A Mediterranean conifer under vegetation shift: seasonal changes of photochemical activity in *Cupressus sempervirens* (L.) and evidence of correlation with temperature models

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**Abstract:** The Italian cypress (*Cupressus sempervirens* L.) is widespread in the entire Mediterranean region and reaches in the Province of Trento high latitude (46° 21' North) and elevation (about 985 m a.s.l.). The human range expansion of this cypress has taken place at the Northern margin of the range in Italy in recent decades, driven by ornamental planting in spite of climatic constraints imposed by low temperature. The aim of the present work was to investigate the effect of low temperature in cypress in a nursery of the Autonomous Province of Trento (North-Eastern Italy). Over a period of two and half years, maximum quantum use efficiency of photosystem II (Fv/Fm) was monitored in order to study the effects of air temperature on the photosynthetic efficiency of 99 different clones and to derive information about the potential distribution of cypress in this province. A significant positive correlation (r = 0.64, p < 0.001) was found between air temperature and Fv/Fm over the 29-month period and especially in spring and autumn. Minimum values of Fv/Fm were observed in winter. Moreover, in order to assess critical environmental factors for cypress, mean values of Fv/Fm were correlated with daily air temperature and temperature-based indices to identify the driving factors of the physiological changes along the annual cycle. The maximum value of correlation (r = -0.85, p < 0.001) was found with 10-day Cold Degree-Day Running Sums before sampling and with a threshold of 2.0 °C for daily minimum air temperature.

**Keywords:** chlorophyll fluorescence, PSII quantum use efficiency (Fv/Fm), low temperature, cold tolerance, Cupressaceae, conifer planting, conifer adaptation, climate change.

**INTRODUCTION**
Together with several meteorological forcing agents, such as radiation, water and nutrient availability, air temperature can play a relevant role in temperate climates as driving factor for the vegetative cycle of plants. It controls phenological development (Schwartz, 2003), particularly bud break and leaf senescence in deciduous species. It is also involved in hardening and de-hardening processes and regulates photosynthesis and evapotranspiration (Farquhar et al., 2001). However, these processes are difficult to predict if, for the investigated species, the relationships between environmental factors and the rate at which photosynthetic capacity is impaired with the onset of winter and regained in the spring are unknown (Nippert et al., 2004).

In evergreen species, air temperature acts on both chlorophyll concentration and efficiency (Ensminger et
Italian cypress is a medium-sized evergreen tree that increasingly grows in highly hostile locations. It is expected to shift its distribution as plants cope with rapid climate change (Abrams, 2011). These changes, already observed also in the Southern Alps, are important areas of natural vegetation of the Alps and other continents like California, Australia, New Zealand, and South Africa. The Pre-Alps, the Alpine foothills and valley bottoms of the Southern slope of the Alps are important areas of natural vegetation of cypress, where it has considerable potential for further spreading in marginal areas with suitable microclimate (Zoëca et al., 2008, Baldi et al., 2012). In Trentino (Italian Alps), cypress was introduced long ago (Bagnoli et al., 2009). The species is currently present within an altitudinal range from 66 m a.s.l. (Lake Garda) up to 985 m a.s.l. in the Fiemme Valley (Fig. 1). An inventory study performed in 2004-2005 recorded about 15,000 individuals higher than 4 m and larger than 5 cm diameter at breast height (DBH). They grow as single trees or in groups scattered along ca. 1900 sites (Zocca et al., 2008), more than 90% of elevations ranging from 50 to 350 m a.s.l., with prevalent aspects S, SW and W (unpublished data). Growing as single trees, the cypress habitus itself might favor the higher aerodynamic exposure to air circulation and consequently trees experiences critically low temperatures in winter. Recently, a multidisciplinary scientific research project (ECOCYPRE) was carried out to evaluate cold tolerance of cypress and to improve planting material by assessing clonal selections (Pedron et al., 2009; Baldi et al., 2011). Cold tolerance itself can indirectly protect the tolerant clones from cypress canker disease (Seiridium cardinale) because it can avoid or limit the entry of the fungal spores represented by small bark micro-wounds due to cold stress. In fact, these cold injuries are often hardly noticeable, but they are sufficient to weaken the plant and open the way for the canker agent (La Porta et al., 2008) and numerous other parasitic diseases, which the trees could otherwise escape (Manion, 1991). The exposure to multiple stress factors would increase the vulnerability of cypress.

