Effect of Extreme Temperature on Quantum Yield of Fluorescence and Membrane Leakage of the Canarian Endemic Pine (*Pinus canariensis*)

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Z. Naturforsch. **54c**, 681–687 (1999); received December 10, 1998/February 20, 1999

Pinus canariensis, Low Temperature, High Temperature, Altitudinal Gradient, Chlorophyll Fluorescence, Electrolyte Leakage

The effect of extreme temperature on the quantum yield of fluorescence and membrane leakage of *Pinus canariensis* growing in 5 stands at different altitudes and orientation in Tenerife (Canary Islands, Spain) was determined. Needles were collected from the field and transferred to the laboratory where they were kept in a closed chamber with water-saturated air overnight. Then they were exposed for 30 minutes in plastic bags in a water bath at temperature treatments with steps of 2K between 56 °C and –24 °C and the effect was determined immediately and 24 hours after the treatment by chlorophyll fluorescence and electrolyte leakage.

Needles presented incipient damage at temperatures ranging from –5 to –10 °C depending on the altitude and orientation of the stand. The results were more evident when the measurements were done 24 hours after the cold treatment and values were consistent with the electrolyte leakage results. Different resistance to high temperature depending on the altitude and orientation was also found, varying the temperature for incipient damage from 42 to 44 °C detected with the fluorescence parameters but not with the leakage of electrolytes which was not found until 50 °C. The amplitude of thermal limits for photosynthetic efficiency alteration in needles of *P. canariensis* was relatively narrow and similar to that of evergreen Canarian laurel forest trees.

Introduction

Heat and cold, depending on their intensity and duration, impair the metabolic activity, growth and viability of plants and thus set limits to the distribution of the species (Larcher, 1995). Disturbances on the photosynthetic apparatus are among the first indicators of extreme temperature damage, as well as changes in the physicochemical state of membranes (Steffen *et al.*; 1989, Hällgren and Öquist, 1990; Larcher, 1995).

Pinus canariensis is an endemic plant of the Canary Archipelago whose natural distribution area at present is restricted to the highest islands. It has been relegated to its current habitat by climate changes in the past (as was pointed out, based on fossils found in Miocene and Pliocene deposits in the western Mediterranean region by some authors, and summarised by Bramwell (1976), Klaus (1988), Climent *et al.* (1996)). Therefore the knowledge of high and low temperature effect on its physiology is of considerable interest, since it could be one of the causes of its confinement to the Islands where the climate is milder than that of the Mediterranean region.

The aim of this study is to determine the effect of extreme temperature on the photosynthetic apparatus of *Pinus canariensis* growing at different altitudes and orientation in Tenerife, evaluated by the measurement of the maximum quantum yield (according to Kitajima and Butler, 1975), combined with measurements of electrolyte leakage as indicator of membrane damage (Burr *et al.*, 1990), and by observing visible symptoms (Larcher, 1990), following a previous work line in our department (Larcher *et al.*, 1991; González-Rodríguez, 1998).

Materials and Methods

The study was carried out with *Pinus canariensis* Chr. Sm. ex DC. in Buch, growing at 5 stands situated at 550, 850, 1500 and 1950 m altitude facing South (Sites I_S, II_S, III_S, and IV_S) and 1900 m facing North (Site V_N) in Tenerife, Canary Islands,

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Spain, covering the natural distribution area and where other ecophysiologycal studies are being done (Jiménez *et al.*, 1997; Tausz *et al.*, 1997, 1998).

During the Autumn, one year old needles were collected from five pines at each stand and transferred to the laboratory, where they were kept in a closed chamber with water saturated air overnight before exposing them, for 30 minutes in plastic bags in a dark water bath, at temperature treatments with steps of 2K between 56 °C and –24 °C, except for the range from 35 °C to 0 °C where the steps were of 5K. The effects were monitored immediately and 24 hours after the treatments by chlorophyll fluorescence, electrolyte leakage and observing visible symptoms. The temperature treatments duration was of 30 minutes as was used in other similar experiments (Sakai and Larcher, 1987; Lösch 1980). We took three samples for every identical temperature treatment and three new ones for the following treatment.

