Source: Maaten-Theunissen M, Spiecker H, Gärtner H, Helle G, Heinrich I (Eds.) (2011): TRACE - Tree Rings in Archaeology, Climatology and Ecology, Vol. 9. GFZ Potsdam, Scientific Technical Report STR 11/07, Potsdam, p. 149 - 157.

$\delta^{13}C$ and δD in tree growth rings of *Pinus pinea* and its relationship with climate in Doñana National Park (Spain)

S. Granados & A. Delgado

Instituto Andaluz de Ciencias de la tierra, CSIC-UGR. Camino del Jueves, s/n, 18100, Armilla, (Granada), Spain
E-mail: soniagranados@ugr.es

Introduction

The Doñana National Park is one of the most important protected coastal areas in Europe (Serrano et al. 2006, Garcia Novo et al. 2007). This area (located in the southwestern Iberian Peninsula) is particularly sensitive to the consequences of Global Change such as increases in temperature and decrease in seasonal rainfall (IPCC 2007). This fact is noted in the Spanish Climate Change Report (2007) where an upward trend in temperatures of 1 to 2 degrees Celsius is reported over the period 1850 to 2005 and a change in rainfall patterns. The effects of this change have been notably severe in the southern Iberian Peninsula (IPCC 2007). In this context it is essential to study the response of the plants to climate change on regional scale. Stable isotopes are a powerful method to study water and carbon fluxes within an ecosystem (Resco et al. 2009) and specifically tree ring analysis offers a unique opportunity for understanding the response of trees to drought stress over decades, over centuries and millennia.

In Mediterranean regions, water is often a limiting factor for plant growth, due to high losses from the plant via evapotranspiration. However, an excess of water (such as in temporary ponds in Doñana National Park) can also cause physiological responses, similar to water stress and can cause stomatal closure (Kozlowski 1984, Ewe & Sternberg 2002). Thus, stomatal movement is an able mechanism that controls water loss from the leaves and optimizes carbon assimilation. Under stress situations (like higher air temperatures, lack or excess of water), the stomata close to avoid losing water and the CO_2 influx becomes scarce and Rubisco (the enzyme responsible for carbon fixation) reduces its discrimination against ^{13}C from the carbon dioxide within the intercellular spaces. This fact, affects to carbon isotopic composition of organic molecules (like glucose) formed during photosynthesis (e.g. Farquhar & Richards, 1984, Farquhar et al. 1989, Ehleringer & Dawson 1992, Saurer et al. 1997). Consequently, plants that have experienced water stress show less negative carbon isotopic values than non-stressed plants.

Hydrogen isotope ratios of stem cellulose of tree rings have been used to reconstruct relative humidity and rainfall patterns (Burk & Stuiver 1981, Yapp & Epstein 1982, Edwards et al. 1985, Edwards & Fritz, 1986). The hydrogen isotopic values in stem cellulose of tree rings preserve a signal related to the fractionation between source water and cellulose such as the isotopic signature of leaf or/and xylem water used to cellulose synthesis and the isotope composition of rainfall, which is mainly dependent of temperature (e.g. Sternberg et al. 1986, Rozanski et al. 1993). But hydrogen isotopes in tree rings are not a direct record of the isotopic composition of rainfall, since there are many steps along the path from source water to cellulose (Roden et al. 2000). For example, during evapotranspiration water molecules containing the lighter isotope are able to diffuse more rapidly than those that include the heavier isotope. The net effect is an enriched in δD values in plant material and vice versa (Roden & Ehleringer 1999b, Waterhouse et al. 2002). However, biochemical fractionations are not fully understood and can result in both enriched and depleted values of the isotope composition in organic molecules (Roden and Ehleringer, 1999b).

In this study we analyzed $\delta^{13}C$ and δD from *Pinus pinea* growth rings in the Doñana National Park to investigate the relationship between isotope variability associated to climate variability to obtain a better understanding of the physiological process underlying tree growth. Consequently, this test provides an indication of whether dendro-isotopes of carbon and hydrogen can be a good climate proxy in areas sensitive to the consequences of global change as Doñana National Park.

Material and methods

Description of study site

Doñana National Park lies between 36º 48´- 37º 08´ N and 6º 16´- 6º 34´ W (Fig. 1). This area is given the highest degree of environmental protection in Spain and it was designated as a Biosphere Reserve in 1980. This region is characterized by two main landscape types: marshland and aeolian sands. The marshes are a place of transit, breeding and wintering grounds for thousands of European and African birds, being the largest ecological reserve in Europe. The aeolian sands cover is composed of several dune generations originally deposited by marine material in the Holocene. Consequently, hundreds of small ponds appear in rainy years when the water table rises above the topographical surface.

