Effect of environmental stress factors on ecophysiological traits and susceptibility to pathogens of five *Populus* clones throughout the growing season

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The variability of ecophysiological traits associated with productivity (e.g., water relations, leaf structure, photosynthesis and nitrogen (N) content) and susceptibility to fungal and insect infection were investigated in five poplar clones (*Populus deltoides* Batr.—Lux clone; *Populus nigra* L.—58-861 clone and *Populus × canadensis* Mönch.—Luisa Avanzo, I-214 and Adige clones) during their growing season. The objective of the study was to determine their physiological responses under summer constraints (characteristic of the Mediterranean climate) and to propose clone candidates for environmental restoration activities such as phytoremediation. Relative water content, the radiometric water index and $\delta^{13}$C isotope discrimination ($\Delta^{13}$C) results reflected improved water relations in Adige and Lux during summer drought. Leaf structural parameters such as leaf area, leaf mass per area, density (D) and thickness (T) indicated poorer structural adaptations to summer drought in clone 58-861. Nitrogen content and $\Delta^{13}$C results pointed to a stomatal component as the main limiting factor of photosynthesis in all clones. Adige and Lux showed enhanced photoprotection as indicated by the size and the de-epoxidation index of the xanthophyll-cycle pool, and also improved antioxidant defence displayed by higher ascorbate, reduced glutathione, total phenolics and $\alpha$-tocopherol levels. Photoprotective and antioxidative responses allowed all clones to maintain a high maximum quantum yield of PSII ($F_{v}/F_{m}$) with the exception of Luisa Avanzo and 58-861 which experienced slight photoinhibition in late spring. The study of susceptibility to rust (*Melampsora* sp.) and lace bug (*Monosteira unicostata* Muls. and Rey) infections showed Adige and Lux to be the most tolerant. Overall, these two clones presented high adaptability to summer conditions and improved resistance to abiotic and biotic stress, thereby making them highly commendable clones for use in environmental remediation programmes.

**Keywords**: biotic stress, chloroplast pigments, photochemistry, poplar, water stress, water use efficiency.

Introduction

Poplars (*Populus* spp.) are fast-growing trees, traditionally used as a source of fuel, fibre, lumber and plywood. They are widely cultivated due to their high adaptability to different climate conditions and their large biomass yield. Poplars can be cultivated under short rotation coppicing, a management regime whereby plants are repeatedly cut back to a low stump (every 2–6 years) with the subsequent production of new shoots from the stump and/or roots (Blake 1983), thus allowing one to obtain 8–10 kg of dry biomass per plant per year (Ceulemans et al. 1992, Scarascia-Mugnozza et al. 1997, Kauter et al. 2003).
Furthermore, poplar is a model tree for forest plant genomics (Jansson and Douglas 2007). The *Populus trichocarpa* (Torr. and Gray) genome was completely sequenced (Tuskan et al. 2006) and a highly informative genetic mapping of *Populus nigra* L. species was carried out (Gaudet et al. 2008), thus representing a valuable tool in the identification of the genetic determinants of biomass production and environmental adaptation. Moreover, several studies have focused on the poplar genome-based analysis of qualitative and quantitative trait loci to evaluate the genetic control of some characteristics such as resistance to rust (Newcombe et al. 1996, Jorge et al. 2005), biomass yield and its distribution throughout the tree (Wullscheleger et al. 2005), adaptation to elevated CO₂ (Rae et al. 2007), response to drought (Street et al. 2006) and cadmium tolerance (Induri et al. 2012). In recent years, the value of poplars in terms of ecological services has been highlighted (Tognetti et al. 2013). Among these, the ability of poplar species to mitigate the environmental impacts of human activities has received particular attention. In fact, the fast growth rate which characterizes these plants is of particular interest for the purposes of the sequestration of atmospheric CO₂ (Calfapietra et al. 2010) or the reduction of metal pollution in soils and water (Laureysens et al. 2004, Fernández et al. 2012). Adaptability to growth under pedo-climatic unfavourable conditions, i.e. low water and nutrient availability, is an important pre-requisite of candidate plants for phytoremediation. Therefore, the evaluation of a plant’s ability to overcome growth factor limitations other than soil pollution is a very important step in order to assess its real potential in terms of phytoremediation in open-field applications. The importance of exploring the large variability which characterizes the *Populus* genus in order to evaluate genotypes with superior potential for phytoremediation has been raised (Dos Santos et al. 2007, Pietrini et al. 2010).

