

Gas Exchange of Irrigated and Non-Irrigated *Pinus canariensis* Seedlings Growing Outdoors in La Laguna, Canary Islands, Spain

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Chlorophyll fluorescence, gas exchange, water potential and relative water content were measured in the needles of five year old seedlings of *Pinus canariensis* in order to know their response to mild water stress. Two trial plots of ten plants per plot, of similar age and characteristics were irrigated daily until the experiment was undertaken, then one of the plots was left without watering while the other one was irrigated as before.

After a week of treatment, the water potential at midday did not change in any of the irrigated or non-irrigated plants maintaining around -0.4 MPa, and the relative water content changed from 93% in irrigated to 84% in non-irrigated seedlings. The stomatal conductance decreased 60% in non irrigated plants and as a result CO_2 assimilation decreased by 50% and transpiration was reduced at a higher proportion (70%), indicating a good control against water loss, before any change in water status in the needles could be observed suggesting an isohydric water economy in this plant.

Introduction

Water supply is a very important factor limiting plant growth in most soils. Inhibitions in plant growth rate, stomatal conductance and leaf photosynthesis as a result of soil drying are commonly observed (e.g. Bradford and Hsiao, 1982; Kaiser, 1987; Davies and Zhang, 1991). Regulation of stomatal aperture to restrict damage to the tissues as a result of dehydration is of major importance for plants. Stomatal closure is one of the first lines of defence against desiccation since it is a quicker and more flexible process than other alternatives which are better suited for long-term adaptation. (Chaves, 1991).

The endemic Canary pine (*Pinus canariensis*) can be seen growing in very wet places oriented to the North with a great supply of water, and also in very dry and rocky places where the amount of water is very low. At present a great reforestation campaign is being carried out in the Tenerife mountains with this pine, so it would be of great interest to know the control mechanism of water stress held by the small seedlings of this tree.

The aim of this study is to understand the response of *Pinus canariensis* seedlings to mild water

stress, by measuring gas exchange, chlorophyll fluorescence and water status of this species. In spite of its scientific and economic interest, studies about its physiology are very scarce.

Materials and Methods

The experiment was performed in seedling of *Pinus canariensis* Chr. Sm. ex DC in Buch, growing in pots outdoors (in La Laguna, Tenerife, Canary Islands, Spain). Two trial plots of ten plants per plot, of similar age (about five years old) and characteristics were irrigated daily until the experiment was undertaken, then one of the plots was left without watering while the other one was irrigated as before. After a week of treatment the following measurements were taken on two consecutive days:

Gas exchange with a portable Infrared Gas Analyser (LCA4, ADC Analytical Development Company, LTD, U. K.) using ten needles at a time, considering the needle projected area as a rectangle given by the length of the chamber, and approximately 1 mm width. Gas exchange rates were calculated using equations of von Caemmerer and



Farquhar (1981). Measurements were taken during the day in different needles of every plant, changing from irrigated to non-irrigated ones.

Chlorophyll fluorescence with a portable fluorometer (Plant Efficiency Analyser, PEA, Hansatech, U. K.) after 30 minutes dark adaptation, using specially designed clips attached to needles, several times a day in every seedling.

Water potential at midday with a pressure chamber (PMS Instrument Company, 2750 N. W. Royal Oaks Drive, Corvallis, Oregon, USA) on individual fascicles (having three needles per fascicle).

Relative water content at midday at the same time as the water potential :

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{SFW} - \text{DW}) \times 100$$

where RWC is relative water content, FW is fresh weight, DW is dry weight and SFW is saturated fresh weight of the needles.

Results and Discussion

After a week of treatment, water potential at midday was similar in irrigated and non-irrigated seedlings, maintaining around -0.40 MPa and relative water content changed from 93% in irrigated to 84% in non-irrigated pine seedlings (Table I.).