The vegetation of Doñana National Park is dominated by Mediterranean scrubland such as *Cistus libanotis, Rosmarinus officinalis, Halimium commutatum, Erica scoparia, Erica ciliaris, Ulex minor, Cistus salvifolius*, being *Pinus pinea* the dominant tree species. The soil where the trees for this study lay is arenosols (FAO soil classification) and the average distance to water table is two meter.

Doñana has a Mediterranean climate with Atlantic influences, classified as dry sub-humid. The mean annual temperature is 18º-19ºC, the seasonal minimum temperature values are seen in winter, and the seasonal maximum values correspond to July with average values between 25 º-29 ºC. Average annual rainfall varies between 500-600mm. These values show a clear seasonal rainfall pattern, with the minimum in summer (June-September) and maximum in December with an occasional secondary maximum in April-May.

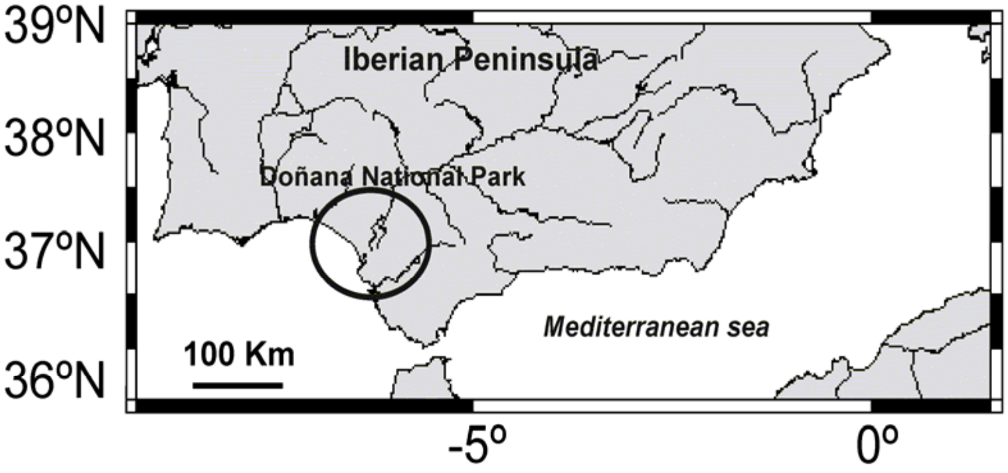

Figure 1: Map of situation. Black circle represents study area (Doñana National Park).

Sample preparation

For this study we selected two stem discs of *Pinus pinea* (DO-33 and DO-36) from Doñana National Park. These samples were previously dried for 48 hours to ambient temperature, polished and cross dated along with ten samples from the same site. To eliminate any influence of the "juvenile effect" observed in young trees (Freyer 1979, Francey & Farquhar 1982), we didn't consider the two first decades to isotopic analysis, so the periods for which data are available are 1925-2007 (for DO-36 individual) and 1945-2007 (for DO-33 individual). An aliquot of 50 mg of wood of each ring was extracted for to carry out cellulose extraction following the method described by Brendel et al. (2000). According to this technique ground sample was treated in acetic acid (80%; v/v) and 0.2 ml of concentrated nitric acid (69%; v/v) for 20 minutes at 120ºC.

Afterwards the samples were washed repeatedly with ethanol, deionised water and acetone. Finally, all samples were freeze dried to remove any contamination.

Carbon isotopic analysis

The isotopic analyses for this study were conducted in the Stable Isotope Laboratory at the Zaidín Experimental Institute (Granada). For carrying out $\delta^{13}C$ analysis an aliquot of 0.5-0.6 mg of cellulose extracted sample was weighed into a tin capsule. The isotopic ratio of $^{13}C/^{12}C$ was determined using a Carlo Erba Elemental Analyzer (NC 1500). The CO_2 produced after combustion was analyzed using a Finnigan Delta^{PLUS} XL isotope ratio mass spectrometer. The δ values are defined as:

$$\delta^{13}C \text{ or } \delta D = [(R_{sample} / R_{standard}) - 1] \times 1000 \text{ (in ‰)}$$

where $R = {}^{13}C/{}^{12}C$ or D/H ratios.

