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Ecophysiological Studies on *Pinus canariensis*

By

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Summary

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In this review we summarize work carried out over the last 10 years on *Pinus canariensis*, an endemic tree species of the Canary Archipelago, in order to find out: how it is able to adapt to living from near sea level up to about 2.200 m; the response to different environmental conditions; the causes which prevent the natural regeneration in reforested areas and the methods needed to achieve nursery quality plants for reforestation.

P. canariensis is able to modulate its physiology, the pigment and antioxidants content, the needle temperature resistance and the structure of its needles depending of the site where it grows, and it has a good stomatal control. The low light in reforested areas influences seedling growth and vigour. Also it has been shown that good quality planting stock obtained with fertilized artificial substrates is important for reforestation success.

Introduction

Pinus canariensis C. Sm. ex DC. is an endemic tree species of the Canary Archipelago whose natural distribution area is restricted to the highest islands. In Tenerife it grows spontaneously from near the sea level up to about 2.200 m (DEL ARCO & al. 1990); it colonizes volcanic soils and is able to live from mesic places in the North of the island to very dry and rocky sites in the South. Although it had been studied from different viewpoints, very little was known about its ecophysiology.

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In this review we summarize the work carried out over the last 10 years, in order to find out how it has been able to adapt to living over such a great altitudinal gradient and its response to different environmental conditions. Furthermore, the causes which prevent the natural regeneration in reforested areas are studied and the methods to obtain nursery quality plants for reforestation are evaluated before and after the seedlings are planted in the field.

Structure and Ultrastructure of *Pinus canariensis* Needles

The structure and ultrastructure of *P. canariensis* needles was studied in order to use the results as reference data for further investigations of physiological, biochemical and ultrastructural responses of this species to environmental variations due to pollutants and different altitudinal and exposure situations. As a three-needled species, they have a triangular shape in transverse section being the abaxial side hemispherical. The deeply sunken stomata and the highly reflective waxes covering the epidermal cells as well as the epistomatal chamber constitute a good adaptation to avoid loss of water and increased protection against high photon flux densities and UV-radiation. The ultrastructure of mesophyll cells, transfusion tissues as well as endodermis and resin ducts was also described (JIMÉNEZ & al. 2000, ZELLNIG & al. 2002, STABENTHEINER & al. 2004). The epistomatal chamber is a spacious cave and the opening is not situated directly above the stomata. This characteristic feature was shown as the result of a 3-D reconstruction of serial sections (ZELLNIG & al. 2002).

The mechanical tissue below the epidermis contributes to a higher drought resistance and to maintain the shape of the long needles as a skeletic tissue. Cross-section areas of different needle tissues were measured in an image analysis system (GRILL & al. 2004), showing that in drought stressed trees the sclerenchymatic tissues in the hypodermis and adjacent to the vascular bundles increased due to large increases in the number - not the size - of sclerenchymatic cells and the ratio of assimilation parenchyma to the vascular bundle (supplied versus supplying tissue) decreased; concluding that these changes, which adapt needles to drought conditions, were triggered by drought stress experienced while needles are growing.

Altitudinal Gradient

A combination of low temperatures with high radiation and high atmospheric ozone concentrations occurs at high altitudes. Many plants can adapt to live in this environment thanks to morphological or physiological traits. But only the plants which can modulate some parameters according to the environmental conditions are able to live in a larger range of altitude. It was shown that *P. canariensis* is able to change pigment and antioxidant contents, as well as the limits to temperature resistance in its needles depending on the site where it lives.

Four natural stands covering an altitudinal range from 200 to 2000 m a. s. l. in the South-East of Tenerife were established in order to investigate differences in antioxidants, chloroplast pigments, chloroplast ultrastructure, and chlorophyll fluorescence in the needles (JIMÉNEZ & al. 1997, TAUSZ & al. 1997, 1999 a, b, JIMÉNEZ & MORALES 2001). These parameters have been used frequently as indicators of oxidative stress. An altitudinal dependence of these parameters was partially masked by different environmental conditions at the sites, but a tendency of decreasing in chlorophyll and increasing carotenoid content and thus, an increase in the carotenoids/chlorophyll ratio with altitude was found. Besides, the α-carotene/β-carotene ratio was lower and the ascorbic acid content was significantly higher showing an increase in the oxidation processes with altitude (Fig. 1). Nevertheless the glutathion and α-tocopherol levels did not vary significantly among the different sites. Since the increase in α-tocopherol is usually related to severe membrane damage, we assume that no damage was produced in the needles in any site. This was confirmed by the ratio of variable to maximal fluorescence (Fv/Fm) measured at the early morning indicating healthy photosynthetic apparatus, and the ultrastructure of the chloroplasts that indicated no severe damage to the thylakoid systems in any of the sites.