Under these conditions, gas exchange measurements were done on two consecutive days, one was clear but the second one very variable with short and frequent intervals of clouds. This and the fact that we used different needles and different plants for each measurement, gave us very variable values. Therefore, for the evaluation we plotted all data of each parameter against every variable and lines were set to the upper surface of data clouds; the points which lay on the surface reflected maximal values of that parameter when other variables were not limiting. These type of results are very common for field measurements and they have been evaluated in this way among other authors

Table I. Relative water content (RWC) and water potential (Ψ_w) at midday in needles of irrigated and non-irrigated seedlings of *Pinus canariensis*. Values are the mean of ten measurements with their standard deviation.

	RWC [%]	Ψ_w [MPa]
Irrigated	93 ± 1.8	-0.40 ± 0.11
Non-irrigated	84 ± 3.0	-0.44 ± 0.22

by Masarovicová and Eliás (1986), Cheeseman *et al.* (1991), Cheeseman and Lexa (1996).

On this way, stomatal conductance (g_s) of well irrigated seedlings presented the normal light response curve, attaining the saturation at the maximum value of $130 \text{ mmol m}^{-2} \text{ s}^{-1}$. Non-irrigated seedlings showed maximum values as low as $50 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Fig. 1), and were not able to maintain this value at high levels of light since at those levels, the VPD was also high and this produced a large decrease of g_s (Fig. 2). In well irrigated plants g_s also decreased at high values of VPD but the decrease was milder. With these differences in g_s the values of transpiration were also very different in well irrigated and non-irrigated plants. The former ones showed values of transpiration as high as $3.7 \text{ mmol m}^{-2} \text{ s}^{-1}$ depending on the evaporative demand and only when the VPD values were so high as to produce some stomatal closing, the values of transpiration decreased a little. As the stomata were partially closed in non-irrigated plants, they regulated the transpiration attaining the maximum value of $1 \text{ mmol m}^{-2} \text{ s}^{-1}$ (Fig. 3).

The light response curve of net photosynthesis (P_n) was very similar in both control and non-irri-

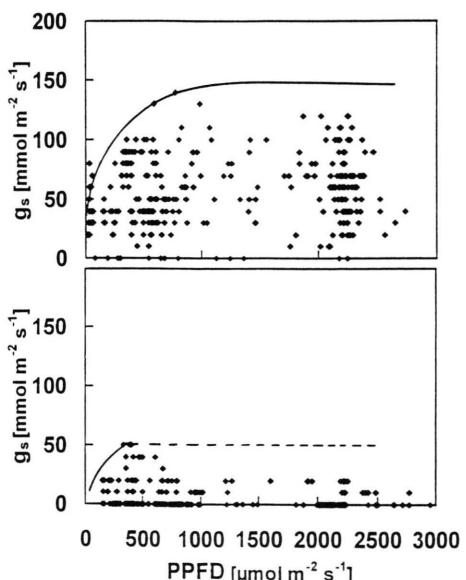


Fig. 1. Comparison between global measurements of stomatal conductance (g_s), in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna, in response to photosynthetic photon flux density (PPFD).

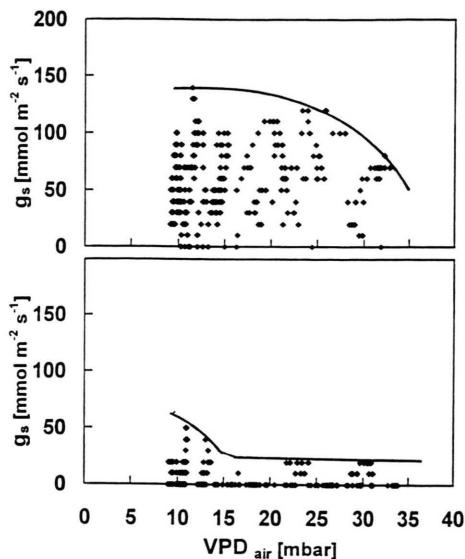


Fig. 2. Comparison between global measurements of stomatal conductance (g_s), in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna, in response to air vapour pressure deficit (VPD).