Stable carbon isotope values are reported relative to the international standard Vienna-Pee Dee Belemnite (V-PDB). Multiple aliquots of in-house standards were analyzed periodically as a check on the analytical precision throughout a run (n=10), which was about ±0.1‰. To eliminate the effects of the general atmospheric decline observed in carbon isotopes of tree rings due to land-use change and the effects of burning fossil fuel, we calculated carbon isotopic discrimination ($\Delta^{13}C$) from equation (1) (Farqhuar et al. 1982)

$$(1) \quad \Delta^{13}C = (\delta^{13}C_{atm} - \delta^{13}C_{wood}) / (1 + \delta^{13}C_{atm}/1000)$$

where $\delta^{13}C_{atm}$ and $\delta^{13}C_{wood}$ are the $^{13}C/^{12}C$ ratios in atmospheric CO_2 and whole wood of each tree ring, respectively, expressed in parts per thousand (‰) relative to the standard Vienna Pee Dee belemnite. For $\delta^{13}C_{atm}$ we used published data from McCarroll & Loader (2004), to 2003 and Leuenberger, (2007) from 2004 to 2007.

Hydrogen isotopic analysis

For hydrogen isotopic analysis, the samples require equilibration or nitration (Ramesh et al. 1988, Schimmelman 1991, Feng et al. 1993) prior to mass spectrometry to remove exchangeable hydroxyl bound hydrogen that constitutes *ca.* 30% of the hydrogen within the α-cellulose monomer. For this study α-cellulose was equilibrated by a method described by Sauer et al. (2009). For hydrogen isotope analysis 0.8 mg of cellulose was weighed into silver perforated capsules. Then, cellulose samples were equilibrated during 8 hours at 115ºC with D-enriched and D-depleted water vapours.

After equilibration, the steam was subsequently replaced by a flow of dry N_2 gas at least 4h at 115ºC to dry the samples. The N_2 supply was dried right away prior to entering the equilibration enclosure by passing the gas through a coil of copper tubing immersed in liquid nitrogen. Finally, the oven was turned off and the equilibration enclosure cooled to room temperature. The equilibrated samples were loaded into the carousel and pyrolised at 1450 ºC using a high temperature elemental analyser (TC/EA) connected to a ThermoFinnigan Delta Plus XL isotope ratio mass spectrometer which was operated in continuous-flow mode. Values of δD were calculated by comparison of sample gases with peaks of H_2 reference gas, and were anchored to the VSMOW scale by comparison with in-house and international standards. Analytical precision throughout a run (n=12) was about ±1‰.

Meteorological data and statistical analysis

Before to investigate the relationship between isotopic data and climate, we compared isotopic series from both trees to each other to see how similar they are. When they are highly correlated, this would indicate a strong common forcing by climate, if they are not, then other factors like competition may be more important.

To investigate the relationship between carbon and hydrogen isotopic composition of growth tree rings and climate we considered climate data (annual and monthly rainfall and annual and monthly temperature) from weather stations near to the study area such as: Palacio de Doñana (36º 59´53´´N - 6º26´37´´E and 6 m.a.s.l.) for the period 1978-2007 and for previous years, meteorological data from Tablada-Sevilla (37º24´10´´N - 6º 0.03´18´´E and 13 m.a.s.l.), Sevilla-Iglesia (Custodio et al., 2005), Gibraltar (36º 8´ 15´´N - 5º 20´ 43´´E and 315 m.a.s.l.) and Rota (36º 39´N - 6º 21´E and 88 m.a.s.l.).

Temporal trends in Δ^{13}C, δD, annual and monthly rainfall, mean annual and monthly temperature were tested by simple linear regression. δD and Δ^{13}C values of growth tree rings were related by linear correlation with annual and monthly climatic variables (rainfall and temperature).

Results and discussion

Variability hydrogen isotopic values in Pinus pinea growth rings.

The δD time series from the individual *Pinus pinea* trees (DO-33 and DO-36) is shown in figure 2a,b. The values range from -68.4‰ to -38.9‰ for the period 1945-2007, for the individual DO-33 and range from -52.2‰ to -25.1‰ for the period 1925-2007, for the individual DO-36 (Tab. 1). The both δD time series were compared to each other in 10-year periods from 1945 to 2005 (ie, the period common to both). We found positive significant relationships only for the periods 1975-1985 ($r=0.62$, $p<0.05$) and 1995-2005 ($r=0.63$, $p<0.05$).This, may indicate a strong common forcing by climate in recent decades in contrast to previous decades where other factors seem more important, like competition or the different rooting depth.

Then, each δD time series from the individual DO-33 and DO-36 was compared with annual and monthly rainfall and with mean annual and monthly temperature from weather stations near study area. We did not find significant correlation between hydrogen isotopic data and temperature. However, we found negative significant relationships between hydrogen isotopic values and mean annual rainfall ($r= -0.25$, $p<0.05$ for DO-33 and $r= -0.27$, $p<0.05$ for DO-36) (Fig. 2a, b) and with monthly rainfall (Table 2a). For both individual trees the summer rainfall (specifically August for DO-36 and June for DO-33) seem to being the most important limiting factor (as inferred of the highest correlation coefficients, Table 2a). However, the rainfall of the major recharge periods is also related to isotopic data. For DO-36 significant correlations were also found with autumn and winter rainfall (November, December, January and February) (Table 2a). On the other hand, significant correlations for DO-33 were also found with spring rainfall (March and April).