Chlorophyll fluorescence was measured with a portable fluorometer (Plant Efficiency Analyser, PEA, Hansatech, U. K.) before (control values), after the temperature treatments (moment values) and after 24 hours of being left in a dark closed chamber with water saturated air (recuperation values). When measured the needles were always dark adapted since they were in darkness during the complete experiment. Then, similar portions of approximately 15 mm from the middle section of the needles were put in 3 ml distilled water for 24 hours and then the electrolyte leakage was measured (C1) with a portable conductivimeter (Dist 4, Hanna Instruments, Mauritius). Later, the same sections were treated with liquid nitrogen for a total destruction of cell membrane integrity, and stored for 24 hours in a freezer, and then the conductivity was measured again (C2). We also measured C1 and C2 in control untreated needle sections (C′1 and C′2, respectively). The results were expressed with the index of injury (I_t%) (Flint *et al.*, 1967):

$$I_t = (Rm\text{-}Rc)/(1\text{-}Rc) * 100,$$

where Rm = C1/(C1+C2) and Rc = C′1/(C′1+C′2). I_t will be near “0” in undamaged tissues and near “100” in totally damaged ones (Read and Hill, 1988). The range of absolute values for the conductivity were from 0.00 to 0.12 mmho mm^{-1}.

Values of chlorophyll fluorescence and electrolyte leakage were plotted against the corresponding temperatures of each treatment. From these graphics we derived the temperature of incipient damage (LT10) and the temperature of irreversible damage (LT50) as the temperatures at which a deviation of 10 and 50% from normal values (i.e. those obtained at 10 to 25 °C) was produced respectively.

Results and Discussion

Potential efficiency of PSII measured as the rate of variable to maximal fluorescence (Fv/Fm) decreased with the decrease of temperature (Fig. 1). This decrease was more evident in the measurements done 24 h after the temperature treatment. In those made just after the treatment, a clear decrease was not seen (the leaves were still frozen). The temperature of incipient damage (LT10) ranged from –6 °C at the lower site to –9 and –10 °C at the higher ones. This parameter attained similar values when it was evaluated in base of chlorophyll fluorescence and electrolyte leakage (Fig. 3), although irreversible damage (LT50) was detected first with the chlorophyll fluorescence measurements (Table I), similar findings were reported earlier by other authors (e.g. Szalai *et al.*, 1996).

In many species the first symptom produced by low temperature is damage in the plasma membrane and as a result, changes in the cellular environment which affect the physiology of organelles such as mitochondria and chloroplasts (Senser and Beck, 1979; Krause *et al.*, 1988, Steffen *et al.*, 1989). In *Pinus canariensis*, PSII activity was affected at the same temperature as the plasma membrane started to be damaged, indicating the great susceptibility of photosynthetic apparatus to this type of stress. Similar results were obtained with Canarian laurel forest species (González-Rodríguez, 1998) but not in some others species in which the PSII started to be affected (LT10) at temperatures where irreversible damage (LT50) was produced in the membranes (Clement and van Hasselt, 1996).

With the increase of temperature, the Fv/Fm values decreased and the decrease was larger in the measurements done just after the temperature treatment, this indicated that some alterations