By assessing the photosynthetic efficiency, the use of chlorophyll fluorescence has been tested for long term monitoring of cold tolerance of several conifer species, including Norway spruce (Lundmark et al., 1998; Montagnani et al., 2004), western red cedar (Weger et al., 1993), lodgepole pine (Lundmark et al., 1988), larch and dwarf mountain pine (Montagnani et al., 2004), Scots pine (Lundmark et al., 1988; Montagnani et al., 2004; Repo et al., 2006), and Phoenician juniper and...
Aleppo pine (Martínez-Ferri et al., 2004). The ratio of variable to maximal fluorescence $F_{v}/F_{m}$ measures the proportion of open photosystem II centres and quantifies the efficiency of energy capture, generally termed the “maximum quantum yield of photosystem II” (Genty et al., 1989). Leverenz and Öquist (1987) found a significant correlation (0.91) between quantum yield and $F_{v}/F_{m}$ in Scots pine. Generally, quantum yield decreases with temperature in the proximity of 0 °C (Leverenz and Öquist, 1987), which indicates photoinhibition, a protective mechanism for dissipating excess energy. In this frame, the aim of the present work was to investigate the effect of low air temperature on $F_{v}/F_{m}$, measured in a collection of 99 selected cypress clones. Genotypes able to tolerate cold temperatures show rapid photosynthetic recovery when temperatures rise in spring (Fracheboud et al., 1999). Chlorophyll fluorescence was used to test suitable temperature-related parameters that respond conveniently to photochemical activity, and to screen the cypress population for frost tolerance. A spin-off of the investigation was the definition of a limiting thermal threshold for cypress distribution and the comparison with thermal thresholds in Trentino, where the species grows at the Northern edge of its range, due to occasionally unfavorable conditions in terms of recurrent low temperatures (Raddi and Panconesi, 1959; Zocca et al., 2008). By this work, we want to assess the best combination of time-lapse, temperature threshold and temperature record for the photochemical response of Italian cypress to cold stress. We hypothesized that i) there are differences in $F_{v}/F_{m}$ annual trends among clones and ii) $F_{v}/F_{m}$ response to temperature is effective and clone specific.

MATERIALS AND METHODS

Research area and planting material

The trees were planted in an experimental plot located about 2 km North of Riva del Garda (Trentino, North-Eastern Italian Alps; 45° 53´ 55,56 Lat. N; 10° 50´ 13,80 Long E) at 105 m a.s.l. of elevation on a deep well-drained fertile mixed soil on a flat site in the bottom of the Sarca river Valley (Fig. 1). Plant material consisted in 99 clones propagated by graft on wild cypress rootstocks. After 3 years growing in pots, ca. 1 m plants were transferred from the nursery to the experimental plot. Ten ramets for each clone were planted in randomized blocks. Each of the blocks contained one ramet of the 99 different clones. The clones represented the result of a 30 years breeding programme for Seiridium cardinale resistance carried out at the Research Institute IPP-CNR (Firenze, Italy) and kindly provided for the experiment trial.

Climatic and micrometeorological conditions

Main meteorological features for the site were derived by the Arco meteorological station located about 4 km away from the nursery, at 83 m a.s.l. The station, managed by the FEM CTT-SIG Unit, has been operating since 1983. Lake Garda and the lower reach of the Sarca River have the lowest elevation among all the South-Central Alps (69 m a.s.l.). The presence of such a large water body in the outlet of the valley into the Po Plain is at the base of regional climatic peculiarities, mainly due to the presence of a regular breeze regime, known as “Ora del Garda”. From a general point of view, the climate of the area can be classified as temperate, with no dry season (“Cfb“ according to Köppen). In spite of its supposed sub-Mediterranean characters, the climate of Riva del Garda is classified as “humid”, like all the low-elevation southern Alpine valleys. However, basing classification uniquely on temperatures and not on wind may be misleading in an area where the latter represents an outstanding climatic feature, and a more reliable framing of humidity/drought regimes should be shifted a little towards a drier class.

