

# Evaluation of flooding tolerance in cuttings of *Populus* clones used for forestation at the Paraná River Delta, Argentina

VMC Luquez<sup>1\*</sup>, FG Achinelli<sup>1,3</sup> and S Cortizo<sup>2</sup>

<sup>1</sup> Instituto de Fisiología Vegetal, CONICET La Plata – FCAYF UNLP, CC 327, 1900 La Plata, Argentina

<sup>2</sup> INTA Delta, Río Paraná de Las Palmas y Canal Laurentino Comas, 2804 Campana, Argentina

<sup>3</sup> Comisión de Investigaciones Científicas, Calle 526 entre 10 y 11, 1900 La Plata, Argentina

\* Corresponding author, e-mail: [vlquez@agro.unlp.edu.ar](mailto:vlquez@agro.unlp.edu.ar)

We analysed the responses to flooding of 14 poplar clones used for forestation at the Paraná River Delta, Argentina. Some are commercial clones planted in the area, and others belong to a poplar breeding program from the National Institute of Agricultural Technology (INTA) in Argentina. Potted plants of 60 cm high growing in a greenhouse were watered (control) or submerged in water 10 cm above soil level (flooding treatment) for 35 d. Flooding reduced growth in height and diameter (combined as the volume index), total leaf area, photosynthesis (measured as electron transport rate) and stomatal conductance. In flooded plants, the number and area of newly formed leaves were also reduced. In some clones, flooding accelerated the senescence of basal leaves and induced the development of adventitious roots. A hierarchical cluster analysis was performed to classify the responses of the clones to flooding. Evaluating flooding tolerance as less reduction in growth and retention of a higher leaf area under flooding conditions, three groups with different degrees of flooding tolerance were identified: clone Alton was the most tolerant; clones A129-60, A106-60, SIA 22-85, Catfish 2 and 150-82 showed intermediate tolerance; and the remainder of the clones had low flooding tolerance. This information will be useful to recommend clones to be planted in flood-prone areas, and to select parents to be used in breeding programs.

**Keywords:** flooding, growth, Paraná Delta, *Populus*

## Introduction

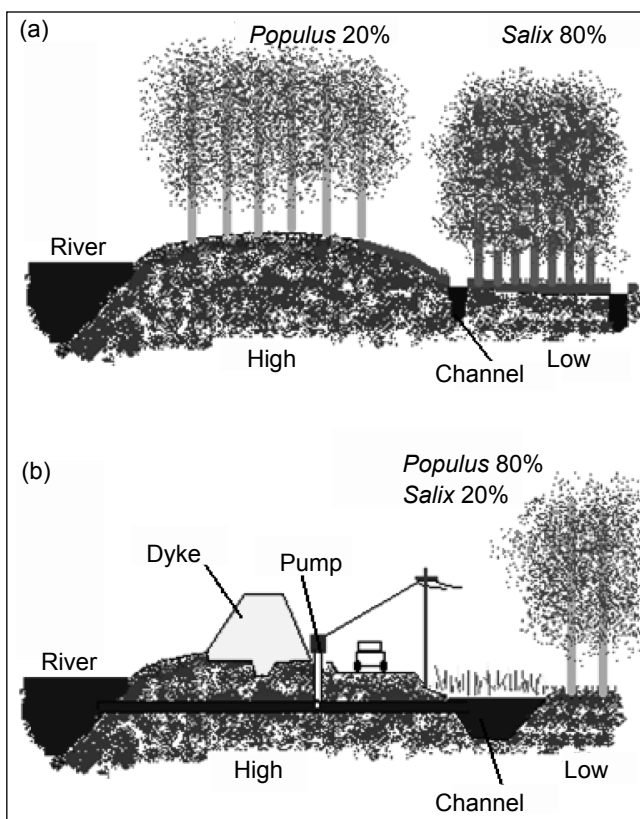
A number of species of the genus *Populus* are important forest trees around the world and sources of timber, pulp and biomass for energy (Taylor 2002). In Argentina, the main area for poplar plantations is the Paraná River Delta, an extensive group of islands located between 32°5' and 34°29' S and 58°22' and 60°45' W, with a land surface of 14 000 km<sup>2</sup> (Bonetto and Hurtado 1998). The main economic activity for the lower Delta area is Salicaceae (poplars and willows) forestry, the total area for poplar plantations being approximately 15 000 ha (Borodowski 2006).

The Delta region experiences episodes of flooding caused by a combination of factors: high water levels of the Paraná River or its tributaries from the Paraná–La Plata basin, storm surge floods caused by southeastern winds over the La Plata River, or excessive local rains (Borodowski 2006). The characteristics of the islands make some areas flood-prone, two being typically distinguished: an upper coastal area of 1–1.5 m high, comprising 20% of the surface, and a lower central area that occupies 80% of the surface. In the higher areas, the natural vegetation is woodland, whereas in the lower areas the main formation is marshland dominated by rushes (*Scirpus* spp.; Malvarez 1999).

When the islands are used for forestry, channels are dug in the lower area to facilitate the drainage of the marshland. As Figure 1a shows, this is an open system with no protection against river floods; the water table is usually very close

to the surface. Flooding episodes are more likely to occur in the lower areas of the islands, so poplars are planted in the upper areas, and the most flood-tolerant willows (*Salix* spp.) in the lower areas (Borodowski 2006). This system resembles the natural stands along Northern Hemisphere rivers, where *Salix* species grow in the lower, flood-prone areas, and *Populus* species grow in the upper part of the river bank (Amlin and Rood 2001).

The islands of the Paraná River Delta have suffered from long periods of flooding of irregular occurrence. During the twentieth century, floods took place in 1905, 1914, 1922, 1940, 1958, 1959, 1966, 1973, 1977, 1982–1984, and 1998 (Borodowski 2006). These extreme flooding episodes caused severe economic losses and prompted local land-owners to build dykes to protect the plantations (Figure 1b). Dykes are built over the highest borders of the islands and the mouth of the channels is closed by a gate to avoid river floods. If the gates are closed, the excess of water that accumulates is removed by pumping; in this closed system poplars are planted in the lower sites, occupying 80% of the area (Figure 1b). The height of the dykes may range from 2 to 6 m above basin level; the highest ones protect the islands from the extraordinary flooding episodes. The dyke-protected areas are no longer wetlands (Bó et al. 2010). Although dykes protect the islands from river water flooding, the occurrence of



**Figure 1:** Schematic illustration of the two main productive systems for forestry at the Paraná River Delta. (a) Open system without dykes, with channels that drain into the river; only the higher part of the island (20% of the surface) is planted with poplars. (b) Closed system with dykes, with draining channels, gates at the end of the channels to avoid the entrance of river flood waters, and pumps to remove the excess of water when gates are closed. In this system, 80% of the surface is planted with poplars

heavy local rains combined with high river levels, which make drainage difficult, can cause flooding episodes lasting several weeks. This situation happened between March and June 2007, and in November–December 2009; extensive dyke-protected areas were flooded or waterlogged even with pumps working at maximum capacity to remove the excess of water.