With regard to biotic stress factors in poplars, leaf rust caused by species of the genus *Melampsora* (Basidiomycota, Uredinales) is considered to be one of the most widespread and devastating diseases (Pinon and Frey 2005). *Melampsora* spp. depend on living host tissues for their development and reproduction. Their infection induces the depletion of sugars and nutrients, which affect growth, produce premature defoliation and predispose trees to other diseases and pests (Feau et al. 2007). Moreover, leaf rust disease produces a decrease in plant photosynthesis leading to lower biomass production (Agrios 2005). Poplars are also commonly affected by small insects such as lace bugs (*Monosteira unicostata* Muls. and Rey (Het: Tingidae; Miller 2004)). Lace bugs are usually host specific and can be very destructive to plants, feeding on the undersides of leaves by piercing the epidermis and extracting the sap which can result in premature leaf abscission. In poplars, pest management is carried out by using resistant clones or certified plant material, and through the use of good culture practices; for this reason, the determination of clone susceptibility is indispensable.

The aim of the work was to study the adaptability of selected poplar clones to environmental constraints typical of the Mediterranean climate, and their resistance to fungus (genus *Melampsora*) and insect (genus *Monosteira*) infections by tracking physiological characteristics and responses during growth. The poplar clones *Populus deltoides* Batr.—Lux clone; *Populus nigra* L.—58-861 clone and *Populus × canadensis* Mönch. Luisa Avanzo and I-214 clones were chosen for their outstanding performances in phytoremediation (Zacchini et al. 2009, Pietrini et al. 2010) while *Populus × canadensis* Mönch. Adige clone was chosen for its remarkable survival rate and resistance to mosaic virus (Facciotto and Frison 1999). Physiological characterization included parameters related to water relations and productivity, Δ¹³C isotopic discrimination and photochemistry. Chloroplast pigments, antioxidants (ascorbate (AscA), reduced glutathione (GSH) and total phenolics (TPhe)) and chlorophyll fluorescence measurements provided information on electron transport characteristics, excess energy dissipation processes and reactive oxygen species (ROS) scavenging and detoxification activities. The evaluation of different responses against abiotic and biotic stress of the selected clones will provide information for the selection of poplar clones that display a high growth capacity under adverse conditions such as those experienced in sites in which environmental restoration is required.

### Materials and methods

**Experimental site and plant material**

Adult plants of five female poplar clones (*Populus deltoides* Batr. (Cottonwood)—Lux clone; *Populus nigra* L. (black poplar)—58-861 clone and *Populus × canadensis* Mönch. (hybrid black poplar)—Luisa Avanzo, I-214 and Adige clones) grown since 2001 in the IBAF-Institute experimental field near Rome in the Tevere valley were used for this experiment performed in 2009. Each clone was represented by 10–30 individuals and localized in multiple parcels characterized by a single-row design with inter-row distances of 220 cm and a spacing of 60 cm between plants within the row. Three randomly selected plants per clone were used for measurements and sampling. The soil is a typical alluvium and the climate is Mediterranean, with a mean maximum temperature recorded in 2009 of 27.6 °C, a mean minimum temperature of 3.63 °C and an annual rainfall of 927.6 mm. Climatic data during the study were recorded at the Monterotondo (Rome) meteorological station (Italian Agrometeorological Network, CMA-EnteCRA, Italy) (Table 1).