Temperature measurements close to the trees were carried out by two Tinytag Ultra data-loggers (Gemini Data Loggers Ltd., Chichester, UK), equipped with a shielded temperature probe. The loggers were placed at 150 cm height among the trees of the experimental plot and were programmed to record hourly temperatures. Data were averaged between the two loggers.
Collection of shoots and needles

Sampling was performed monthly in the first period of the experiment (January 2004 – September 2005) and biweekly in the second one (October 2005 – May 2006). For each clone, an amount of 20-30 g of apical shoots was collected from the South-exposed part of the crown at 1.3 m from the ground. To avoid the negative effect of recurrent cuttings, different trees of the same clone were selected in different block each time. The sampled leaves were wrapped in aluminium foil, packed and sealed in a plastic envelope, in order to avoid dehydration. All the envelopes were placed into a portable cooling box and brought to the plant physiology laboratory. The measurements were performed within two hours after cutting. The shoots were dark adapted at 20 °C for at least 1 h before the fluorescence measurements.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured at room temperature using a modulation fluorometer (PAM-2000 Portable Chlorophyll Fluorometer Walz, Effeltrich, Germany). Fully developed shoots were fastened in the leaf clip, which maintains a fixed angle and an adjustable distance from the leaf surface to the end of the optic fibre cable. This distance was kept constant.
at 5-7 mm during all measurements. The leaves were exposed to a 0.8 s saturated flash of approximately 6000 μmol m⁻² s⁻¹ to obtain the maximum fluorescence, Fₘ. The ratio between variable and maximum fluorescence, Fᵥ/Fₘ, was calculated automatically according to measured minimum and maximum fluorescence yield, F₀ and Fₘ respectively. All measurements of F₀ were performed with the measuring beam set to a frequency of 600 Hz, whereas all measurements of Fₘ were performed with saturating flash automatically switching to 20 kHz. The Fᵥ/Fₘ ratio \( \frac{F_v}{F_m} = \frac{(F_m - F_0)}{F_m} \) was used as a measure of the potential quantum yield. For each clone, chlorophyll fluorescence measurements were repeated on three shoots.

**Environmental parameters**

For the assessment of critical environmental parameters for cypress, the mean values of quantum use efficiency were correlated with the corresponding daily air temperature data and temperature-based indices in order to identify driving factors of physiological changes. Based on previous works (Bergh et al., 1998; Montagnani et al., 2004), for each sampling day (i) the values of many parameters for the day before (i-1) were retrieved or calculated from daily air minimum, maximum and average temperature (Tab. 1).

Running means were used to smooth out daily variability and to provide a clear trend line. Trials were done by varying length of the averaging period, 3 to 10 days before the sample collection, and using minimum, mean and maximum daily values of air temperature.

The number of days (n+1) of moving sum ranged from 3 to 15 while TS ranged between -5 and 25 °C. Cold Degree-Day Running Sums (CDDRS) were calculated based on Kira's coldness index (Kira, 1945; Krestov and Nakamura, 2007) using minimum, mean and maximum daily air temperature below a threshold temperature (TS).

**Statistical analysis**

Simple linear correlation analysis between air temperature-based indices (Table 1) and the mean values of Fᵥ/Fₘ of all cypress clones was performed using the software Statistica 8.0 (StatSoft Inc., Tulsa OK, USA). Pearson correlation coefficients (r) and the significance level (p) were calculated for each micrometeorological parameter.

**RESULTS**

**Climate and micrometeorology**

The average annual air temperature of the nursery area over the last 25 years was 12.7 ± 0.1 °C, a rather high value, for a site within the edge of the alpine region, and is due to the low elevation of the station (70 m a.s.l.). The coldest month is January (3.0 ± 0.1 °C) while the warmest is July (22.7 ± 0.1 °C). The pluviometric regime, indeed, cannot be thought of as a “quasi-Mediterranean” one, since neither a true dry summer nor a rainy winter occurs. On the contrary, winter is the driest season (Fig. 2), while maxima of yearly rain supply are mostly recorded in autumn (principal maximum) and in spring (secondary maximum). The average yearly precipitation amount is about 830 mm.

The main micrometeorological parameters were similar during the two years of observation (Fig. 3). In 2004 and 2005 the mean value of air temperature was 13.1 °C and 12.6 °C, respectively, absolute minimum air temperature -5.4 °C and -8.9 °C, absolute maximum air temperature 36.5 °C and 33.2 °C. Yearly precipitation amount reached 980 and 845 mm, respectively.