Climate change models predict an increasing frequency of excessive local rains and storm surge floods in the area (Barros et al. 2006). With the increased risk of flooding as a consequence of climate change, it will be important to know the degree of flooding tolerance of the poplar clones to be planted in the area, for both the open system and the dyke-protected areas.

The tolerance to flooding of woody plants varies according to species and genotypes, the age of the plant, the degree of covering by water, the flood duration and the conditions of the floodwater (Kozłowski 1997, Glenz et al. 2006). Among the most conspicuous responses to flooding are: growth reduction, stomatal closure and a decrease of photosynthetic activity; development of adventitious roots and aerenchyma formation; accelerated leaf senescence

and abscission; changes in the absorption and availability of mineral nutrients; and several metabolic changes caused by hypoxic or anoxic conditions (Kozłowski 1997, Vartapetian and Jackson 1997, Braendle and Crawford 1999, Glenz et al. 2006). Previous works showed differences in flooding tolerance between *Populus* species, hybrids and clones, for instance in *Populus trichocarpa* (Smit 1988), *Populus deltoides* (Cao and Conner 1999), *P. ×canadensis* (Béjaoui et al. 2006, Gong et al. 2007, Du et al. 2008, Guo et al. 2011), *P. trichocarpa* × *P. deltoides* (Gong et al. 2007, Guo et al. 2011) and *P. trichocarpa* × *P. nigra* (Guo et al. 2011). In this work, we analysed flooding tolerance in poplar clones used for forestation at the Paraná River Delta. These species are mostly *P. deltoides* and a lower quantity of *P. ×canadensis*, which were introduced as there are no native *Populus* species in the area (Ragonese et al. 1987). Some genotypes are commercial clones planted in the Paraná Delta area, and others are experimental clones from a breeding program developed at the National Institute of Agricultural Technology (INTA) and have not been released for commercial use yet. The experimental clones tested in this work were selected based on growth rates, wood quality and pest resistance, on field trials conducted in an experimental field protected from flooding by dykes, hence their response to flooding was unknown (Cortizo et al. 2009). Two of the most-planted commercial clones (A129 and A106) were selected in Australia (Pryor and Willing 1983). We expected to find different degrees of flooding tolerance in the analysed poplar clones and to identify tolerant clones to be planted in flood-prone areas.

## Material and methods

### Plant material, growing conditions and flooding stress treatment

Information about the clones used in this experiment is detailed in Table 1. The experiment was carried out in a greenhouse located in La Plata, Argentina (34°54' S). One-year-old cuttings of 60 cm length were obtained from stool beds and stored at 4 °C until planting in 7 l pots filled with soil. The planting date was 20 July 2007. The pots were placed in a greenhouse in a completely randomised factorial design, with 14 clones and two treatments (control and flooded), and with four replicates for each clone and treatment. The photoperiod was the normal for the area at this time of the year, irradiance inside the greenhouse on clear days reached a maximum value of 1 228  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Temperature values ranged between 15 °C (minimum) and 34 °C (maximum). Bud flush occurred between 4 September and 18 September. A slow-release commercial fertiliser (N 12%, P 12%, K 17%, Mg 2%, S 15%, Ca 5%, Zn 0.01%, Fe 0.2%, B 0.02%, and traces of Mo, Mn and Cu) was added to the pots on 20 September and 12 October 2007; the dose was 1 g fertiliser per pot on each date. To avoid fungal diseases, the trees were treated once a week with two commercial fungicides (Benomyl 50% WP and Carbendazim 50% SC).

Before the beginning of treatment, trees were pruned and only one shoot was retained, in order to minimise the variability induced by different shoots per tree; this unique shoot is denoted as the 'main stem'. Flooding was induced

**Table 1:** The *Populus* clones used in this study. Origin refers to the geographical origin of the parents in the case of clones obtained by a controlled cross, and of the mother in the case of open-pollinated provenances. Sex: M = male, F = female. The clones highlighted in bold have not been released for commercial planting yet

Clone full name	Abbreviation	Species	Parents	Place of origin	Sex
Alton	ALT	<i>P. deltoides</i>	Unknown	USA	M
A106-60	A106	<i>P. deltoides</i>	Selected mother, open-pollinated	Texas, USA	F
A129-60	A129	<i>P. deltoides</i>	Selected mother, open-pollinated	Texas, USA	F
Catfish 2	CF2	<i>P. deltoides</i>	Selected mother, open-pollinated	USA	M
Stoneville 67	ST67	<i>P. deltoides</i>	Selected mother, open-pollinated	Issaquena County, Mississippi, USA	M
Carabelas INTA	CAR	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	M
2-82	2-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	M
20-82	20-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	F
21-82	21-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	F
89-82	89-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	F
149-82	149-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	M
150-82	150-82	<i>P. deltoides</i>	Selected mother, open-pollinated	Mississippi Delta, USA	F
Conti 12	CT12	<i>P. ×canadensis</i>	Unknown	Italy	F
SIA 22-85	SIA	<i>P. ×canadensis</i>	<i>P. deltoides</i> 'Lux' × <i>P. nigra</i> 'N10,11'	Illinois, USA (mother); Aragón, Spain (father)	F

when the shoots were 60 cm long by placing the potted trees inside a sealed 10 l pot filled with tap water up to approximately 10 cm above soil level; water was added when necessary to maintain this level. The type of flooding stress applied is of common occurrence in the Paraná Delta area, with only roots and part of the trunks covered by floodwater. The control plants were watered regularly to field capacity. The flooding stress treatment started on 16 October 2007 and lasted for 35 d.

#### Growth measurements and morphological observations

Total shoot height (height; cm) was measured with a graduated stick, and basal diameter (diameter; mm) was measured with a digital caliper. The volume index (VI; dm<sup>3</sup>) was calculated as follows:

$$VI = (\text{basal diameter})^2 \times \text{total height}$$

The total number of leaves on the main stem (LNms) was counted at the beginning and at the end of the experiment.

Initially, the latest completely expanded leaf was tagged with a colour wire; this leaf was used as a reference to calculate the leaf number increase rate (LNI; number d<sup>-1</sup>) as follows:

$$LNI = (La_2 - La_1) / t_2 - t_1$$

where  $La_1$  was the number of leaves above the latest fully expanded leaf at the beginning of the experiment ( $t_1$  = day 0), and  $La_2$  at time  $t_2$  (day 35), as mentioned above.