In general, the maximum hydrogen isotopic values coincide with the driest years and *vice-versa* (Fig.2). The reason could be that in dry periods the evaporation from water source and soil is very important and this causes a strong enrichment in heavy isotopes (D). On the one hand, the evapotranspiration from plants cause enrichment in heavy isotopes (D) in water located in the inside of the leaves (Lawrence & White 1984, Krishnamurthy & Epstein 1985). This can be observed, for instance, during the droughts in 1954, 1981, 1992-1995, 2005 (Fig.2). On the other hand is interesting to emphasize that during very rainy years as the beginnings of the 1960s the isotopic values are more negative. This is reflected in δD values from each individual tree (-55.6‰ ±4.4 and -41.8‰ ± 3.3 for DO-33 and DO-36, respectively, averages during the wet period 1960-1963). It is also remarkable that after a strong drought like last one (1992-1995) followed by a very rainy year as 1996 (1181mm) the tree responds with a high isotopic fractionation (δD: -57.6‰ for DO-33 and -49.0 for DO-36) (see Fig. 2). These so negative values could be due to "account effect" which means that more precipitation is corresponded with more negative isotopic values for water (Rozanski et al. 1993).

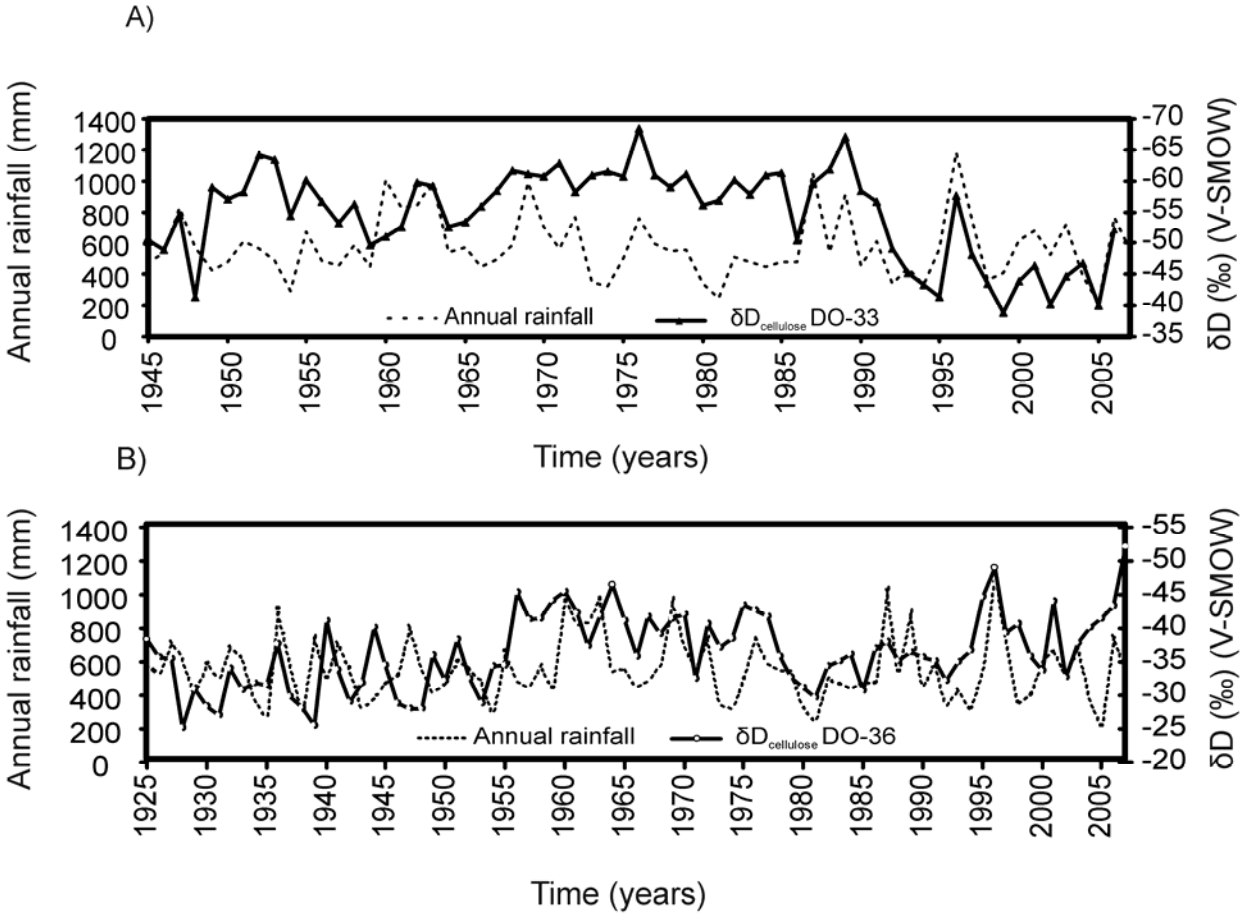

Figure 2: Hydrogen isotopic composition of cellulose from Pinus pinea individual trees: DO-33 (A) and DO-36 (B) in contrast to annual rainfall (doted line). The deuterium scale was inverted.