**Chlorophyll fluorescence**

Fᵥ/Fₘ showed a typical seasonal variation with a maximum in summer and minimum values in winter for all 99 clones (Fig. 4). The lowest mean values of Fᵥ/Fₘ occurred in February 2005. Cluster analysis failed to recognize significant well-separated groups. Clone 1 and 31 showed the largest Euclidean distance (Fig. 4). On the contrary, interannual variability was high.

**Correlations between environmental parameters and Fᵥ/Fₘ values**

Based on the air temperature data collected at the nursery, the daily values of the micrometeorological parameters and indices (Tab. 1) were calculated and

**Fig. 2 - Bagnouls-Gaussen's diagram representing both mean monthly temperature and precipitation in conventional scales, where 1 °C corresponds to 2 mm of rainfall (data from the meteorological station of Arco; time series 1983-2008).**

**Fig. 2 - Diagramma di Bagnouls-Gaussen che rappresenta la temperatura media mensile e le precipitazioni in scala convenzionale, dove 1 °C corrisponde a 2 mm di precipitazione (dati della stazione meteorologica di Arco; periodo 1983-2008).**
calculation of cooling degrees from the maximum (A), medium (B) and minimum (C) air temperature. Most of the correlations between air-temperature based indices and the mean values of $F_V/F_M$ proved to be highly significant ($p<0.001$; Tab. 2). Only the relationship between the 3-day running mean of maximum daily air temperature ($T_{\text{max}}$RM3) and $F_V/F_M$ showed higher p value (0.038), but still significant ($p<0.05$). The highest correlation ($r = -0.852; p<0.001$) was found between ($F_V/F_M$) and 10-day cold-degree-

used to correlate the $F_V/F_M$ data and to estimate a threshold temperature for cypress photochemical activity.

Fig. 5 shows the trend of the Pearson correlation coefficient performances of micrometeorological parameters and the mean values of $F_V/F_M$ of the cypress clones, with changes in temperature threshold for the calculation of cooling degrees from the maximum (A), medium (B) and minimum (C) air temperature. Most of the correlations between air-temperature based indices and the mean values of $F_V/F_M$ proved to be highly significant ($p<0.001$; Tab. 2). Only the relationship between the 3-day running mean of maximum daily air temperature ($T_{\text{max}}$RM3) and $F_V/F_M$ showed higher p value (0.038), but still significant ($p<0.05$). The highest correlation ($r = -0.852; p<0.001$) was found between ($F_V/F_M$) and 10-day cold-degree-

Fig. 5 - Correlation performances with changes in temperature threshold for the calculation of cooling degrees from the maximum (A), medium (B) and minimum (C) air temperature. Fig. 5 - Risultati di correlazione con valori variabili delle soglie di temperatura per il calcolo dei gradi di freddo con le temperature massime (A), medie (B) e minime (C).
Night frosts in the early autumn resulted in minor and temporary declines in FV/FM measured in cypress clones grown in Trentino. However, by early November a permanent decrease in FV/FM occurred, correlated to a decline in mean air temperatures. This pattern was consistent for all clones. A similar reduction in photosynthetic capacity during winter has been observed in many other conifer species, including white spruce (Binder and Fielder, 1996), Norway spruce (Lundmark et al., 1988; Bolhàr-Nordenkampf and Öquist, 1993; Adams and Perkins, 1993; Westin et al., 1995; Weng et al., 2005), red spruce (Lawson et al., 2000), black spruce (Gaumont-Guay et al., 2003), Douglas fir (Rose and Haase, 2002; Nippert et al., 2004), western redcedar (Weger et al., 1993; Nippert et al., 2004), and Scots pine (Porcar-Castell et al., 2008; Robakowski and Wyka, 2009), Engelmann spruce (Nippert et al., 2004), grand fir (Nippert et al., 2004), ponderosa pine (Nippert et al., 2004) and lodgepole pine (Nippert et al., 2004).