**Sampling and measurements**

Sampling was performed in spring (18–25 May), early summer (6–13 July) and late summer (14–21 September) at midday (13.30–16.30 h) local time. Samples were obtained by
selecting fully developed leaves from the entire flush of growth which were exposed to sun irradiance, omitting leaves formed with bud break and the youngest leaves of the growth flush. Two samples of 12–21 south-facing and fully developed leaves were randomly selected between 2 and 4 m height from three plants of each *Populus* clone (six samples per clone). Samples were immediately frozen in liquid nitrogen, stored in the laboratory at −80 °C, lyophilized (Virtis Lyophiliser, Freezemobile 6EL, Gardiner, NY, USA) and milled in a Cyclotec 1093/Foss Sample Mill (Tecator, Höganäs, Sweden) until analyses were carried out. Measurements of the relative water content (RWC), leaf biomass parameters, chlorophyll fluorescence and leaf reflectance were performed during the same sampling weeks at midday on three south-facing leaves of three trees per clone.

### Relative water content and leaf biomass parameters

Relative water content was determined as \(\frac{(M_i - M_d)}{(M_{fs} - M_d)} \times 100\), with \(M_i\) being plant fresh mass; \(M_{fs}\) plant fresh saturated mass (after rehydrating samples for 24 h in darkness at 4 °C); and \(M_d\) plant dry mass (after oven-drying at 60 °C until a constant weight was achieved). Leaf area (LA) was determined with a Laser Leaf Area Meter (CI-203) (CID, Inc., Camas, WA, USA). Leaf mass per area (LMA) was determined as \(M_i/LA\), and its components leaf thickness (T) and leaf density (D) were calculated as \((M_i/LA)\) and \(((M_d/M_i) \times 100)\), respectively (Niinemets 1999).

### Leaf reflectance measurements

The analysis of foliar reflectance (Peñuelas and Filella 1998) allowed for non-destructive evaluation of hydric responses by means of the calculation of the water index (WI). Leaf reflectance was measured with a portable spectral analysis system with artificial light (USB4000, Oceanoptics), operated with Spectrasuite (Oceanoptics) software. Ten scans per sample were integrated (integration time 50 ms). The WI was derived from the spectra and calculated as \(R_{970}/R_{900}\), where reflectance at 970 nm is associated with water absorption and 900 nm is a reference wavelength (Peñuelas et al. 1997). Methodological problems did not allow us to obtain WI values in May.

### Leaf carbon isotope composition and nitrogen content

Carbon isotopic composition (δ\(^{13}\)C) and total nitrogen (N) content were determined with ~1 mg of lyophilized material with an elemental analyser (EA1108, Series 1, Carlo Erba Instrumentazione, Milan, Italy) coupled to a mass spectrometer (Delta C, Finnigan MAT, Bremen, Germany). δ\(^{13}\)C values were determined using a standard reference calibrated against Pee Dee Belemnit carbonate and used to estimate δ\(^{13}\)C as: \(\Delta^{13}C = 1000 \times (\delta_p - \delta_a)/(1 + \delta_p)\), where \(\delta_a\) and \(\delta_p\) are values for air (δ\(^{13}\)C = −10.5%) and the plant, respectively (Farquhar et al. 1989).

### Chlorophyll fluorescence

Minimum fluorescence yield (\(F_o\)), maximum fluorescence yield (\(F_m\)) and maximum quantum yield of PSII photochemistry (\(F_o/F_m\)) (equivalent to \(F_{m}-F_o/F_m\)) were determined in dark-adapted leaves (at least 40 min) with a Mini-Pam Photosynthesis Yield Analyser (Walz, Effeltrich, Germany).

### Chloroplast pigments

Light-exposed leaf samples were collected for chloroplast pigment analysis from the same flush where chlorophyll fluorescence measurements were made. Chloroplast pigments and α-tocopherol (α-Toc) were extracted from 50 mg of lyophilized material with pure acetone buffered with CaCO\(_3\) and centrifuged twice at 12,000 × g. The supernatants were filtered through 0.2-μm PTFE filters (Teknokroma, Barcelona, Spain). The pigments were separated by high-performance liquid chromatography on a reversed-phase C18 column (Waters Spherosorb ODS1, 4.6 × 250 mm, Milford, MA, USA) and detected with a photodiode array detector, according to the method by García-Plazaola and Becerril (1999, 2001). Tocopherol detection and quantification were performed with a Scanning Fluorescence Detector (Waters 474) that was operated in series with the photodiode array detector according to García-Plazaola and Becerril (1999, 2001). The relative de-epoxidation state of the xanthophyll-cycle pigments was estimated by the ratio \((A + Z)/(V + A + Z)\), abbreviated AZ/VAZ.