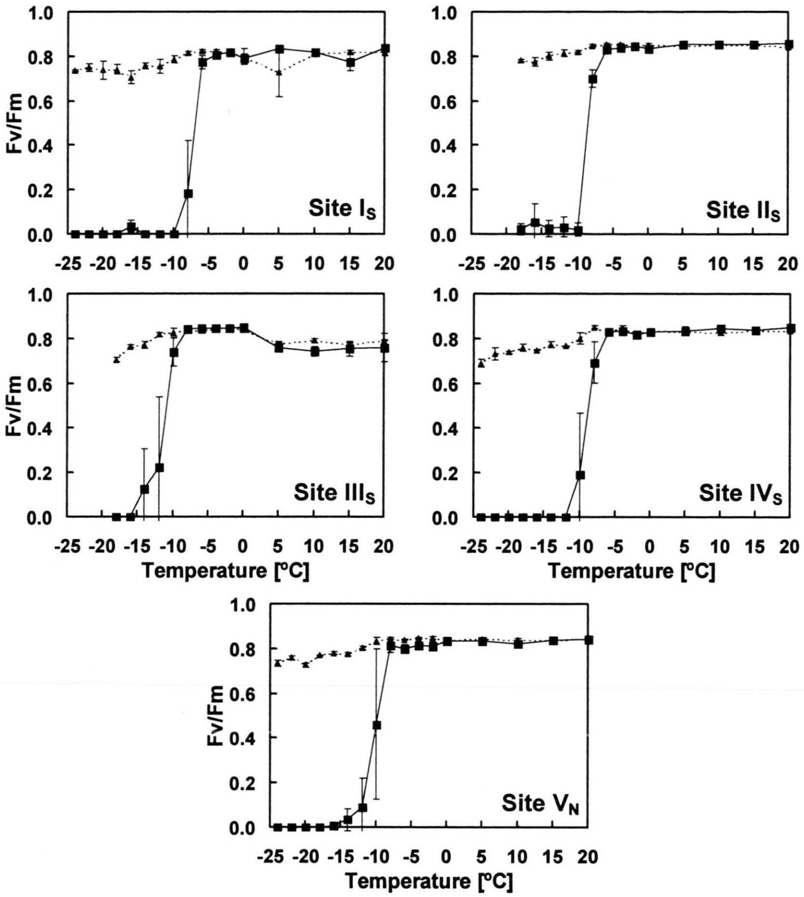

Fig. 1. Changes in the variable to maximum fluorescence ratio (Fv/Fm) in *Pinus canariensis* needles with the decrease of temperature, at the five experimental sites. The points represent the mean of three measurements monitored at the moment (-▲-) and 24 hours after the temperature treatments (-■-), with their standard deviations.

Table I. Values of LT10 and LT50 (temperatures at which the damage produces a deviation of 10 and 50% of the normal values, respectively) based on fluorescence after 24 hours of the treatment (Fv/Fm) and electrolyte leakage (EL) in needles of pines living at different sites and orientations.

	Low temperatures				**High temperatures**			
	LT10		LT50		LT10		LT50	
	Fv/Fm	**EL**	**Fv/Fm**	**EL**	**Fv/Fm**	**EL**	**Fv/Fm**	**EL**
Site I_S	−7	−5	−8	−9	44	–	52	–
Site II_S	−8	−6	−9	−9	44	53	48	56
Site III_S	−10	−10	−11	−14	44	51	46	54
Site IV_S	−8	−8	−9	−11	42	50	47	53
Site V_N	−9	−9	−10	−11	44	53	50	54

produced at the moment could disappear after 24h, so that, we considered the latter values for the evaluation of damage, since they indicated more permanent damage (Fig. 2). Incipient damage (LT10) produced by high temperatures was found at 42 °C in most elevated sites (IV_S and V_N), while in the other sites it was found at 44 °C. In these high temperature treatments the damage was de-