Previous meteorological data were necessary to predict current photosynthetic capacity of cypress in the wintertime, the maximum correlation being observed with the cold-degree-days sum of minimum air temperature (TminCDDRS) with a threshold value (TS) of 2 °C (Fig. 6) with a mean air temperature the strongest correlation was found at 12 °C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pearson coefficient</th>
<th>p</th>
<th>Parameter</th>
<th>Pearson coefficient</th>
<th>p</th>
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<tbody>
<tr>
<td>Tmin</td>
<td>0.675</td>
<td>&lt;0.001</td>
<td>TminCDDRS, TS=2°C</td>
<td>-0.836</td>
<td>&lt;0.001</td>
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<tr>
<td>Tmean</td>
<td>0.640</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=10°C</td>
<td>-0.852</td>
<td>&lt;0.001</td>
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<tr>
<td>Tmax</td>
<td>0.587</td>
<td>&lt;0.001</td>
<td>TmaxCDDRS, TS=2°C</td>
<td>-0.824</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TminRM3</td>
<td>0.636</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.738</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TmeanRM3</td>
<td>0.670</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.764</td>
<td>&lt;0.001</td>
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<tr>
<td>TminRM7</td>
<td>0.686</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=15°C</td>
<td>-0.805</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TmeanRM7</td>
<td>0.673</td>
<td>&lt;0.001</td>
<td>TminCDDRS, TS=12°C</td>
<td>-0.778</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TminRM10</td>
<td>0.679</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.788</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TmeanRM10</td>
<td>0.670</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.774</td>
<td>&lt;0.001</td>
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<tr>
<td>TmeanRM15</td>
<td>0.659</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.773</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TmeanRM10</td>
<td>0.670</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=12°C</td>
<td>-0.774</td>
<td>&lt;0.001</td>
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<tr>
<td>TmeanCDDRS, TS=2°C</td>
<td>-0.762</td>
<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=20°C</td>
<td>-0.679</td>
<td>&lt;0.001</td>
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<td>TmeanCDDRS, TS=5°C</td>
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<td>&lt;0.001</td>
<td>TmeanCDDRS, TS=20°C</td>
<td>-0.690</td>
<td>&lt;0.001</td>
</tr>
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</table>

Tab. 2 - Pearson correlation coefficients (r) and significance level (p) of the correlations between the reported micrometeorological parameters and the mean values of FV/FM of the cypress clones. The best performing model (with minimum, mean, and maximum temperature) is highlighted in bold characters.

Tab. 2 - Coefficienti di correlazione di Pearson (r) e livello di significatività (p) delle correlazioni tra i parametri micrometeorologici riportati e i valori medi di FV/FM per i danni di cipresso. Il modello migliore (con le temperature minime, medie e massime) è evidenziato in neretto.

DISCUSSION

Night frosts in the early autumn resulted in minor and temporary declines in FV/FM measured in cypress clones grown in Trentino. However, by early November a permanent decrease in FV/FM occurred, correlated to a decline in mean air temperatures. This pattern was consistent for all clones. A similar reduction in photosynthetic capacity during winter has been observed in many other conifer species, including white spruce (Binder and Fielder, 1996), Norway spruce (Lundmark et al., 1988; Bolhàr-Nordenkampf and Öquist, 1993; Adams and Perkins, 1993; Westin et al., 1995; Weng et al., 2005), red spruce (Lawson et al., 2000), black spruce (Gaumont-Guay et al., 2003), Douglas fir (Rose and Haase, 2002; Nippert et al., 2004), western redcedar (Weger et al., 1993; Nippert et al., 2004), and Scots pine (Porcar-Castell et al., 2008; Robakowski and Wyka, 2009), Engelmann spruce (Nippert et al., 2004), grand fir (Nippert et al., 2004), ponderosa pine (Nippert et al., 2004) and lodgepole pine (Nippert et al., 2004). Previous meteorological data were necessary to predict current photosynthetic capacity of cypress in the wintertime, the maximum correlation being observed with the cold-degree-days sum of minimum air temperature.
air temperature below 2 °C. Schaberg et al. (1995) reported the highest correlation between winter net photosynthesis in red spruce and maximum daily temperature occurring 4–6 days before measurement, and Lawson et al. (2000) found the patterns of winter Fv/Fm measurements of the same species to be significantly correlated with current air temperatures over the preceding 72 h. Tanja et al. (2003) found that photosynthetic rates estimated from eddy flux data were best correlated with a 5-day running mean temperature in boreal forests of Finland, Sweden and central Siberia (Russia). Medlyn et al. (2002) reported the strongest correlation between the maximum rate of carboxylation and the potential rate of electron transport in maritime pine to mean daily minimum temperature over the previous 30 days. Nippert et al. (2004) found similar delays in the response of photosynthetic capacity to changing ambient temperature, but the nature of the relationship and length of the delay differed among several conifer species in northern Idaho (USA). Mechanisms controlling photosynthetic machinery during winter are slow to damage or repair and vary among species. Clones differed in susceptibility to winter photoinhibition, which was probably related to their specific stress-tolerance mechanisms and different light requirements (Baldi et al., 2012). Winter photoinhibition in cypress was not associated with permanent damage to photosynthetic machinery, because recovery of Fv/Fm was observed with increasing air temperature in all clones. The absence of persistent winter damage was further verified during consecutive growing seasons. Presumably the long-lived “needles” of this species (functioning for several years) experience recurrent slight winter photoinhibition, triggering acclimation mechanisms, which makes their spring recovery possible each year. It may be hypothesized that the low-temperature photoinhibition observed in the present study played a photoprotective role (Somersalo and Krause, 1990; Robakowski, 2005).