Total leaf area (TLA; cm<sup>2</sup>) was measured at the end of the experiment with a LICOR LI 3100 leaf area meter. Individual leaf area (LAI; cm<sup>2</sup>) was measured on the latest expanded leaf at the end of the experiment.

The leaf expansion rate was measured over a leaf expanded after starting the flooding treatment. On 2 November, a leaf at least 3 cm long was tagged with typing corrector as previously described (Luquez et al. 2006). On 8 November and 12 November this leaf was photographed with a digital camera, including a reference of known length on the background. The leaf area was

determined with Image J software (available at <http://rsb.info.nih.gov/ij/>) following the instructions displayed at the website. The leaf expansion rate (LER; cm<sup>2</sup> d<sup>-1</sup>) was calculated as the difference in area measured on both dates divided by the number of days between them.

Adventitious root formation was observed after 33 d of flooding and included in the cluster analysis as a presence/absence trait.

#### Physiological observations

Stomatal conductance ( $g_s$ ; mmol m<sup>-2</sup> s<sup>-1</sup>) was measured with a LICOR LI 1600 steady state porometer. Stomatal conductance was measured on three different dates on the abaxial surface of the same leaf; the first measurement was made when the leaf had just completed expansion and the last measurement was recorded 18 d later. The measurements were carried out without any artificial light supplements, from 9:30 to 12:30 on three days with clear skies (1, 6 and 19 November); the average irradiance during the measurements was 928  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

The apparent electron transport rate (ETR;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was measured with a Hansatech FMS2 modulated fluorescence meter. The measurement was performed 35 d after the beginning of the flooding treatment, on the last expanded leaf adapted to light with similar irradiance values to  $g_s$  measurements, from 10:00 to 11:30, in accordance with the manufacturer's instructions.

Chlorophyll content was measured non-destructively with a SPAD Minolta 502 on the last expanded leaf at the beginning of the experiment. With the subsequent expansion of new leaves, this leaf was in a basal position at the end of the experiment.

#### Statistical analysis

All of the statistical analyses were carried out with R software version 2.8.1 (R Development Core Team 2011). A two-way analysis of variance (ANOVA) was carried out using the *anova* and *lm* functions to determine the effects of clone, flooding treatment and the interaction between them.

The clones were classified into groups by means of an agglomerative hierarchical cluster analysis. For variables

measured several times, one representative measurement was chosen, so each variable was included only once in the analysis. Chlorophyll values were those of day 35, when the differences between clones and treatments were larger. Stomatal conductance was measured on the same leaf three times; the first measurement was chosen because the stomatal conductance in most control plants had the highest value on that date. The variables used are those included in Table 3 plus presence/absence of adventitious roots (Table 2). The cluster was built with the agnes function in the cluster R package (Seefeld and Linder 2007) with the complete linkage method.

## Results

The presence/absence of adventitious roots was assessed 33 d after the start of flooding, when roots had developed in some but not all clones (Table 2).

**Table 2:** Development of adventitious roots and hypertrophied lenticels in flooded plants of 14 poplar clones, recorded as presence/absence. The observations were made after 33 d of flooding

Clone name	Hypertrophied lenticels	Adventitious roots
Alton	Yes	Yes
A106-60	Yes	No
A129-60	Yes	No
Catfish 2	Yes	No
Stoneville 67	Yes	Yes
Carabelas INTA	Yes	No
2-82	Yes	Yes
20-82	Yes	Yes
21-82	Yes	Yes
89-82	Yes	Yes
149-82	No	No
150-82	Yes	Yes
Conti 12	Yes	No
SIA 22-85	Yes	Yes

Flooding reduced growth, measured as VI (Figure 2a), therefore the effects of both clone and flooding were highly significant, whereas the interaction was marginally significant (Table 3). The degree of VI reduction differed between clones, the least affected clone was Alton with a 20% growth reduction, and the most sensitive clone was 21-82 with a 70% reduction (Figure 2b).

All leaf traits were reduced by flooding, i.e. TLA (Figure 3a), LAI (Figure 3b); LER (Figure 3c), LNms (Figure 3d) and LNI (Figure 3e). For all leaf traits, the effects of flooding were significant, but the clone effects were significant only for LN, LAI and TLA (Table 3). There were clonal differences in leaf traits; *P. deltoides* clones had a lower number of leaves (LN) with higher areas (LAI) than *P. ×canadensis*. Clone SIA had a TLA similar to the *P. deltoides* clones, because the smaller individual leaf area was compensated by a higher number and a faster production of leaves (i.e. higher LN and LNI).

Photosynthetic activity, measured as ETR, was reduced by flooding in most clones, except for A129 and ST67 (Figure 3f). The flooding and interaction effects were significant for ETR (Table 3).

Stomatal conductance was reduced in flooded plants for most clones on all measuring dates (Figure 4). Flooding effects were significant on all measurement dates, but clone effects were significant only on day 34, and interaction effects were not significant on any date (Table 3). In the last measurement, there was a reduction in  $g_s$  in both control and flooded plants, compared with previous dates (Figure 4). The same leaf was measured on the three dates; on the first date it was the last fully expanded leaf, but this was not the case three weeks later, in the last measurement, when younger leaves had completed their expansion. Stomatal resistance increased (i.e. stomatal conductance decreased) from the apical leaves to the basal leaves of plants, in parallel with an increase of water flow resistance through and into the leaf blades, which appears to be part of an internally programmed change in the hydraulic architecture of plants (Neumann and Stein 1984). The

**Table 3:** Two-way ANOVA of the effects of clone, flooding treatment and the interaction clone × flooding on the variables measured. All the increase rates were calculated for the entire period of treatment (35 d). Variables and dates highlighted in bold were included in the cluster analysis

Variable name	Abbreviation and units	Effect of clone	Effect of flooding	Clone × flooding
<b>Volume index, day 35</b>	<b>VI; cm<sup>3</sup>.</b>	****	****	*
<b>Leaf number on main stem, day 35</b>	<b>LN</b>	**	****	ns
<b>Leaf number increase</b>	<b>LNI; d<sup>-1</sup></b>	ns	****	ns
<b>Area of last expanded leaf, day 35</b>	<b>LAI; cm<sup>2</sup></b>	***	****	ns
<b>Total leaf area, day 35</b>	<b>TLA; cm<sup>2</sup></b>	**	****	ns
<b>Leaf expansion rate</b>	<b>LER; cm<sup>2</sup> d<sup>-1</sup></b>	*	****	ns
Chlorophyll content, day 7	Chl; SPAD units	****	ns	ns
Chlorophyll content, day 14	Chl; SPAD units	****	***	ns
Chlorophyll content, day 21	Chl; SPAD units	***	****	ns
Chlorophyll content, day 28	Chl; SPAD units	****	***	ns
<b>Chlorophyll content, day 35</b>	<b>Chl; SPAD units</b>	***	***	**
<b>Electron transport rate, day 35</b>	<b>ETR; mmol m<sup>-2</sup> s<sup>-1</sup></b>	ns	****	**
<b>Stomatal conductance, day 16</b>	<b><math>g_s</math>; mmol m<sup>-2</sup> s<sup>-1</sup></b>	ns	****	ns
Stomatal conductance, day 21	$g_s$ ; mmol m <sup>-2</sup> s <sup>-1</sup>	ns	****	ns
Stomatal conductance, day 34	$g_s$ ; mmol m <sup>-2</sup> s <sup>-1</sup>	***	****	ns

\*  $P < 0.10$ ; \*\*  $P < 0.05$ ; \*\*\*  $P < 0.01$ ; \*\*\*\*  $P < 0.001$ ; ns, non-significant

observed decrease in  $g_s$  in both control and flooded plants was likely to be caused by a similar ontogenic change on the measured leaf.