Variability of carbon isotopic values in Pinus pinea growth rings.

The carbon isotopes ratios of tree rings in this study were expressed in terms of discrimination against ^{13}C to avoid the observed negative trend of δ ^{13}C in atmospheric CO_2 for the 20th century due to increased CO_2 emissions from burning fossils fuels. This atmospheric trend is apparent in most δ^{13}C tree ring series (e.g. Freyer & Belacy 1983, Epstein & Krishnamurthy 1990, Leavitt & Lara 1994, Feng & Epstein 1995a, b, February & Stock 1999, Treydte et al. 2001). Consequently, we used equation 1 (Farquhar et al. 1982) to express carbon isotopes values.

The Δ^{13}C values range from 16.2‰ to 20.5‰ for DO-33 and from 14.4‰ to 19.8‰ for DO-36 (see Fig.3a and Tab.1) for the periods 1945-2007 and 1925-2007, respectively.

High correlation between both carbon isotopic series were found ($r=0.75$, $p<0.001$). This may indicate that the factors that regulate the stomatal conductance are the same for the two trees.

From 1950 to 2007, a negative linear tendency in Δ^{13}C tree ring values was observed (Fig. 3a). This change was about 0.03‰ per year (as suggested from the slope of the regression line, (Fig. 3a).This trend concords with the increase of the mean annual temperature during the last century (Fig. 3b). In fact, we found a negative significant relationship between Δ^{13}C values and average annual temperature ($r= -0.36$, $p<0.05$ for DO-33 and $r= -0.55$, $p<0.05$ for DO-36). There is a period of about 20 years (from 1925 to ~1945) where Δ^{13}C values are higher (using data from DO-36, which have an average of 18.0‰ ±0.7) indicating more favorable weather conditions during this period. In fact the climatic data from meteorological stations considered in this study indicates lower mean annual temperatures during that period (~17.3ºC) (Fig.3). From 1950, we observed a trend towards a decrease in Δ^{13}C values of tree rings with a minimum in the period 1988-1991, which is also the period with the highest annual temperatures (~18.7ºC).

Monthly linear correlations were also investigated between $\Delta^{13}C$ values and monthly temperature and monthly rainfall (Table 2b). Summer temperature (in particular July temperature) seem to being the most important factor that regulate the stomatal conductance for the two trees (as inferred of the highest correlation coefficients, r= -0.36, $p<0.001$ for DO-36 and r= -0.20, $p<0.1$ for DO-33). In regards to monthly rainfall, spring and summer rainfall are the most influential on the carbon isotopic composition of these tree rings (specifically March rainfall for DO-36 and March, July and August rainfall for DO-33).

These results suggest that in years when temperatures are higher (particularly, summer temperature) and rainfall are scarce (specifically spring and summer rainfall), plant stomata close to minimize water loss (stress defence mechanism). Consequently, the concentration of CO_2 inside the leaves decreases and therefore discrimination against CO_2 molecules with the heavy isotope ($^{13}CO_2$) is smaller (Francey & Farqhuar 1982, Ehleringer et al. 1993, Saurer et al. 1995).

Table 1: Isotopic variability between individual trees. Maximum, minimum and average hydrogen data and carbon isotopic discrimination values.

Tree	**δD(‰) (V-SMOW)$_{max}$**	**δD(‰) (V-SMOW)$_{min}$**	**δD(‰) (V-SMOW)$_{aver}$**	**$\Delta^{13}C$(‰) (V-PDB)$_{max}$**	**$\Delta^{13}C$(‰) (V-PDB)$_{min}$**	**$\Delta^{13}C$(‰) (V-PDB)$_{aver}$**
DO-33	-38.9	-68.4	-54.5±7.3	20.5	16.2	18.3±0.9
DO-36	-25.1	-52.2	-36.5±5.4	19.8	14.4	16.9±1.1