In situ experiments would have probably revealed less between-clone variation as evidenced by the present common garden experiment, because of environmental determinism due to clone-site interaction. Indeed, while differences in phenological traits observed in situ reflect both environmental and genetic variations, common garden experiments are related to genetic variation only. Higher exposure to air of scattered trees in the field would make them experience critically low temperatures. Nevertheless, the observed intraclonal variability of the studied functional trait indicates substantial genetic diversity within genotypes, which could potentially facilitate rapid adaptation to changing environmental conditions. Local adaptations, however, influence phenotypic variability in situ.

Winter photosynthetic rates were not measured directly in this study. However, the relatively smaller reduction in Fv/Fm in several clones in winter compared with the others may be explained by a relatively higher winter photosynthetic capacity, which provides a sufficient sink for absorbed solar energy, thereby preventing photo-oxidative stress. Throughout the winter, from November until the beginning of April, photochemical activity was relatively low and stable with Fv/Fm values ranging from values below 0.3 to above 0.6, depending on the clone. Light-demanding pioneer species are generally less susceptible to photoinhibition than shade-tolerant climax species (Krause et al., 2001). In cypress, susceptibility to photoinhibition in the winter and early spring depended on prevailing air temperature and genotype more than light (Baldi et al., 2012), occurring at temperatures well above the lethal minimum temperature (Öquist and Huner, 2003).

In winter, photosynthesis can eventually be constrained by photoinhibition (Öquist and Huner, 2003), desiccation due to extracellular freezing (Neuern and Fransohler, 2006), xylem blockage due to freezing of the stem, roots or soils (Pittermann et al., 2010), and inhibition of the dark reactions of photosynthesis (Adams and Perkins, 2004), acting independently or in combination as a correlated set of responses through the acclimation process (Öquist and Huner, 2003). Each would have different controls and different lag times, and each would have different effects on Fv/Fm. Therefore empirical descriptions of the temperature–response curves may offer clues as to which are most important in a given system.

Although the weather during winter was occasionally mild period, recovery of the photochemical activity to high values was not observed in any of the clones. Because of the time interval between measurements during winter, it is possible that the actual variation in Fv/Fm was larger than reported here. Extreme events such as frost, with temperature lower than -10 °C, were recorded at the nursery, but consecutive frost days were infrequent and the effect of these episodes on the photochemical activity of cypress was not completely investigated. Most conifers tolerate much colder temperatures than the night-time minima reported here (Strand and Lundmark, 1987), and it is unlikely that such episodes act as the major control agent in acclimated vegetation (Strand et al., 2002), and in orchard conditions. However, if dehydration due to extracellular freezing were the mechanism of loss, then Tmin would better predict Fv/Fm than Tmax.
because the freezing would be unaffected by light and would proceed furthest at the lowest temperatures reached. Moreover, because the initial cytoplasmic freezing would be rapid and the recovery from freezing damage would probably be slow (Schwarz et al., 1997), we might be unable to distinguish between photoinhibition and extracellular freezing as potential mechanisms leading to the loss of photosynthetic capacity (Nippert et al., 2004).