Chlorophyll content is shown in Figure 5. Flooding greatly accelerated chlorophyll degradation in some clones (20-82 and 21-82), whereas in others there were no differences with control plants (2-82, 89-82, 149-82 and 150-82), and in ALT and CT12 the degradation was slightly slower in flooded than in non-flooded plants. On day 7, only clone effects were significant (Table 3). On days 14, 21 and 28 both clone and flooding effects were significant, whereas on day 35 clone, flooding and interaction effects were significant (Table 3).

A hierarchical cluster analysis was carried out (Figure 6). Three groups of clones were clearly recognisable: clone Alton was the most flood tolerant, clones A106, A129, CF2, SIA and 150-82 were moderately tolerant, and the remainder of the clones showed low flood tolerance.

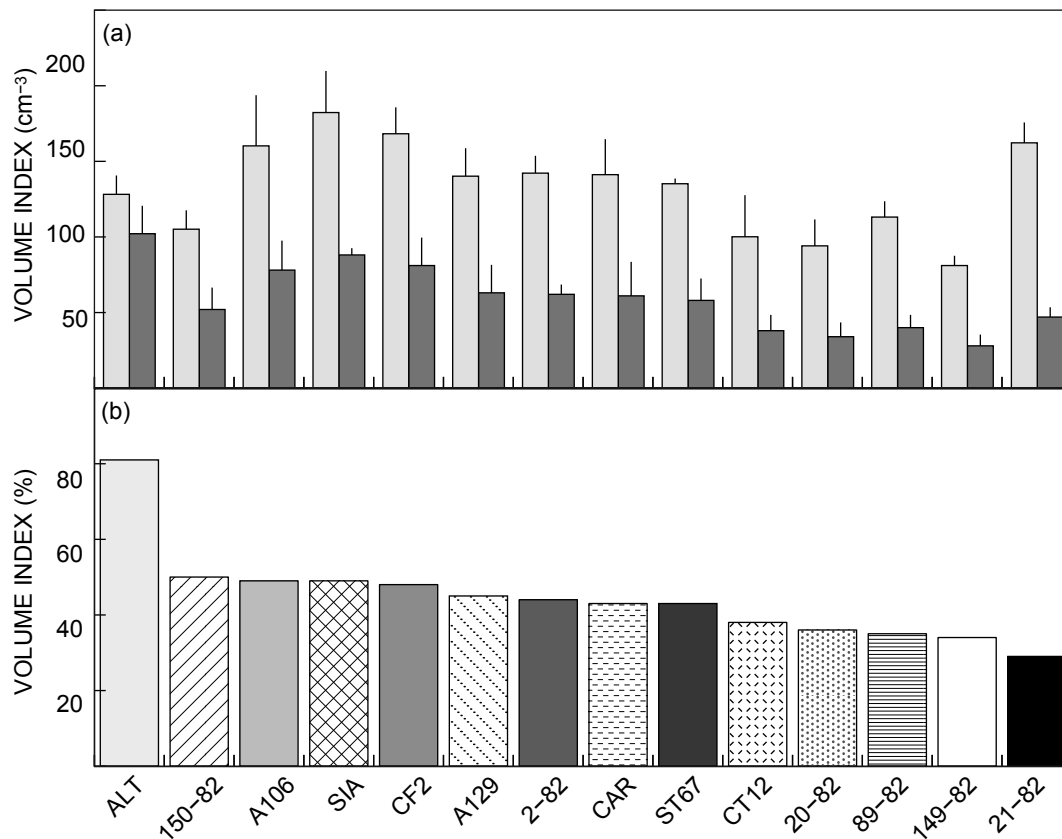
**Discussion**

We measured flooding effects on aboveground growth and leaf traits related to productivity in 14 *Populus* clones, some of which were already planted in the Paraná River Delta and others that had shown a good response in preliminary field trials, and were candidates to be released for commercial forest plantations.