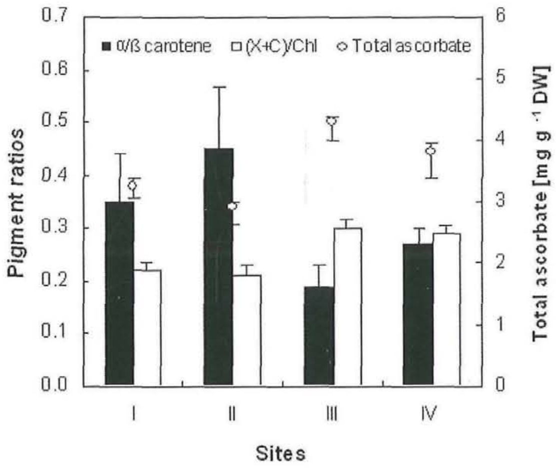

Fig. 1. Total ascorbate content, α-carotene/β-carotene and carotenoids/ chlorophyll ratios (x+c)/chl, of one year old *Pinus canariensis* needles at four field stands situated in the south-eastern slope of Tenerife at 550 (Site I), 850 (Site II), 1500 (Site III) and 1950 (Site IV) m of altitude. Data from JIMÉNEZ & al. 1997 and TAUSZ & al. 1998.

The effect of extreme temperature on the quantum yield of fluorescence and membrane leakage (as first indicators of damage) was determined in needles sampled in the same experimental stands as above, when they were exposed for 30 min at different temperatures treatments (PETERS & al. 1999). Needles of pines living at higher sites, where the temperature during winter can attain values below zero and sometimes snow is present, were more sensitive to higher temperatures (limit was 42 °C) and less to low temperatures (limit was from -8 to -10 °C). The opposite tendency was found in needles of pines living in the much warmer lower places (limits of 44 °C and -5 to -7 °C), showing acclimation to the specific environment where they had developed.

Needle Nutrient Content

The foliar nutrient content was studied all over the range of this species in Tenerife. Compared to available data from related pine species (*P. halepensis*, *P. pinaster*, *P. radiata*), *P. canariensis* needles had lower N and P contents, higher Ca and Mg, and about equal K contents. N/nutrient ratios were in the range considered optimal for pine species. Micronutrients were in the range considered adequate for pines except for low Cu values (TAUSZ & al. 1998, 2004). Na and Cl concentrations were high at low elevations. Only at few plots sulphur concentrations indicated possible pollutant impact. Cluster and correlation analyses identified a related group of V, As, Cr, Fe, Mo, Ni, Cu, Pb, and Al, possible related to traffic exhaust aggregated with soil particles. In conclusion, seawater and soil particles explained most of the element distribution pattern in pine needles in Tenerife, but strong indications for some effect of local sources of air pollutants were detected (TAUSZ & al. 2005).

Behaviour of *P. canariensis* in Relation to Environmental Conditions

One experimental plot was established in an open 50-year old *P. canariensis* forest to measure climatic conditions permanently and to carry out physiological measurements in adult trees. It is situated in Morro de Isarda (28°35′ N, 27°15′ W), Tenerife, Canary Islands at an altitude of 1650 m a. s. l. facing NE. The site is characterized by a Mediterranean climate with a mean annual temperature of 12.6°C (absolute minima -4.2°C, absolute maxima 31.2°C), a mean relative humidity of 52% and an annual sum of precipitation ranging from 460 mm (ABOAL & al. 2000) to 930 mm (PETERS & al. 2003). Opposite to the typical Mediterranean climate, summer drought is often mitigated by a high relative humidity of the air and a high frequency of clouds due to the north-east trade winds. A wooden tower of 20 m height gives access to the needles. Further details concerning stand and climatic conditions are given in ABOAL & al. 2000, LUIS & al. 2001 and PETERS & al. 2003.

Gas exchange of *P. canariensis* needles was measured during a whole year; exhibiting high values of carbon assimilation rate (A, with maximal value of 17 μmol m^{-2} projected leaf area s^{-1}) and high optimal needle temperature for photosynthesis (25 °C), which were at the upper limit of the values given for conifers.