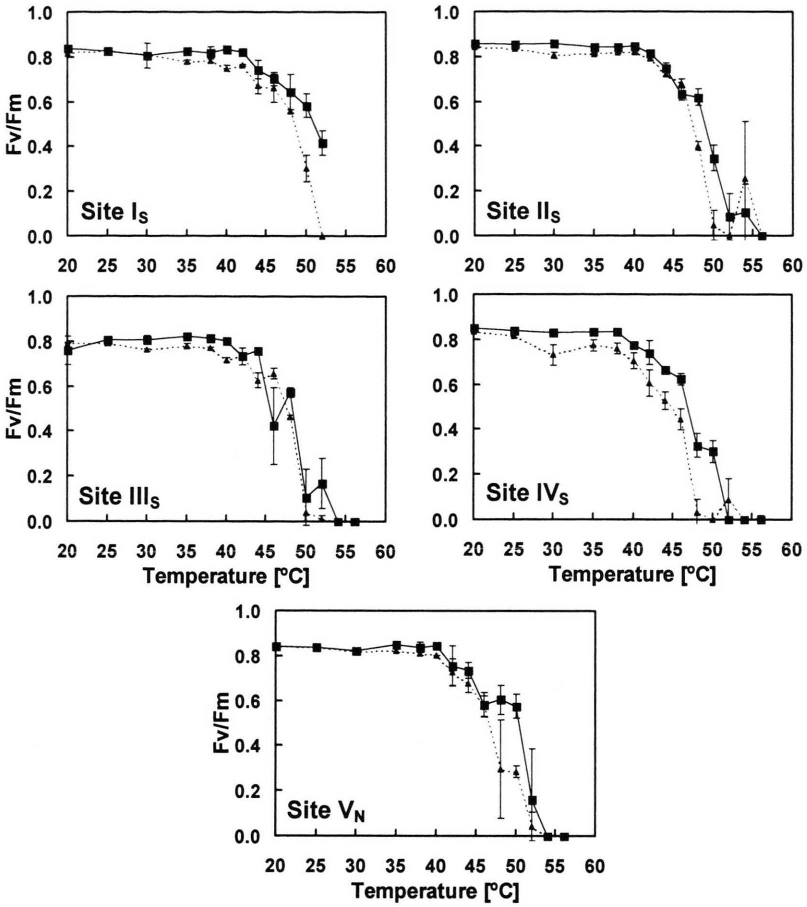

Fig. 2. Changes in the variable to maximum fluorescence ratio (Fv/Fm) in *Pinus canariensis* needles with the increase of temperature, at the five experimental sites. The points represent the mean of three measurements monitored at the moment (-▲-) and 24 hours after the temperature treatments (-■-), with their standard deviations.

tected first with the chlorophyll fluorescence measurements while the leakage of electrolytes took place always at much higher temperatures (Fig. 3 and Table I). Even irreversible damage (LT50) detected by chlorophyll fluorescence could be seen when still no damage was detected in the membranes. It is known that photosynthetic membranes are much more sensitive to high temperatures than other cell membranes, and the first affected site is the PS II (Yamashita and Butler, 1968; Krause and Santarius, 1975; Berry and Raison, 1981) therefore many authors consider changes in chlorophyll fluorescence as a sensitive indicator for high temperature damage to the photosynthetic apparatus (Renger and Schreiber, 1986; Havaux *et al.,* 1991; Krause and Weis, 1991; Larcher, 1994).

While in broad-leaved species the damage can be seen by observing visible symptoms at similar temperatures as is detected through fluorescence parameters (Larcher *et al.,* 1991; González-Rodríguez, 1998), it has never been found in the needles of *Pinus canariensis* at the range of temperatures used in our study. Therefore, this was not a good method to use on this species.

From our results we can deduce that the photosynthetic apparatus of *Pinus canariensis* is chilling resistant but freezing and high temperature sensitive. The temperature at which the photosynthetic apparatus started to be affected varied depending of the stand altitude. Needles of pines living in higher places, where the temperature during winter can attain values below zero and sometimes snow is present, are more sensitive to higher tem-