The spring recovery in $F_v/F_m$ values was rapid in all clones and occurred when air temperatures increased in April-May, which is in accordance with recovery studies in Scots pine (Ottander and Öquist 1991) and Norway spruce (Westin et al., 1995). In springtime, the reversal of photoinhibition may be slow at low temperatures (Lamontagne et al., 1998), leading to long lag times, when bright light and low temperature combine their effects during daytime (presumably in association with $T_{max}$).

Trees cannot sustain water losses due to transpiration if their stems or roots (or soils) are frozen. Therefore, xylem (and soil) freezing may be a major control over winter photosynthetic processes (Schwarz et al., 1997; Jarvis and Linder, 2000). Although we cannot address this question directly with our data, and a direct correlation between xylem freezing and changes in fluorescence has yet to be documented, the cold-degree-days sum of minimum air temperature below 2 °C represented the best correlation with $F_v/F_m$. The rate of dark reactions of photosynthesis are strongly dependent on temperature, especially as temperature approaches 0 °C (Strand et al., 2002; Gaumont-Guay et al., 2003), and control photosynthetic rates during the wintertime. Dark-acclimated chlorophyll fluorescence measures a photochemical trait, though this effect could still be detected if induced by feedback inhibition (Öquist and Huner, 2003). While all these four mechanisms have the potential to limit wintertime photosynthesis, our results suggest that photoinhibition, and perhaps xylem freezing, are the most probable candidates.

The extent to which cypress clones are able to adapt to environmental changes will depend on the level and distribution of genetic variation between genotypes and phenotypic plasticity, other than on seed dispersal and establishment rate. Our results show that, despite gene flow between clones due to their proximity, genotypes may nonetheless display genetic differentiation for functional traits due to diversifying selection along temperature gradients. As large within-genotype differentiation is possible for functional traits, due to selection occurring during past natural warming, we suspect that substantial adaptive evolutionary changes are likely to occur in response to current climate change. The migration of cypress will be facilitated by the extensive genetic diversity within populations and phenotypic plasticity. However, the lack of systematic, long-term monitoring of species distribution, morphology, physiology and other critical responses of Mediterranean species is hampering our ability to predict the impact of climate change on biodiversity and productivity in these changing landscapes.

The duration of seasonal growth influences the annual estimates of conifer productivity (Bergh et al., 1998). To reflect annual production accurately, a more detailed understanding of seasonal growth and temperature response is needed (Leverenz and Öquist, 1987; Medlyn et al., 2002). Leverenz and Öquist (1987) reported seasonal differences in the response of quantum yield to varying temperature that make it difficult to define maximum quantum yields based on temperature alone. Predictions may be improved with accounts of cold acclimation and winter differences in photo inhibition (Gaumont-Guay et al., 2003), direct reduction in photosynthesis (Linder and Flower-Ellis, 1992; McMurtrie et al., 1994; Bergh et al., 1998), variation in soil temperatures (Bergh and Linder, 1999), and seasonal differences in photosynthetic recovery (Linder and Flower-Ellis, 1992; McMurtrie et al., 1994; Bergh et al., 1998; Lundmark et al., 1998; Medlyn et al., 2002; Zunzunegui et al., 2011). The model describing the best correlation between environmental parameters and $F_v/F_m$ illustrates the complexity of this system in a Mediterranean environment even during the wintertime. However, fluorescence measurements provide a quick and reliable method to describe this complication for future analyses and model predictions (Nippert et al., 2004), particularly in screening studies in nursery conditions.

This study at the nursery scale aimed at providing insight into the specific factors determining the patterns of species distribution associated with air temperature gradients. We observed similar trends for 99 clones from distant sites, indicating that local drivers of functional leaf traits may be consistent at the regional scale. Nevertheless, cypress is distributed across all the latitudinal range of the Mediterranean Basin and can display substantial population differences, as observed in other conifers (Zhang and Marshall, 1994; 1995).

As global warming impacts are predicted to increase in the next decades, it is likely that the cypress will expand its range in the Alps. Under this perspective, a much wider spread of cypress due to human plantations is also expected and the availability of adapted genotypes will be a key issue in the future use of cypress as ornamental tree at the Northern margin of its range.
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