The stomatal conductance (gs) was reduced when vapour pressure deficit (VPD) values increased; allowing this pine to have high values of intrinsic water use efficiency (A/gs, near 150 μmol mol^{-1}) during high evaporative demand conditions. The instantaneous water use efficiency (WUE, carbon assimilation rate/transpiration), varied over the year attaining values as high as 7 mmol mol^{-1} during late autumn and winter, being lower during summer (2 mmol mol^{-1}), see Fig. 2. The photosynthetic activity was present during the whole year and more dependent on the particular meteorological conditions of each day, but in general the higher rates were registered during the spring and the autumn. The ratio Fv/Fm varied only slightly during the day and always recovered in the evening showing that the photosynthetic machinery was not damaged by any stress factor (PETERS 2001, PETERS & al. 2003). In general, there were not observed significant differences in physiological parameters between needle age classes, but they were between needles of the upper and the lower canopy level. This indicates a good acclimation of needles to the different canopy environmental conditions. The relative water content was always above 80 %, even at midday indicating that the needles were not submitted to severe water stress during the whole year. This was corroborated by data of chlorophyll content and predawn F_v/F_m (PETERS 2001). Additionally, one experiment was carried out in order to find out the responses of potted *P. canariensis* seedlings to mild water stress. This led to changes not only in the stomatal conductance and carbon assimilation rate with the drought (MORALES & al. 1999), but also in the redox state of the glutathione pool, whereas the state of ascorbate remained stable (TAUSZ & al. 2001).

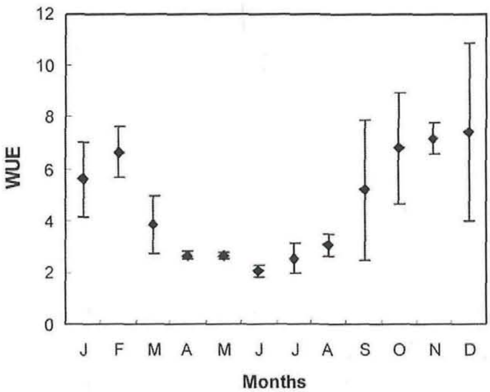

Fig. 2. Variation of the instantaneous water use efficiency (WUE, mmol mol^{-1}) on *Pinus canariensis* over the year. Data from PETERS 2001.

Gas exchange measurements at the leaf level in the upper crown give detailed information how transpiration is influenced by environmental variables.

However, on the long term under the climatic conditions in the Canary Islands this assessment of transpiration is a difficult task because crowns are periodically wet due to the influence of the trade winds bringing moisture from the sea (PETERS 2001). Sap flow sensors by contrast, give long term records and enable the automatic recording of whole canopy transpiration on the tree level. Additional measurements of changes in stem radius with point dendrometers at breast height reflect diurnal patterns of transpiration. Such measurements in combination with data on soil water relationships contributed to have a better knowledge of the water balance of *P. canariensis* forests (WIESER & al. 2002).

Transpiration in the *P. canariensis* stand was variable and totally influenced by meteorological conditions. The amount of transpired water for the stand during the year was 252.3 mm and maximum daily transpiration for the period was of 1.85 mm day^{-1}. Two models for calculation of transpiration were established based on soil water content and air temperature showing the amount of not available water during the dry period (LUIS & al. 2002).

Problems of Pine Forest Regeneration

During the last 20 years there have been problems related to the natural regeneration of pine seedlings in some reforested areas of Tenerife. Therefore thirty eight experimental plots with different PAR attenuation (from 8% to 48 %) were established in a reforested pine forest with different thinning treatments, in order to study problems in natural regeneration. Statistical analyses showed that seedlings survival was not correlated either with the initial seedlings density or with PAR in each plot (LUIS & al. 2001). Two experiments, quantifying growth and mortality of seedlings growing in pots at different light intensities, were carried out (PETERS & al. 2001) concluding that an incident light above the 7 % might not be decisive factor for seedlings mortality in the forest, although it influences seedlings growth and vigour (Fig. 3).

Table 1. Differences between natural substrates (with forest soil and without fertilizers) and artificial ones (with peat and fertilizers) in height (cm), diameter (mm), needles nitrogen content (% of dry mass) and survival, one year after the plantation (Means ± SE). Data from LUIS & al. 2004a, 2004b.

Substrates	Height (cm)	Diameter (mm)	N (%)	Survival (%)
Natural	8.1 ± 0.16	23.4± 0.03	1.0 ± 0.10	65.54 ± 0.04
Artificial	20.1 ± 0.34	40.4 ± 0.03	2.2 ± 0.10	95.78 ± 0.01

At present, a reforestation campaign has started in Tenerife in order to complete the pine forest belt around the highest summits of this island. Good quality planting stock is an essential precondition for reforestation success. The influence of different nursery practices, which may affect final plant quality was tested to determine their influence on morphological, physiological and performance attributes of *P. canariensis* and also the survival in the field , showing significantly better results plants grown in artificial substrates with fertilizers, see Table 1, (LUIS & al. 2004a,b).