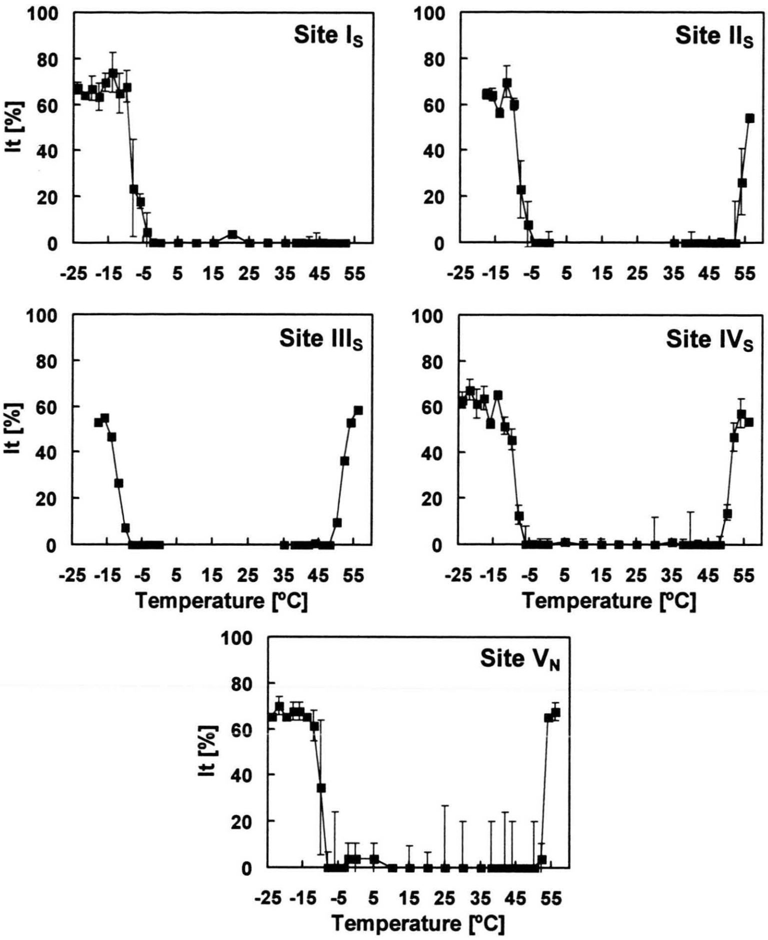

Fig. 3. Changes in the electrolyte leakage in *Pinus canariensis* needles with extreme temperatures at the five experimental sites. The points represent the mean of three measurements, with their standard deviations.

peratures (42 °C) and less to low temperatures (−8 to −10 °C). The opposite feature was found in needles of pines living in the much warmer lower places (44 °C and −5 to −7 °C). It is well known that high and low temperature resistance can change along the time, that is, a hardening process occurs based on phenotypic adjustments to temporary changes in temperature known as acclimation (Sakai and Larcher, 1987; Hällgren and Öquist, 1990; Larcher, 1995). One year old needles of *P. canariensis* showed acclimation to the specific environment where they were developed, in this way the extreme temperature resistance was slightly different depending on the altitudinal gradient.

Obviously changes along the year would be expected also due to seasonal acclimation. The potential frost resistance of conifer leaves has been studied deeply by Sakai (1983), Sakai and Larcher (1987), they showed a range for cold hardiness temperature for different *Pine* species, which go from −15 °C in *P. patula* in coastal regions with mild winter, to −70 °C in *P. banskiana* from regions with very cold winters. It is evident that although our measurements were done in Autumn when a strong hardiness process had still not taken place, the values of frost resistance were in a lower region than that found for *Pinus* species.

The amplitude in the thermal limits for the decrease of photosynthetic efficiency in needles of

P. canriensis was similar to that of the evergreen Canarian laurel forest trees determined in the same way in our laboratory (González-Rodríguez,1998). The threshold temperature for cold injury evaluated by chlorophyll fluorescence (LT50, −7 to −11 °C) was in the range found in other evergreen woody subtropical species but for heat injury (LT50, 46 to 52 °C) it was lower and more similar to evergreen woody plants of coastal regions with mild winter in temperate zone (ranges of −8 to −12 °C and 46 to 50 °C respectively in data, from different authors, shown by Larcher, 1995). Therefore the amplitude was relatively narrow and similar to that of Canarian laurel forest species (Larcher *et al.,* 1991; González-Rodríguez, 1998) also relic of the Terciary Mediterranean flora and now confined to this region too. This could be one of the causes of the small distribution area of this species, although many other aspects of its physiology could be responsible for that. Many studies considering these aspects should be done to better understand the ecophysiology of this beautiful and interesting tree.

Acknowledgements

We want to thank Agueda M^a González-Rodríguez, M^a Inmaculada Rodríguez-Jiménez and M^a del Carmen González Silgo for their kind help during the experiments. Also we express our gratitude to the "Consejería de Educación, Cultura y Deportes" of the Canarian Government for the concession of a grant to Juliane Peters which has allowed the realisation of this work. Thanks to Neil Abrey for his help with the English style.

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