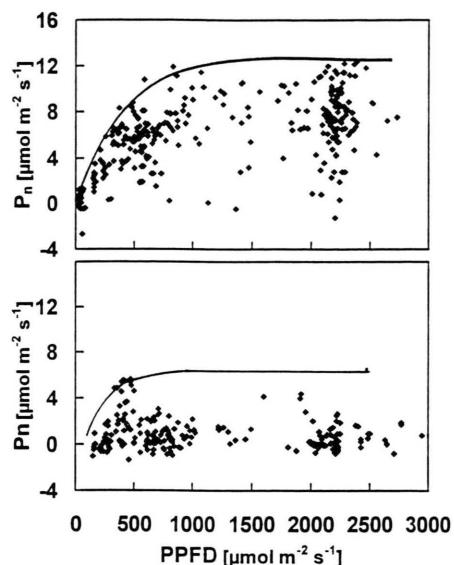


Fig. 4. Comparison between global measurements of net photosynthesis (P_n) in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna, in response to photosynthetic photon flux density (PPFD).

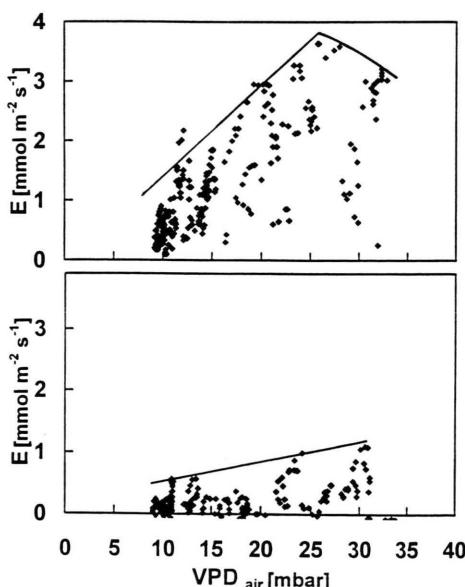


Fig. 3. Comparison between global measurements of transpiration (E), in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna, in response to air vapour pressure deficit (VPD).

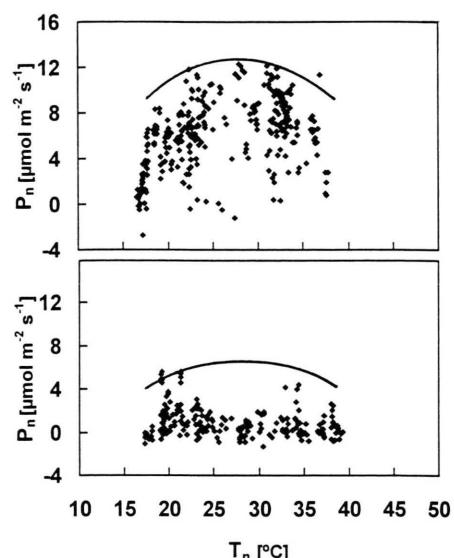


Fig. 5. Comparison between global measurements of net photosynthesis (P_n) in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna, in response to needle temperature

gated plants, although as a result of lower values of g_s in the latter, the values of photosynthesis were also lower. So in control plants Pn_{max} was of $12 \mu\text{mol m}^{-2} \text{s}^{-1}$, while in non-irrigated plants it was attained at $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ and the light saturation point changed from 700 to 500 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ respectively (Fig. 4). In both types of plants the temperature response curve presented its optimal values between 20 and 35 °C, but with lower values in non-irrigated plants (Fig. 5).