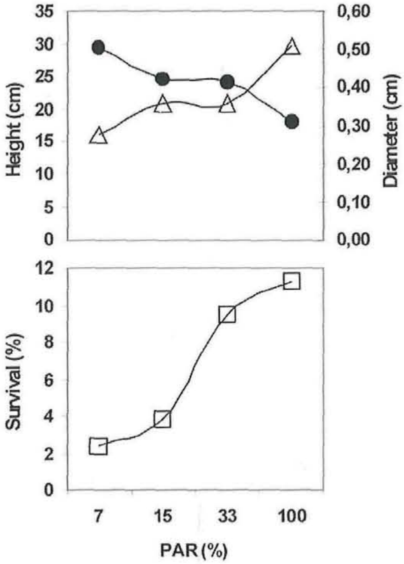

Fig. 3. Height (circles), diameter (triangles) and percentages of survival (squares), for seedlings grown into greenhouses with different values of PAR. Data from PETERS & al. 2001.

Conclusions

P. canariensis is able to modulate its physiology and the structure of its needles depending on the site where it grows. The deeply sunken stomata with highly reflective waxes and the narrow epistomatal chamber comprise an efficient adaptation to avoid water loss and a good stomatal control, which has been shown by gas exchange and sap flow measurements. Furthermore, it is able to change its pigment and antioxidants content and temperature resistance of its needles which show acclimation to the specific environment where they have developed and making it capable of growing over a wide altitudinal gradient. The nutritional status is within the range considered optimal for pine species; moreover, seawater and soil particles explained most of the element distribution pattern in pine needles in Tenerife. However, strong indications were also found of the effect of local sources of air pollutants. The low light in reforested areas influences seedling growth and vigour. Finally, it has been shown that good quality planting stock obtained with fertilized artificial substrates is important for reforestation success.

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References

ABOAL J.R., JIMÉNEZ M.S., MORALES D & GIL P. 2000. Effects of thinning on throughfall in canary islands pine (*Pinus canariensis* Chr.Sm. ex DC) forest. - Journal of Hydrology 238: 218-230.

DEL ARCO M., PÉREZ DE PAZ P.L., RODRÍGUEZ O. & WILDPRET W. 1990. Atlas cartográfico de los pinares de Canarias II. Tenerife. - Dir. Gral. de Medio Ambiente y Cons. de la Naturaleza. Gobierno de Canarias. Sta. Cruz de Tenerife, 228 pp.

GRILL D., TAUSZ M., PÖLLINGER U., JIMÉNEZ M.S. & MORALES D. 2004. Effects of drought on needle anatomy of *Pinus canariensis*. - Flora 199: 85- 89.

JIMÉNEZ M.S. & MORALES D. 2001. Pino Canario. Ejemplo de adaptación. - Investigación y Ciencia 23-24. Noviembre.

— , TAUSZ M., ZELLNIG G., PETERS J., GRILL D. & MORALES D. 1997. Environmental stresses and antioxidative responses of *Pinus canariensis* at different field stands in Tenerife. - Phyton 37: 109-114.

— , ZELLNIG G., STABENTHEINER E., PETERS J., MORALES D & GRILL D. 2000. Structure and ultrastructure of *Pinus canariensis* needles. - Flora 195: 228-235.

LUIS V.C., JIMÉNEZ M.S., GIL P. & MORALES D. 2001. Influencia de los factores ambientales en la mortalidad de plántulas de *Pinus canariensis* en condiciones naturales. - Actas del III Congreso Forestal Español. Tomo I, pp. 357-362. Ed. Junta de Andalucía. Consejería de Medio Ambiente. Granada.

— , KUCERA J., JIMÉNEZ M.S. & MORALES D. 2002. Medidas de flujo de savia en un pinar repoblado con *Pinus canariensis*. - II Simposio Internacional sobre Manejo Sostenible de Ecosistemas Forestales. Universidad de Pinar del Río. Cuba. Abril, 2002.

— , PETERS J., GONZÁLEZ-RODRÍGUEZ A. M., JIMÉNEZ M. S. & MORALES D. 2004a. Testing nursery plant quality of Canary Islands Pine seedlings grown under different cultivation methods. - Phyton 44: 231-244.

— , CLIMENT J., PETERS J., PÉREZ E., PUÉRTOLAS J., MORALES D., JIMÉNEZ M. S. & GIL L. 2004b. Evaluación de la calidad de plántulas de *Pinus canariensis* cultivadas con diferentes métodos en la supervivencia y crecimiento en campo. - Cuad. Soc. Esp. Cien. For. 17: 63-67.

