period, it was assumed that Dorskamp was exposed to a greater stress than Luisa Avanzo. These differences between clones resulted from the greater transpiration of Dorskamp cuttings due to the larger initial leaf development. Ψ_{wp} of Dorskamp and Luisa Avanzo stressed cuttings tended to decline when soil water contents were less than 0.11 v/v and 0.13 v/v, respectively (*figure 1*). These values can be considered as a threshold for stress initiation [24], because the best fit linear regressions (one for the steep decline at low soil water content and one for the asymptotic plateau at high soil water content) intersected at these values. Despite the initial and more rapid decrease in soil water content

noted for Dorskamp, the threshold of stress initiation was lower for Dorskamp than for Luisa Avanzo. Differences between clones, in the relation [soil water content/Ψ_{wp}], could be explained by differences in the depth of rooting or in the distribution of soil water [8]. In addition, Ψ_{wp} of stressed cuttings could reversibly reach –3.5 MPa and –2.5 MPa for Dorskamp and Luisa Avanzo, respectively. This last result demonstrated that Dorskamp can tolerate more negative water potentials than Luisa Avanzo.
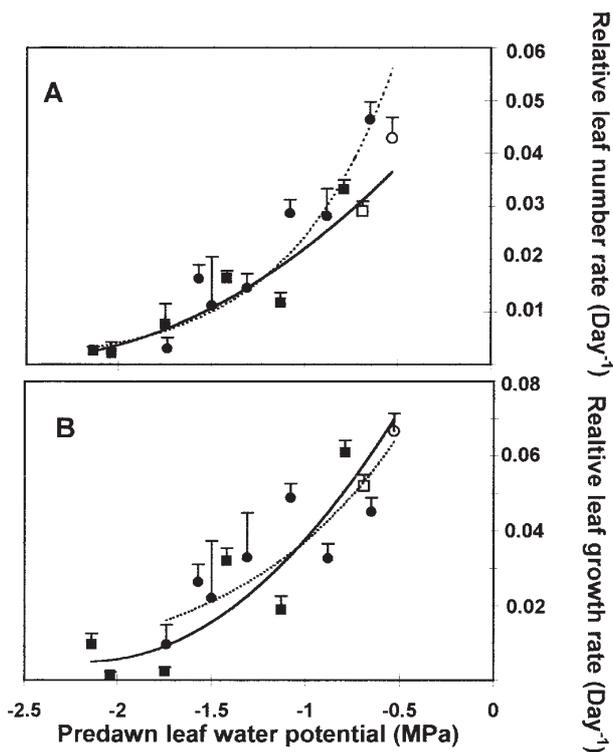
3.2.2. Growth of the two hybrid poplar clones

Under well watered conditions, the total number of leaves reached c.a. 270% and 370% of initial values for Dorskamp and Luisa Avanzo, respectively (*figures 2A and B*). Similar differences were also observed for the increase in total leaf area: c.a. 550% and 800%, respectively (*figures 2C and D*). These results were corroborated by significantly greater relative rates of increase in number of leaves and leaf growth in Luisa Avanzo than in Dorskamp (*figures 3A and B*). These growth differences detected between clones in greenhouse conditions showed that Luisa Avanzo is a faster growing clone than Dorskamp. Similar differences were also observed in large-scale plantations, on a trunk circumference basis [23].

In response to water stress, the total number of leaves reached c.a. 200% and 280% of initial values for Dorskamp and Luisa Avanzo, respectively (*figures 2A and B*). Moreover, both in the case of Dorskamp and Luisa Avanzo, the total leaf area reached c.a. 350% of their initial values (*figures 2C and D*). For similar Ψ_{wp}
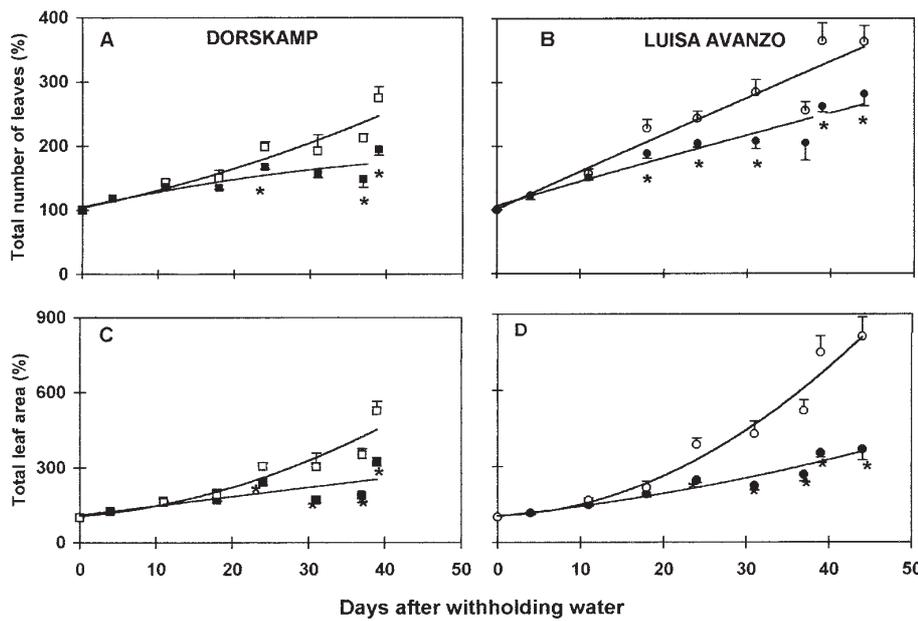
Figure 2. Time course of total number of leaves (A and B) and total leaf area (C and D) in control (□/○) and stressed cuttings (■/●) of Dorskamp (□/■) and Luisa Avanzo (○/●). Means (± standard error) were compared between control and stressed cuttings at each date of measurement; (∗): for each clone, significant differences ($P \leq 0.05$) between control and stress conditions.

values, the relative rates of increase in number of leaves and leaf growth did not significantly differ between clones (*figures 3 A and B*).

The differences between clones, observed in well watered conditions, regarding the relative rates of increase in number of leaves and leaf growth, were not maintained under limiting water supply. Hence, the advantage of Luisa Avanzo under well watered conditions was counterbalanced by its higher susceptibility to water stress. This conclusion is corroborated by a greater decrease in total leaf area in response to stress for Luisa Avanzo than for Dorskamp (–450% and –200%, respectively), since the latter was exposed to the highest stress intensity throughout the experiment. Therefore, cuttings of these two clones constitute a relevant model for study of drought tolerance and for further research of markers associated with water stress resistance.

Figure 3. Relation and regression lines between predawn leaf water potential (Ψ_{wp}) and relative leaf number (A) - and leaf growth rate (B), in control (□/○) and stressed cuttings (■/●) of Dorskamp (——) and Luisa Avanzo (-----). Otherwise as for *figure 2*.

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