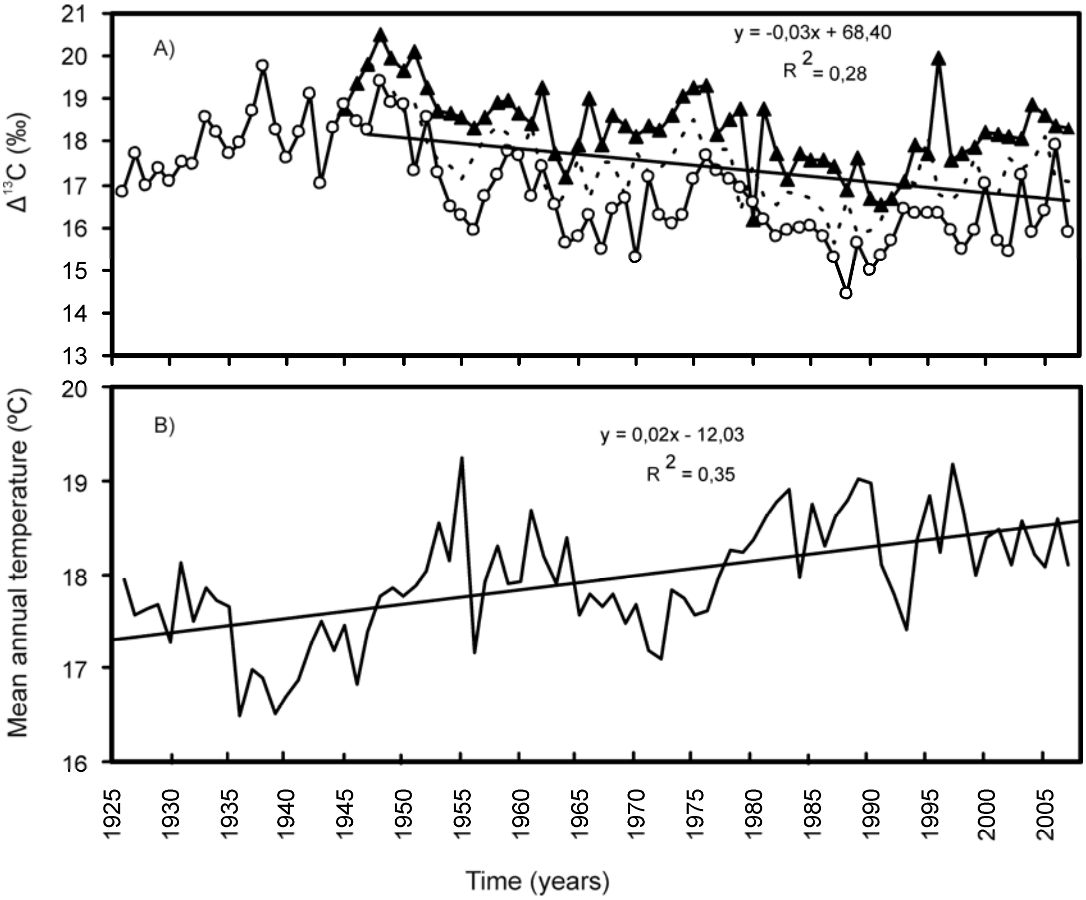

Figure 3: A) Carbon isotopic discrimination of cellulose from DO-33 individual (black triangles) and DO-36 individual (white circles). Regression line from the average values of the two individuals (straight black line). B) Mean annual temperature of the study area and regression line (straight black line).

Table 2: A) Correlation coefficient (r) and significance (p) between hydrogen isotopic data of the two trees (DO-36 and DO-33) with monthly rainfall. B) Correlation coefficient (r) and significance (p) between carbon isotopic data of the two trees (DO-36 and DO-33) with monthly rainfall and monthly temperature. In bold letter, the highest correlation coefficient.

A)

Rainfall	**Months**	**r**	**p**
DO-36	January	-0.20	p<0.1
	February	-0.23	p<0.05
	August	**-0.24**	p<0.05
	November	-0.20	p<0.1
	December	-0.20	p<0.05
DO-33	March	-0.22	p<0.1
	April	-0.25	p<0.05
	June	**-0.26**	p<0.05
	August	-0.22	p<0.1

B)

Temperature	**Months**	**r**	**p**
DO-36	May	-0.22	p<0.05
	June	-0.20	p<0.1
	July	**-0.36**	p<0.001
	August	-0.22	p<0.05
DO-33	July	-0.20	p<0.1
Rainfall	**Months**	**r**	**p**
DO-36	March	0.27	p<0.05
DO-33	March	**0.34**	p<0.01
	July	0.22	p<0.1
	August	0.23	P<0.1

Conclusions

The results obtained in this study suggest that the isotopic composition of tree rings of *Pinus pinea* from Doñana National Park is a useful tool to understand the isotopic variability associated to climate shifts and with global change on Mediterranean regions.