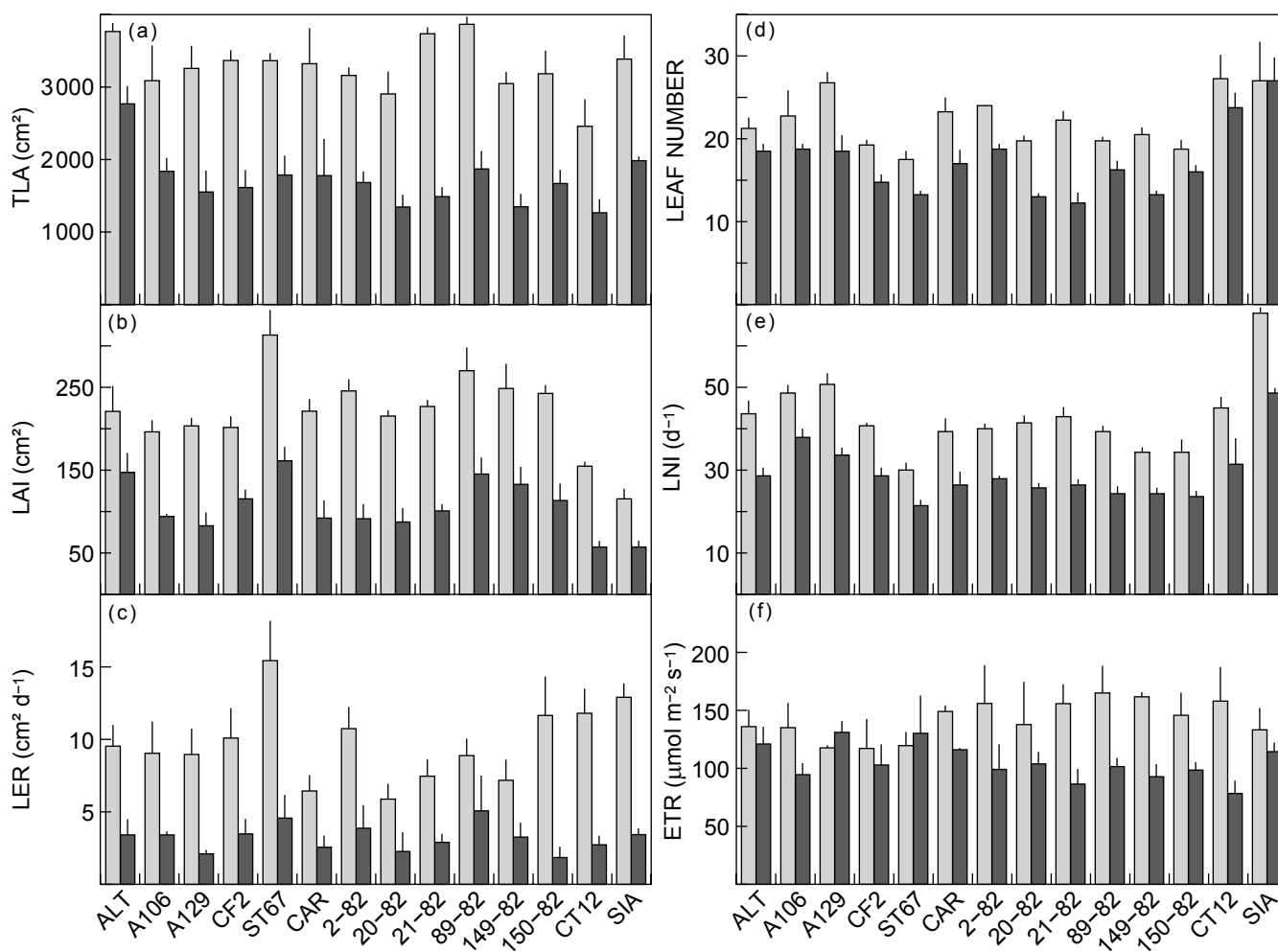
Although flooding reduced growth expressed as VI in all clones, there was an important clonal variation in the extent of the decrease. If we analyse the clonal response to flooding in terms of relative productivity, Alton was the clone least affected by flooding. In absolute terms, several clones, such as A106, ST67, A129, CF2, SIA, CAR and 21-82, are more productive than Alton under normal conditions (Figure 2a). However, the greenhouse data must be treated with caution; in a five-year field trial the experimental clones 149-82 and 150-82 grew better than A129, A106 and ST67 (Cortizo et al. 2009), but the opposite occurred in the greenhouse.

Flooding caused a reduction in total leaf area. Two main factors can be attributed to this: the reduction in the number and area of newly expanded leaves, and the acceleration of senescence and abscission of older leaves. In our experiment, there was a decrease in the area of the last expanded leaf on day 35 in flooded plants in all clones; this agrees with the reduction in leaf expansion rate caused by flooding. The total number of leaves of the main stem on day 35 decreased in all clones except SIA, in which there were no differences between control and flooded plants for this trait. Accordingly, the increase rate of the number of leaves during the experiment was reduced in flooded plants.

The senescence of basal leaves showed a variable response. Chlorophyll degradation in the basal leaf



**Figure 2:** Volume index of 14 *Populus* clones, watered to field capacity (control plants) or flooded for 35 d. (a) Volume index of flooded plants (dark grey bars) and control plants (pale grey bars). (b) Volume index expressed as a percentage of control values. The full names of the clones are detailed in Table 1. Vertical bars represent the SE of the mean



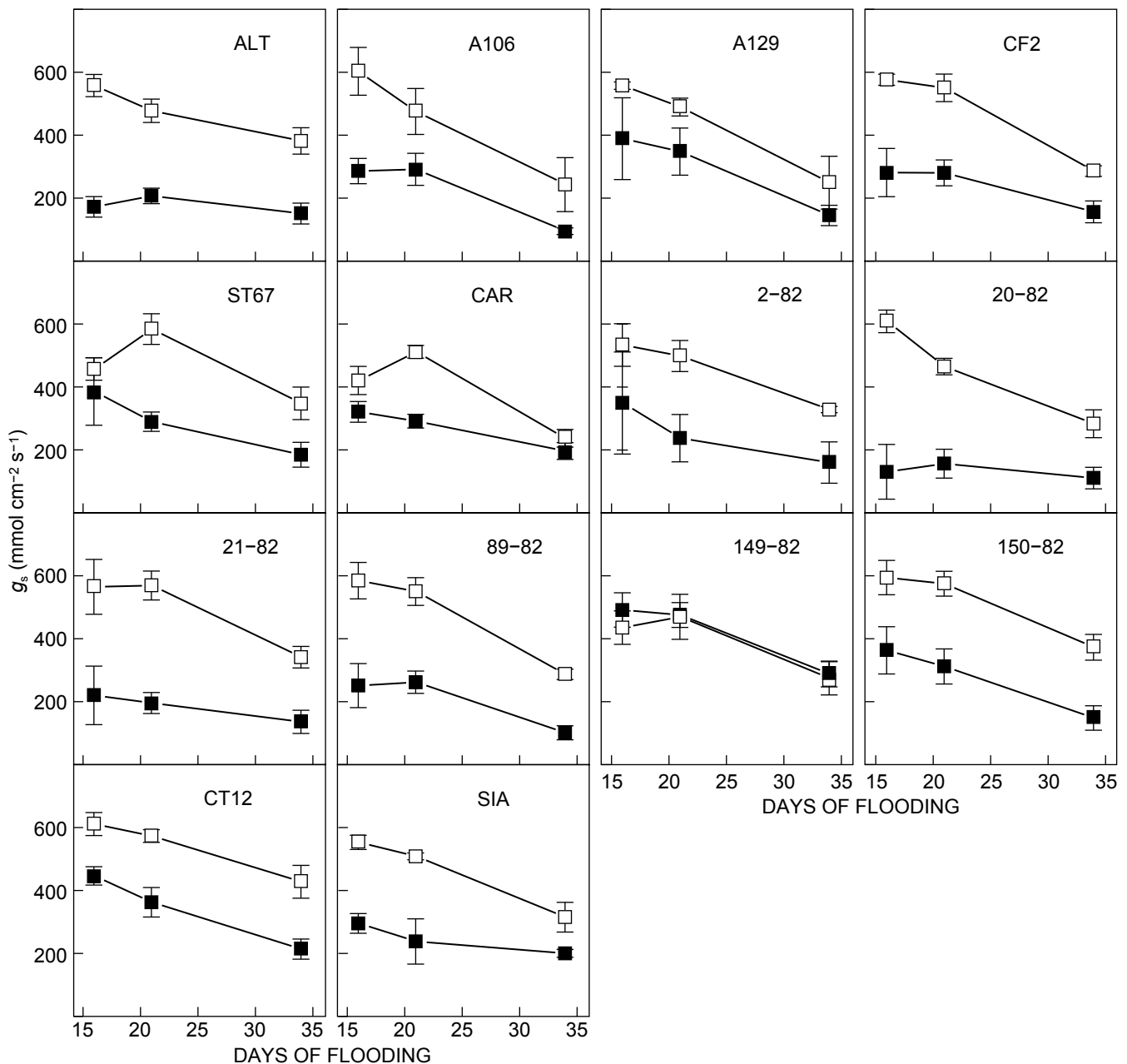
**Figure 3:** Leaf traits responses of 14 poplar clones watered (pale grey bars) and flooded for 35 d (dark grey bars). (a) Total leaf area (TLA; day 35). (b) Area of the last expanded leaf (LAI; day 35). (c) Expansion rate of an individual leaf (LER). (d) Number of leaves on the main stem (day 35). (e) Leaf number increase (LNI) during the stress period. (f) Apparent electron transport rate (ETR; day 34). Vertical bars represent the SE of the mean