MORALES D., PETERS J., JIMÉNEZ M.S., TAUSZ M., WONISCH A. & GRILL D. 1999. Gas exchange of irrigated and non-irrigated *Pinus canariensis* seedlings growing outdoors in La Laguna, Canary Islands, Spain. - Zeitschrift für Naturforschung 54c: 693-697.

PETERS J. 2001. Ecofisiologia del pino canario. - Tesis doctoral. Univ. La Laguna.

— , JIMÉNEZ M.S. & MORALES D. 1999. Effect of extreme temperature on quantum yield of fluorescence and membrane leakage of the Canarian Endemic Pine (*Pinus canariensis*). - Zeitschrift für Naturforschung 54c: 681-687.

— , MORALES D. & JIMÉNEZ M.S. 2003. Gas exchange characteristics of *Pinus canariensis* in a forest stand in Tenerife, Canary Islands. - Trees 17: 492-500.

— , LUIS V.C., JIMÉNEZ M.S., GIL P. & MORALES D. 2001. Influencia de la luz en el crecimiento y mortalidad de plántulas de *Pinus canariensis*. - Actas del III Congreso Forestal Español. Montes para la Sociedad del Nuevo Milenio. Tomo I, pp, 363-368. Ed. Junta de Andalucía. Consejería de Medio Ambiente. Granada

STABENTHEINER E., PFEIFHOFER H., PETERS J., JIMÉNEZ M.S., MORALES D. & GRILL D. 2004. Different surface characteristics of primary and secondary needles of *Pinus canariensis*. - Flora 199: 90-99.

TAUSZ M., GRILL D., JIMÉNEZ M.S. & MORALES D. 1997. A physiological comparison between environmental effects on Canary pine in Tenerife and Norway spruce in Austria. - In: MOHREN G.M.J., KRAMER K. & SABATÉ S. (Eds.), Impacts of global change on tree physiology and forest ecosystems, pp.103-110. - Kluwer Academic Publishers (Dordrecht, Boston, London).

— , MORALES D., JIMÉNEZ M.S. & GRILL D. 1999a. Photoprotection in forest trees under field conditions. - Phyton Ann Rei Bot 39: 25-28.

— , JIMÉNEZ M.S., MORALES D., GRILL D. & PETERS J. 1998. Element contents and stress-physiological characterization of *Pinus canariensis* trees in mediterranean type forest stands in Tenerife. - Chemosphere 36: 1019-1023.

— , WONISCH A., PETERS J., JIMÉNEZ M.S., MORALES D. & GRILL D. 2001. Short-term changes in free-radical scavengers and chloroplast pigment in *Pinus canariensis* needles as affected by mild drought tress. Journal of Plant Physiology 158: 213-219.

— , WONISCH A., PETERS J., JIMÉNEZ M.S., MORALES D., BYTNEROWICZ A., ARBAUG M.J. & GRILL D. 1999b. Complex patterns of the antioxidative defense systems in pine species at field plots subjected to different environmental stress combinations. - In: DENKE A., DORNISCH K., FLEISCHMANN F., GRAβMANN J., HEISWER I., HIPPELI S., OβWALD W. & SCHEMPP H. (Eds.), Different pathways through life. Biochemical aspects of plant biology and medicine, pp. 181-189. - Lincom Europa.

— , TRUMMER W., WONISCH A., GOESSLER W., GRILL D., JIMÉNEZ M.S. & MORALES D. 2004. A survey of foliar mineral nutrient concentrations of *Pinus canariensis* at field plots in Tenerife. - Forest Ecol. Manag. 189: 49- 55.

— , TRUMMER W., GOESSLER W., WONISCH A., GRILL D., NAUMANN S., JIMÉNEZ M. S. & MORALES D. 2005. Accumulating pollutants in conifer needles on an Atlantic island - a case study with *Pinus canariensis* on Tenerife, Canary Islands. - Environmental Pollution 136: 397-407.

WIESER G., PETERS J., LUIS V.C., MORALES D. & JIMÉNEZ M.S. 2002. Ecophysiological studies on the water relations in a *Pinus canariensis* stand, Tenerife, Canary Islands. - Phyton 42: 291-304.

ZELLNIG G., PETERS J., JIMÉNEZ M.S., MORALES D., GRILL D. & PERKTOLD A. 2002. Three-dimensional reconstruction of the stomatal complex in *Pinus canariensis* needles using serial sections. - Plant Biology 4: 70-76.