Despite analytic difficulty of hydrogen stable isotopes in tree rings cellulose of *Pinus pinea,* significant correlation exist between climate data of Doñana National Park and isotope data. The driest years agree with the less negatives isotopic values, due to ground and surface water evolution evaporation effects which are reflected in more positives values of δD of tree rings cellulose. On the other hand, the very rainy years corresponded to more negatives isotopic values.

The most important monthly rainfall in the isotopic composition of hydrogen is summer rainfall for the two trees, however the rainfall of the major recharge periods (like winter and spring) is also reflected in the hydrogen isotopic composition of cellulose.

At the study area an increase in mean annual temperature during the last century was observed which seem to be causing stress on the tree mass as is manifested by a decrease in carbon isotopic discrimination. In fact, we found significant relationships between mean annual temperature, summer temperature (May, June, July and August) with Δ^{13}C data. The spring rainfall (March rainfall) and summer rainfall (July and August rainfall) was also significantly correlated with Δ^{13}C data. This means that temperature (specifically summer temperature) and seasonal rainfall (particularly summer and spring rainfall) are the main factors that controls stomatal conductance on *Pinus pinea* from Doñana National Park.

Acknowledgments

This study was supported by Junta de Andalucía projects P06-RNM-02362 and Ministerio de Medio Ambiente projects 107/2003. We acknowledge El Equipo de Seguimiento de Doñana for meteorological data provided from the measuring station Palacio de Doñana. Special thanks are given to Fernando Hiraldo (manager of Estación Biológica de Doñana, CSIC) and David Paz for their help in the selection of sampling areas.

References

Brendel, O., Iannetta, P. P. M., Stewart, D. (2000). A Rapid and Simple Method to Isolate Pure Alpha-Cellulose. *Phytochem. Anal.* 11: 7–10

Burk, R. L., Stuiver, M., (1981). Oxygen isotope ratios in trees reflect mean annual temperature and humidity. *Science* 211 (4489): 1417– 1419.

Edwards, T. W. D., Aravena, R. O., Fritz, P., Morgan, A. V. (1985). Interpreting paleoclimate from ^{18}O and ^{2}H in plant cellulose: Comparison with evidence from fossil insects and relict permafrost in south-western Ontaio. *Can J Earth Sci* 22**:** 1720–1726.

Edwards, T.W.D., Fritz, P. (1986). Assessing meteoric water composition and relative humidity from ^{18}O and ^{2}H in wood cellulose Paleoclimatic implications for southern Ontario, Canada. *Appl Geochem* 1**:** 715–723.

Ehleringer, J.R., Dawson, T.E., (1992). Water uptake by plants: perspective from stable isotope composition. *Plant Cell Environ* 15: 1073–1082

Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (1993). Introduction: water use in relation to productivity. In: Stable isotopes and plant carbon–water relations. (Ed Ehleringer, J.R., Hall, A.E., Farquhar, G.D.). *Academic Express*, New York, 3–18.

Epstein, S., Krishnamurthy, R.V., (1990). Environmental information in the isotopic record in trees. *Philos T R Soc* 330A: 427–439.

Ewe, S.M.L., L.D.L. Sternberg. (2002). Seasonal water-use by the invasive exotic, *Schinus terebinthifolius*, in native and disturbed communities. *Oecologia* 133: 441–448.

Farquhar, G.D., Richards, R.A., (1984). Isotopic composition of plant carbon correlates with water use efficiency of wheat genotypes. *Aust J Plant Phisyol* 11: 539–552.

Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., (1989). Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Phys* 40: 503-537.

February, E.C., Stock, W.D., (1999). Declining trends in the ^{13}C/^{12}C ratio of atmospheric carbon dioxide from tree rings of South African *Widdringtonia cedarbergensis*. *Quaternary Res* 52: 229–236.

Feng, X., Epstein, S., (1995a). Carbon isotopes of trees from arid environments and implications for reconstructing atmospheric CO_2 concentration. *Geochim et Cosmochim Ac* 59: 2599–2608.

Feng, X., Epstein, S., (1995b). Climatic temperature records in δD data from tree rings. *Geochim et Cosmochim Ac* 59: 3029–3037.

Feng, X.H., Krishnamurthy, R.V., Epstein, S., (1993). Determination of D/H ratios of non-exchangeable hydrogen in cellulose: a method based upon the cellulose-water exchange reaction. *Geochim et Cosmochim Ac* 57: 4249–4256.

Francey, R. J., Farquhar, G. D. (1982). An explanation of ^{13}C/^{12}C variations in trees. *Nature* 297**:** 28–31.

Freyer, H.D., (1979). On the ^{13}C record in tree rings. Part I. ^{13}C variation in Northern hemispheric trees during the last 150 years. *Tellus* 31: 124–137.