### Ascorbate and glutathione

Fifteen mg of lyophilized leaf samples were extracted with metaphosphoric acid (6% (w/v)) and centrifuged at 10,000 g at 4 °C. Ascorbate content was determined according to Foyer et al. (1983) and measured by the change in \(A_{265}\) using a spectrophotometer (Cecil Aquarius, CE7400, Cecil Instruments, Cambridge, UK). Glutathione content was obtained according to the Noctor and Foyer protocol (1998). Reduced glutathione was determined and measured by the change in \(A_{412}\).
**Total phenolics**

Lyophilized leaves were homogenized with 20 volumes of an 80% methanol solution, extracted twice and centrifuged at 14,000 g for 10 min. Total phenolics content was determined according to Singleton and Rossi (1965) and measured by $A_{735}$ (with a spectrophotometer; Perkin Elmer, Norwalk, CT, USA). Total phenolics were expressed as gallic acid equivalents.

**Incidence and severity of rust infection and incidence of lace bugs**

The incidence of *Melampsora* sp. and *M. unicostata* was evaluated in each clone on the basis of the percentage of individuals showing visual symptoms (presence of rust pustules/lace bug faeces). The incidence in each infected individual was evaluated by the percentage of infected leaves from 15 randomly selected leaves per individual and in three individuals per clone. The degree of severity was established from the percentage of LA covered by *Melampsora* pustules (uredinia) as shown in Table 2. The study of both pathogens was performed in September when rust infection typically reaches a more advanced stage.

**Statistical analysis**

Statistical procedures were performed using SPSS for Windows vs. 15.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance was used to test the main effects against appropriate error terms, of clone and season (spring, early summer, late summer) on the measured parameters. A multiple comparison test of the means using the Duncan post hoc test was applied.

**Results and discussion**

**Water relations, $\Delta^{13}C$ and structural attributes of leaves**

The Mediterranean climate is characterized by summer droughts with significant consequences for plant growth (Mitrakos 1980, Camarero et al. 2010). In May, for 3 weeks before the start of our study, rainfall was scarce (~4.6 mm) and climatological data indicated that the lowest precipitation and the highest temperatures occurred in late summer (Table 1). The decline in water availability during the summer drought was observed by the results obtained for hydric and leaf structural parameters. In fast-growing species, such as poplar, productivity is highly dependent on water availability (Tschaplinski et al. 1994) and leaf structure (Ceulemans 1990). Improved water relations were observed in Adige and Lux during growth. These two clones showed the highest RWC at the end of the summer (September) (Figure 1a). A decrease in RWC from May to September was observed in all clones except in Lux, which showed constant values. Relative water content in May (above 90% in all clones) might be related to stomatal closure and to a reduction in cellular expansion. In September, RWC was <80% in L. Avanzo, I-214 and especially in 58-861, which was reflected in a leaf water potential equal to or lower than ~1.5 MPa and pointed to a high water deficit in plants and consequent metabolic changes such as photosynthesis disruption, an increase in respiration, and proline and abscisic acid accumulation (González and González-Vilar 2001). Since the radiometric index WI is related to stomatal conductance ($g_s$) (Serrano et al. 2010), WI results showed

### Table 2. Degrees of severity of the foliar infection produced by *Melampsora* sp. Each percentage of affected LA corresponds to a degree of rust severity.