(Figure 5) was very variable, from no degradation in some clones to complete degradation and abscission of the leaves in others. Overall, it seems that the decrease in leaf area in flooded plants was primarily because of the reduction of the number and area of the newly formed leaves, rather than to the increase in senescence and abscission of basal leaves.

A reduction in leaf area is most likely a response to reduced water flow in roots. Resistance to water flow through roots increases with low  $O_2$  and high  $CO_2$ , conditions that occur as a consequence of flooding (Smit and Stachowiak 1988). In this context, a lower leaf area will reduce water loss from the plant, and it contributes to maintain the water status of the shoot when combined with a lower stomatal conductance (Smit and Stachowiak 1988, Cao and Conner 1999). As frequently occurs with traits that enhance survival under stress conditions, the reduction in leaf area reduces plant growth. Total leaf area, individual leaf area and the number of leaves in the main stem have shown a good correlation with productivity in different poplar pedigrees and environments (Rae

et al. 2004, Monclus et al. 2005, Marron et al. 2007). In our experiment, all of these variables were significantly reduced by flooding. In addition to leaf area reduction, photosynthetic activity (measured as ETR) and stomatal conductance were also reduced by flooding in most clones. These results are similar to previous reports that have shown that flooding causes stomatal closure and reduction of photosynthetic activity in poplars (Regehr et al. 1975, Liu and Dickmann 1993, 1996), and also accounts for the observed reduction in growth.

The development of adventitious roots and aerenchyma are common responses to flooding (Kozlowski 1997, Glenz et al. 2006). These structures contribute to gas exchange in the roots, allowing oxygen diffusion from the atmosphere to the roots and an output for volatile compounds that may accumulate in roots of flooded plants (Kozlowski 1997, Vartapetian and Jackson 1997). Adventitious root formation has been reported in *Populus* clones under flooding (Cao and Conner 1999, Gong et al. 2007, Guo et al. 2011). We found similar results: adventitious roots appeared in some



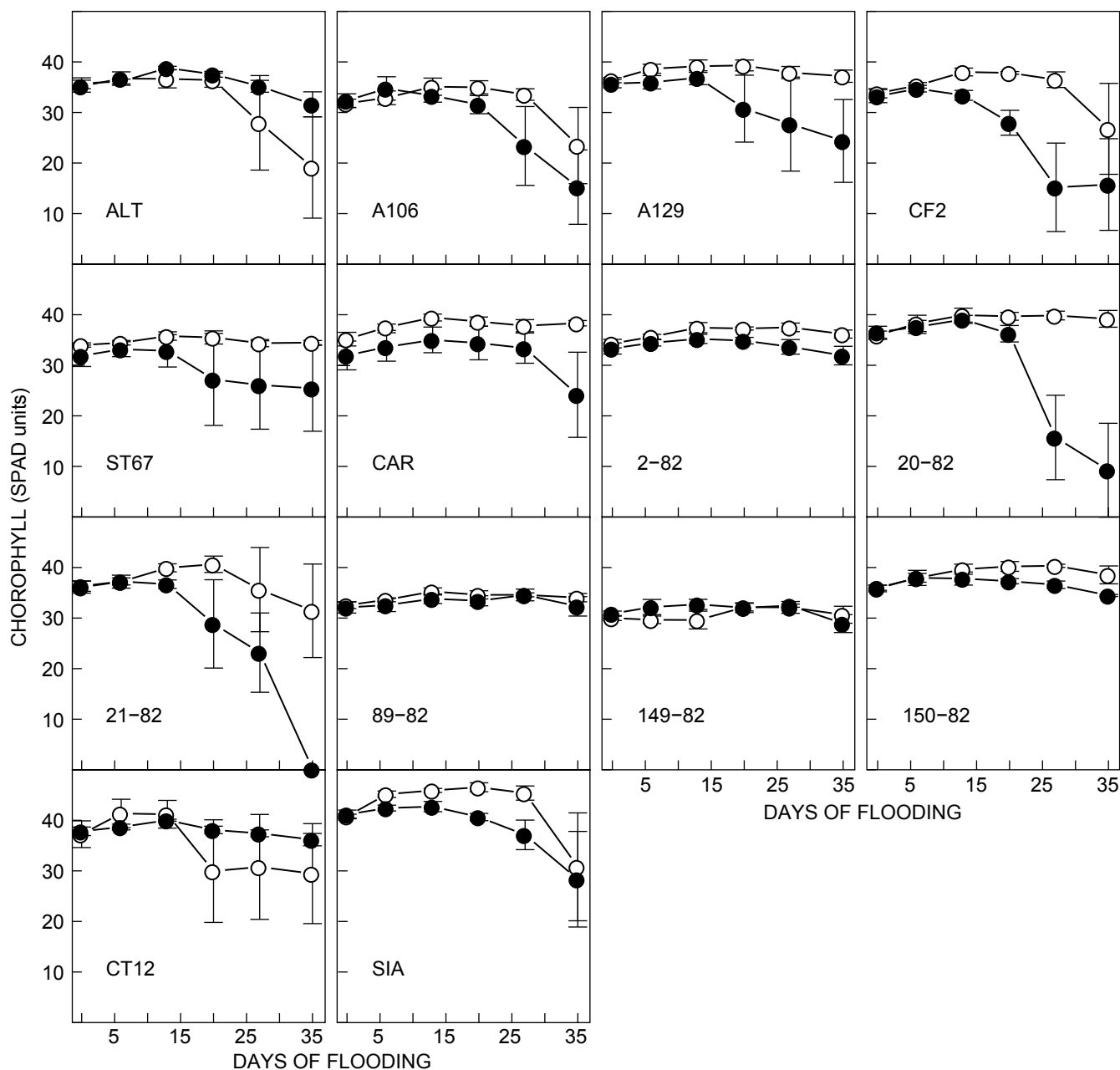
**Figure 4:** Stomatal conductance ( $g_s$ ) in a set of 14 poplar clones watered (white squares) and flooded for 35 d (black squares). Vertical bars represent the SE of the mean

clones, but their presence did not always strictly correlate with higher flooding tolerance.