Freyer, H.D., Belacy, N., (1983). ^{13}C/^{12}C records in Northern Hemispheric trees during the past 500 years- anthropogenic impact and climate superpositions. *J Geophys Res* 88: 6844-6852

García Novo, F., Martín, A., and Toja, J., (2007). La frontera de Doñana. *Universidad de Sevilla*, Seville. 317 pp.

IPCC, (2007). Climate change, fourth assessment report. (*Ed Cambridge University Press*). London.

Kozlowski, T.T. (1984). Flooding and plant growth. *Academic Press*, New York.

Krishnamurthy, R.V., Epstein, S., (1985). Tree ring D/H ratio from Kenya, East Africa and its palaeoclimatic significance. *Nature* 317: 160–162.

Lawrence, J.R., White, J.W.C., (1984). Growing season precipitation from D/H ratios of Eastern White Pine. *Nature* 311: 558–560.

Leavitt, S.W., Lara, A., (1994). South American tree rings show declining δ^{13}C trend. *Tellus* 46B: 152–157.

Leuenberger, M. (2007). To what extent can ice core data contribute to the understanding of plant ecological developments of the past? In: *Stable isotopes as Indicators of ecological change* (T.E. Dawson and R.T.W.Siegwolf Eds) 14**:** 211-232*.*

McCarroll, D., Loader, N.J., (2004). Stable isotope is tree rings. *Quaternary Sci Rev*
23, 771–801.

Ramesh, R., Bhattacharya, S.K., Gopalan, K., (1988). Climatic significance of variations in the widths and stable isotope ratios of tree rings. *British Archaeological Reports, British Series*, vol. 196 (ii): 591–609.

Resco, V., Querejeta, J.I., Ogle, K., Voltas, J., Sebastia, M.T., Serrano-Ortiz, P., Linares, J.C., Moreno-Gutierrez, C., Herrero, A., Carreira, J.A., Torres Canabate, P., Valladares, F., (2009). Stable isotopes views on ecosystem function: challenging or challenged? *Biology Letters* 6: 287-289.

Roden. J.S., Lin, G., Ehleringer, J.R., (2000). A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochim et Cosmochim Ac*, Vol. 64, No. 1: 21–35,

Roden J. S., Ehleringer J. R. (1999b) Leaf water δD and δ^{18}O observations confirm robustness of Craig–Gordon model under wide ranging environmental conditions. *Plant Physiol.* 120: 1165–1173.

Rozanski, K., Araguás–Araguás, L., Gonfiantini, R. (1993). Isotopic patterns in modern global precipitation. In: Climate Change in Continental Isotopic Records (ed. P. K. Swart et al.), *Geophysical Monograph* 78, pp. 1–36. American Geophysical Union.

Sauer, P.E., , Schimmelmann, A., Sessions, A., Topalov, K., (2009). Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. *Rapid Commun. Mass Spectrom.* 2009; 23: 949–956

Saurer, M., Borella, S., Schweingruber, F., Siegwolf, R.T.W., (1997). Stable carbon isotopes in tree rings of beech: climatic versus site-related influences. *Trees* 11: 291–297.

Saurer, M., Siegenthaler, U., Schweingruber, F., (1995). The climate–carbon isotope relationship in tree rings and the significance of site conditions.*Tellus* 47B: 320–30.

Schimmelman, A., (1991). Determination of the concentration and stable isotopic composition of non-exchangeable hydrogen in organic matter. *Analy. Chem.* 63, 2456–2459.

Serrano, L., Reina, M., Martıín, G., Reyes, I., Arechederra, D., León, D. and Toja, J. (2006). The aquatic systems of Doñana (SW Spain): watersheds and frontiers. *Limnetica*, 25: 11–32.

Sternberg, L. S. L., DeNiro, M. J., Savidge, R. A., (1986*).* Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis *Plant Physiology* 82: 423–427.

Treydte, K., Schleser, G.H., Schweingruber, F., Winiger, M., (2001). The climatic significance of δ^{13}C in subalpine spruce (Lötschental, Swiss Alps)—a case study with respect to altitude, exposure and soil moisture. *Tellus* 53B, 593–611.

Waterhouse, J.S., Switsur, V.R., Barker, A.C., Carter, A.H.C., Robertson, I., (2002). Oxygen and hydrogen isotope ratios in tree rings: how well do models predict observed values? *Earth Planet Sc Lett* 201: 421–430

Yapp, C. J., Epstein, S., (1982). A re-examination of cellulose carbon bound hydrogen -D measurements and some factors affecting plant-water D/H relationships. *Geochim et Cosmochim Ac* 46**:** 955–965.