<table>
<thead>
<tr>
<th>Severity degree</th>
<th>% Affected LA</th>
<th>Infection level</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td>1.5</td>
<td>&lt;1</td>
<td>Traces</td>
</tr>
<tr>
<td>2</td>
<td>1–5</td>
<td>Mild</td>
</tr>
<tr>
<td>3</td>
<td>6–25</td>
<td>Moderate</td>
</tr>
<tr>
<td>4</td>
<td>26–50</td>
<td>Severe</td>
</tr>
<tr>
<td>5</td>
<td>&gt;50</td>
<td>Very severe</td>
</tr>
</tbody>
</table>

![Figure 1.](https://example.com/figure1.png)
lower \(g_s\) in Adige and Lux in July and September. Water index values also indicated that there was a decline in leaf water content in September in all clones (Figure 1b).

\(^{13}\text{C}\) isotope discrimination is negatively correlated with time-integrated water-use efficiency (WUE) in C3 plants (Knight et al. 1994), defined as the ratio of biomass accumulation to transpiration. The lower \(\Delta^{13}\text{C}\) in Adige and Lux and higher \(\Delta^{13}\text{C}\) in L. Avanzo (Figure 1c) reflected their differences in WUE, as was also demonstrated in RWC and WI results, previously discussed. In Adige and Lux, a \(\Delta^{13}\text{C}\) of \(\sim 2\) per mil lower than that of other clones accounted for the increase in WUE of \(\sim 30\%\) (Ehleringer et al. 1992). \(^{13}\text{C}\) isotope discrimination varies with the relative contribution of enzymatic and diffusional fractionation processes (Farquhar and Richards 1984). Results confirm that Adige and Lux displayed the lowest \(g_s\) as expected from WI data. High WUE in May (except in I-214) and in September in 58-861 and Lux would indicate changes in \(g_s\) that could contribute to maintaining a high RWC.

The seasonal reduction in water availability was also reflected in leaf structure (Peña-Rojas et al. 2005). Lux, Adige and I-214 displayed improved leaf structural adaptations during their growth period in order to maintain high water content as RWC, WI and \(\Delta^{13}\text{C}\) results demonstrated, particularly in the two first clones. The highest LA was observed in Lux and the lowest in 58-861 (Figure 2a). High-yielding clones display a larger total LA and individual LA in a range of \(P.\) deltoides \(\times\) \(P.\) nigra clones (Marron et al. 2005, Monclus et al. 2005). In L. Avanzo, Lux and Adige, a notable increase in LA occurred from May to July; whereas in L. Avanzo and LA, the trend showed a decline from July to September. A decline in LA reflects the limitations due to water loss through transpiration during summer. On the other hand, Adige and Lux showed the highest LMA during the study (Figure 2b), where the increase in LMA during ontogeny is related to an increase of leaf dry mass accumulation after expansion ceases, cuticle thickening and secondary cell wall development (Marron et al. 2008). Leaf mass per area increased in September in all clones except in I-214. The two components of LMA, thickness (\(T\)) and density (\(D\)), are not necessarily interdependent and may be controlled by different environmental variables (Witkowski and Lamont 1991). The increase in \(T\) in 58-861, Lux and Adige during growth (Figure 2d) explains the additional mesophyll layers which imply a higher photosynthetic capacity (Hanba et al. 2002). From May to July, clones showed a decrease in \(D\) whereas after July it increased (Figure 2c). An increase in \(D\) is associated with thicker cell walls and with smaller and more tightly packed cells (Ninemets 2001). Increases in LMA are due to an increased \(D\) as reported for greenhouse-grown cuttings during drought (Marron et al. 2003).

Nitrogen content, considered an indicator of productivity (Ceulemans 1990), declined in all clones (Figure 2e), reflecting its remobilization during plant ontogeny. We observed a negative correlation between N and LMA values in Lux \((r^2 = -0.75\), data not shown\), whereas no relationship was obtained between \(\Delta^{13}\text{C}\) and LMA values in any clone. The observed independence of \(\Delta^{13}\text{C}\) (and therefore of WUE) from biomass parameters would indicate that there is a potential to improve WUE in poplar without necessarily reducing the overall productivity (Monclus et al. 2005, Chamaillard et al. 2011).