The clone Alton has clearly the highest flood tolerance, albeit not the highest growth in non-flooded conditions. The good performance of Alton can be explained by the maintenance of a higher leaf area and photosynthetic activity (measured as ETR) under flooding stress conditions; both traits contribute to the availability of photosynthates for growth. In addition, Alton retains a higher root biomass than flood-sensitive clones. Flooding reduced the root:shoot ratio in all clones compared to control plants, but in Alton the reduction was only 36% compared with 50% in 149-82 and ST67 (Rodríguez et al. unpublished results). The retention

of a higher root biomass improves the absorption of water and nutrients, thus enhancing the ability to maintain growth under flooding conditions (Kozłowski 1997).

In adult poplar plantations, the fall of trees as a consequence of strong winds is more common in areas that have been flooded, thus reducing stand productivity (FGA pers. obs.). This fact can be a consequence of root dieback caused by flooding, which underscores the importance of the roots as an anchorage system for trees. The maintenance of a higher root biomass might enhance tree stability after a flood episode and, consequently, better productivity at plantation level at the end of the rotation. This fact requires further exploration, especially regarding the



**Figure 5:** Chlorophyll content (SPAD units) of a basal leaf in a set of 14 poplar clones watered (white circles) and flooded (black circles). Vertical bars represent the SE of the mean

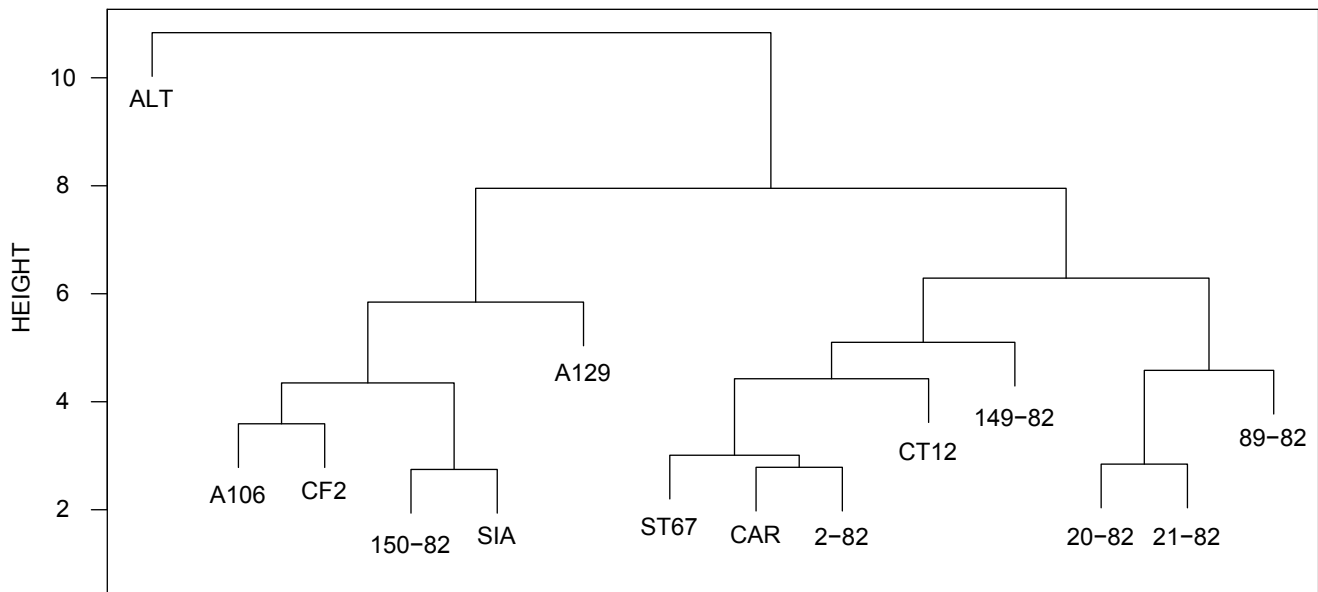
retention of a high root biomass under flooding as a possible trait to be selected in breeding programs.

### Conclusions

In China, areas with different risks of flooding have been defined and different clones are recommended for planting in each site (Guo et al. 2011). A similar method needs to be developed in the Paraná River Delta; the criteria to breed clones for both productive environments (the open and closed systems) regarding flooding tolerance should be different.

Poplar plantations are mostly located in dyke-protected areas, where they can suffer flooding events as a consequence of local heavy rains, especially in the lowest areas near to the drainage channels. Poplar plantations in the Paraná River Delta have a strictly commercial purpose, and a 50% reduction in growth after 5 weeks of flooding for most planted commercial clones (A129, A106 and ST67) is an undesirable outcome. Clones that combine high productivity with improved flooding tolerance are necessary for those areas. A possible strategy to achieve this aim could be to use the highly flood tolerant clone Alton as a parent in crosses with other clones with good growth traits (such as





**Figure 6:** Cluster analysis of the relative flooding tolerance of the 14 clones analysed in this work. The variables included are those listed in Table 3, plus presence/absence of adventitious roots. Height = a vector indicating the distances between merging clusters at the successive stages of the clustering process

A129 and 21-82) to obtain new clones that combine good growth and flood tolerance.

From our results, we cannot recommend any of the experimental clones tested to be planted in the open system. The occurrence of extreme flooding events lasting months makes the improvement of both survival and productivity equally important. The rotation period for poplars in the area is between 12 and 15 years. Although it is possible that an extreme flooding event will not occur in this period, if it does, it will be important that plantations survive and resume growth immediately afterwards. The open system might be uncertain, but it has a lower impact on the natural wetland ecosystem than the closed system (Bó et al. 2010). The availability of more flood-tolerant clones for the open system will favour the development of poplar plantations with less environmental impact than those growing in the dyke-protected sites.

**Acknowledgements** — VMCL is a researcher from CONICET, Argentina. Thanks to M Delgado and S Martínez for helping with measurements. This work was funded by grants from FONCyT (PICT 00487) and CONICET (PIP 0269) to VMCL.