A small decrease of the ratio of variable to maximal fluorescence (F_v/F_m) at midday was observed on both irrigated and non-irrigated seedling. This reduction was higher in non-irrigated plants, although it was not significant in any case and a recuperation could be seen in the evening values (Fig. 6). Some other authors (Sharkey and Seemann, 1989; Vassey and Sharkey, 1989; Lal *et al.*, 1996) have found that the primary mechanism for the inhibition of photosynthesis under both mild and severe water stress is reduced supply of CO_2 to Rubisco, via a stomatal limitation and no damage to chloroplast reactions. Our measurements with chlorophyll fluorescence agree with this finding since the small decrease of F_v/F_m at midday, indicative of photoinhibition (Björkman and Demming, 1987; Osmond, 1994), was recuperated during the night.

Up to now no gas exchange parameters in *Pinus canariensis* were registered, so these present data are the first insight of this plant behaviour. Pn_{max} of $12 \mu\text{mol m}^{-2} \text{s}^{-1}$ was a relatively high value if we compare it with the majority of coniferous species including *Pinus* spp, although lower than some sun adapted species as *Pinus sylvestris* and *Pinus radiata* (Ceulemans and Saugier, 1991). The maximum stomatal conductance of $150 \text{ mmol m}^{-2} \text{s}^{-1}$, was within the normal range of low values given by Körner (1994) and Larcher (1995) for conifer species, and the maximum transpiration rate was very high if we compare with other coniferous species (Larcher, 1995).

When *Pinus canariensis* was submitted to a mild water deficit in the soil the needle water balance was not significantly altered, only a slight decrease of RWC at midday was found. On the contrary, the stomata responded with great sensitivity to lack of water in the soil so that stomatal conductance decreased by about a 60% in non-irrigated pine seedlings and as a result, the CO_2 assimilation rate

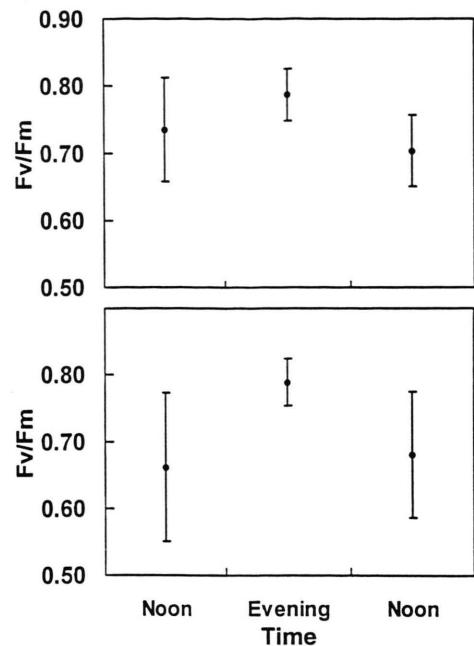


Fig. 6. Changes in the variable to maximum fluorescence (F_v/F_m) along the day in irrigated (upper part) and non-irrigated (lower part) *Pinus canariensis* seedlings growing outdoors in La Laguna.

decreased by 50% and the transpiration rate was reduced at a higher proportion (70%). As demonstrated by Zhang and Davies (1989), when drought is moderate, stomatal responses can be more closely linked to soil drying than to leaf water status. In such cases stomatal closure or even altered growth may be more sensitive indicators of drought than loss of turgor (Chaves, 1991). Evidence for direct effects of soil drying on conductance, not mediated by any change in leaf water status, has also been reported (Davies and Sharp, 1981; Blackman and Davies 1985; Gollan *et al.*, 1986).

We can conclude that this tree has a hydrostable (also called "isohydric") water balance because the great sensitivity of its stomata at shortage of water in the soil in opposition to other plants, as many herbs of sunny habitats and also trees, which have the hydrolabile variant of water balance and can afford to risk quite large losses of water (Larcher, 1995). Following Levitt (1980) and also Larcher (1995), this plant could be classified as a "drought avoider of the saving type", the high degree of stomatal control enables it to maintain

high leaf water potentials for extended periods of drought or, in other words, to postpone desiccation.

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