**Photoprotection and antioxidant response**

The maximum quantum yield of PSII \((F_v/F_m)\) has been used as an indicator of the functional state of the photosynthetic apparatus. Most of our \(P.\) deltoides clones showed values within the typical range for non-severely stressed plants (0.75–0.85, Björkman and Demmig 1987) and maximum values were reached in September (Figure 3a). Throughout the period of study, Adige showed the highest \(F_v/F_m\) values followed by I-214 and Lux. The slightly but significantly lower values of \(F_v/F_m\) for

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**Figure 2.** (a) Leaf area, (b) leaf mass per area, (c) leaf density (\(D\)), (d) leaf thickness (\(T\)) and (e) N content of the different clones through-out the growing season (May, July, September). Different letters correspond to significant differences at \(P < 0.05\) between clones (A–C) or between sampling periods (a–c). Values are the mean ± SE of three replicates on three plants per clone.
of heat during the whole growing season, while I-214 showed the lowest AZ/VAZ values. September was shown to be the month with the highest energy dissipation in clones except for L. Avanzo and Lux. Concomitantly, the highest VAZ/Chl ratio was reached in September for most of the clones, with Lux and Adige showing the highest levels of all the clones throughout the growing season (Figure 3c). Lux presented markedly lower photosynthetic pigment content in May (e.g., Chl_a + Chl_b, Figure 3d) probably due to an immature state of the leaves. At this stage, the high VAZ/Chl and AZ/VAZ observed might indicate that zeaxanthin (Z) offers protection against excessive light during leaf development (Schindler et al. 1994).

Total chlorophyll content declined over the course of the growing season in all clones (Figure 3d). This fact occurred in parallel with the remobilization of N and also with an increase of leaf D. Lux showed the highest Chl_a/Chl_b ratio (Figure 3e) during growth, indicating an improved adaptation to high light intensities.

In addition to its role in excess energy dissipation, Z together with other lipophilic molecules of the chloroplast, such as β-carotene (β-Car) and α-Toc, plays a fundamental role in the plant antioxidant response. Zeaxanthin and β-Car are efficient quenchers of triplet Chl and ¹O₂ (Young and Britton 1990, Havaux et al. 2007, Gill and Tuteja 2010, Dall’Osto et al. 2012), while α-Toc scavenges lipid peroxyl radicals in photosynthetic membranes (Trebst et al. 2002). Furthermore, the three antioxidants can interact synergistically as radical scavengers (Bohm et al. 1997, Havaux and Niyogi 1999). In our study, no remarkable differences were noticed in β-Car content among clones and the months of study, but considerable differences were noticed in α-Toc content (Figure 4a and b). A marked increase occurred in September in all clones especially in Adige and 58-861 probably as a result of both an enhanced antioxidant response and ontogenic accumulation of α-Toc during leaf ageing (Garcia-Plazaola et al. 2003).

Among the hydrophilic antioxidants, ascorbate and glutathione are considered to be the most important intracellular defences against ROS-induced oxidative damage and are localized in the main cell compartments (Gill and Tuteja 2010, Gest et al. 2013). On the other hand, phenolics, mainly localized in vacuoles and the cell wall, are series of diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin) that are abundant in plant tissues (see the review by Grace and Logan 2000), and which play a double role as antioxidants and on plant–herbivore interactions (Reichardt et al. 1991). Ascorbate is quantitatively the most abundant antioxidant in plant tissues (Noctor and Foyer 1998) and interacts with ROS, particularly with H₂O₂, and is required for the regeneration of α-Toc (Beyer 1994) and the formation of Z (Müller-Moulé et al. 2002). Moreover, effective resistance to prolonged stress requires increased AscA regeneration, a process partially dependent on GSH in higher plants (Foyer and Halliwell 2009).
This was further correlated in Adige and Lux clones (with high values of AZ/VAZ and α-Toc), which displayed the highest AscA content during the growth period and (however only in the case of Lux) also the highest GSH content (Figure 4c and d). On the other hand, 58-861 displayed low GSH values in September, whereas total glutathione increased (data not shown), indicating that most glutathione was in an oxidized state and that this clone had more difficulties than others in maintaining the glutathione pool in a reduced state under long-term stress conditions.