## References

- Amlin N, Rood SB. 2001. Inundation tolerance of riparian willows and cottonwoods. *Journal of the American Water Resources Association* 37: 1709–1720.
- Barros V, Menéndez A, Natenzon C, Kokot R, Codignotto J, Re M, Bronstein P, Camilloni I, Ludueña S, González S, Ríos DM. 2006. Vulnerability to floods in the metropolitan area of Buenos Aires under future climate change. *AIACC Working Paper* no. 26. Washington, DC: AIACC Project. Available at [http://www.aiaccproject.org/working\\_papers/working\\_papers.html](http://www.aiaccproject.org/working_papers/working_papers.html) [accessed 12 May 2011].
- Bejaoui Z, Albouchi A, Abassi M, El Aouni MH. 2006 Influence d'une hydromorphie modérée ou sévère sur la production de biomasse et les échanges gazeux de plants de peuplier euraméricain. *Canadian Journal of Forest Research* 36: 2654–2665.
- Bó RF, Quintana RD, Courtalón P, Astrada E, Bolkovic ML, Lo Coco G, Magnano A. 2010. Efectos de los cambios en el régimen hidrológico por las actividades humanas sobre la vegetación y la fauna silvestre del Delta del Río Paraná. In: Blanco DE, Mendez FM (eds), *Endicamientos y terraplenes en el Delta del Paraná*. Buenos Aires: Wetlands Internacional. pp 33–63.
- Bonetto A, Hurtado S. 1998. Delta Paranaense. Región I. Cuenca del Plata. In: Canevari P, Blanco D, Bucher E, Castro G, Davidson I (eds), *Los humedales de la Argentina: situación actual, conservación y legislación*. Wetlands International Publication no. 46. Buenos Aires: Wetlands Internacional. pp 31–72.
- Borodowski E. 2006. Álamos y sauces en el Delta del Paraná: situación del sector y silvicultura. *Actas de las Jornadas de Salicáceas* 2006: 61–70. Available at <http://64.76.123.202/new/0-0/forestacion/biblos/JS%202006/pdf%20tt/Borodowski.pdf> [accessed 2 April 2012].
- Braendle R, Crawford RMM. 1999. Plants as amphibians. *Perspectives in Plant Ecology, Evolution and Systematics* 2: 58–78.
- Cao FL, Conner WH. 1999. Selection of flood-tolerant *Populus deltoides* clones for reforestation projects in China. *Forest Ecology and Management* 117: 211–220.
- Cortizo S, Abbiatti M, Mema V. 2009. Nuevas posibilidades para ampliar la diversidad clonal de las plantaciones de álamo del Delta del Paraná. *Actas de las Jornadas de Salicáceas Mendoza* 2009. Available at <http://64.76.123.202/new/0-0/forestacion/biblos/JS%202009/Trabajos/2%20Trabajos%20Técnicos/2-2%20Gen%20E9tica,%20Mejoramiento%20y%20Conservaci%20n%20de%20Recursos/Cortizo1.pdf> [accessed 2 April 2012].
- Du KB, Shen BX, Xu L, Tu BK. 2008. Estimation of genetic variances in flood tolerance of poplar and selection of resistant F<sub>1</sub> generations. *Agroforestry Systems* 74: 243–257.
- Glenz C, Schlaepfer R, Iorgulescu I, Kienast F. 2006. Flooding tolerance of Central European tree and shrub species. *Forest Ecology and Management* 235: 1–13.
- Gong JR, Zhang XS, Huang YM, Zhang CL. 2007. The effects

- of flooding on several hybrid poplars clones in northern China. *Agroforestry Systems* 69: 77–88.
- Guo XY, Huang ZY, Xu AC, Zhang XS. 2011. A comparison of physiological, morphological and growth responses of 13 hybrid poplar clones to flooding. *Forestry* 84: 1–12.
- Kozłowski TT. 1997. Responses of woody plants to flooding and salinity. *Tree Physiology Monograph* no. 1. Victoria, Canada: Heron Publishing.
- Liu Z, Dickmann DI. 1993. Responses of two hybrid *Populus* clones to flooding, drought and nitrogen availability. II. Gas exchange and water relations. *Canadian Journal of Botany* 71: 927–938.
- Liu Z, Dickmann DI. 1996. Effects of water and nitrogen interaction on net photosynthesis, stomatal conductance and water-use efficiency in two hybrid poplar clones. *Physiologia Plantarum* 97: 507–512.
- Luquez VMC, Sasal Y, Medrano M, Martín MI, Mujica M, Guiamét JJ. 2006. QTL analysis of leaf and plant longevity in *Arabidopsis thaliana*. *Journal of Experimental Botany* 57: 1363–1372.
- Marron N, Dillen SY, Ceulemans R. 2007. Evaluation of leaf traits for indirect selection of high yielding hybrid poplars. *Environmental and Experimental Botany* 61: 103–116.
- Malvárez AI. 1999. El Delta del Río Paraná como mosaico de humedales. In: Malvarez A (ed.), *Tópicos sobre humedales subtropicales y templados de Sudamérica*. Montevideo: UNESCO. pp 35–53.
- Monclus R, Dreyer E, Delmotte FM, Villar M, Delay D, Boudouresque E, Petit JM, Marron N, Bréchet C, Brignolas F. 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides* × *Populus nigra* clones. *New Phytologist* 167: 53–62.
- Neumann PM, Stein Z. 1984. Relative rate of delivery of xylem solute to shoot tissue: possible relationship to sequential leaf senescence. *Physiologia Plantarum* 62: 390–397.
- Pryor LD, Willing RR. 1983. *Growing and breeding poplar in Australia*. Canberra: Canberra Publishing and Printing Co.
- R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.
- Rae AM, Robinson KM, Street N, Taylor G. 2004. Morphological and physiological traits influencing biomass productivity in short-rotation coppice poplar. *Canadian Journal of Forest Research* 34: 1488–1498.
- Ragonese AE, Rial Alberti F, Ciocchini RG, García A. 1987. Fitotecnia de Salicáceas en el Centro Nacional de Investigaciones Agropecuarias Castelar (INTA). *Anales de la Academia Nacional de Agronomía y Veterinaria* 41: 5–30.
- Regehr DL, Bazzaz FA, Bogess WR. 1975. Photosynthesis, transpiration and leaf conductance of *Populus deltoides* in relation to flooding and drought. *Photosynthetica* 9: 52–61.
- Seefeld D, Linder E. 2007. Statistics using R with biological examples. Available at <http://cran.r-project.org/other-docs.html> [accessed 12 May 2011].
- Smit B, Stachowiak M. 1988. Effects of hypoxia and elevated carbon dioxide concentration on water flux through *Populus* roots. *Tree Physiology* 4: 153–165.
- Smit BA. 1988. Selection of flood-resistant and susceptible seedlings of *Populus trichocarpa* Torr. & Gray. *Canadian Journal of Forest Research* 18: 271–275.
- Taylor G. 2002. *Populus*: Arabidopsis for forestry. Do we need a model tree? *Annals of Botany* 90: 681–689.
- Vartapetian BB, Jackson MB. 1997. Plant adaptations to anaerobic stress. *Annals of Botany* 79 (Supplement): 3–20.