Following a similar trend to that of AscA and GSH content, high TPhe levels were observed in all clones in September and also in L. Avanzo in May (Figure 4e). This clone showed the highest TPhe levels of all clones throughout the growing season. The hydrophilic antioxidant levels and the previously mentioned increase in the photoprotective mechanisms in L. Avanzo in May point to a greater requirement in this clone for protection mechanisms in this period.

Overall, *Populus* clones showed higher antioxidative responses in September probably due to the water deficit conditions and the advanced ontogenic state of the plants. High $F_v/F_m$ (>0.8) in this period might indicate that antioxidant protection mechanisms were working efficiently in all clones. High AscA and GSH levels in September could be related to α-Toc and Z synthesis.

### Susceptibility to infection

All clones showed a remarkable presence of rust infection except for Adige in which only 20% of individuals were affected and ~14% of their leaves showed signs of infection (Table 3). Luisa Avanzo displayed signs of severe rust infection (levels 4–5) in ~86% of leaves, whereas Adige was the most resistant clone revealing the lowest degree of infection only presenting traces of the fungus (level 1.5) in 11% of leaves. Lux

### Table 3. Lace bugs and rust incidence and rust severity degree in *Populus* clones in September.

<table>
<thead>
<tr>
<th></th>
<th>L. Avanzo</th>
<th>58-861</th>
<th>Lux</th>
<th>I-214</th>
<th>Adige</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>% Lace bug incidence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Individuals</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>% Leaves</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>93.3</td>
<td>0</td>
</tr>
<tr>
<td><strong>% Rust incidence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Individuals</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>% Leaves</td>
<td>100</td>
<td>86.6</td>
<td>73.3</td>
<td>93.3</td>
<td>14.1</td>
</tr>
<tr>
<td><strong>Degree of rust severity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1.5</td>
<td>25.0</td>
<td>40.3</td>
<td>44.4</td>
<td>20.8</td>
<td>11.1</td>
</tr>
<tr>
<td>2</td>
<td>23.6</td>
<td>19.4</td>
<td>15.3</td>
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<td>13.9</td>
<td>30.6</td>
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<td>4</td>
<td>45.8</td>
<td>1.4</td>
<td>2.8</td>
<td>19.4</td>
<td>0.0</td>
</tr>
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<td>5</td>
<td>40.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
showed a low degree of severity of the infection and 58-861 and I-214 were affected mildly. Melampsora rust has been reported to affect chlorophyll, water relations and photochemical processes in sensitive clones (G. Elena, J. Fernàndez-Martínez, M. Zacchini, A. Moret and I. Fleck, unpublished data). With respect to lace bugs (M. unicostata), we observed a marked presence of the insect in 58-861 and I-214 leaves (Table 3), whereas in L. Avanzo, Lux and Adige the faeces of the insect were not found. The susceptibility of L. Avanzo and 58-861 clones to infection by these pathogens could be related to increased ROS formation in September (Tiedemann 1997) and/or to their lower pool of glutathione that can play an important role in biochemical reactions against the development of the disease (Kuzniak and Sklodowska 1999).

Concluding remarks

We conclude that changes in leaf structure in Adige and Lux contributed to maintain highest RWC during summer drought. This fact, in addition to their improved photoprotective and antioxidant responses, contributed to the preservation of PSII functionality under periods of water deficit. Furthermore, these two clones showed very low susceptibility to Melampsora sp. and M. unicostata infections. The evaluation of the adaptation to environmental constraints represents a primary step for the selection of poplar clones for different uses of this multifunctional wood species. This study highlighted the suitability of Lux and Adige for cultivation in unfavourable abiotic and biotic conditions, opening up interesting prospects for their use in environmental restoration in the Mediterranean region.

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Conflict of interest

None declared.

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