Hydrogen and Carbon Isotope Ratios of Selected Species of a Mediterranean Macchia Ecosystem

R. Valentini, G. E. Scarascia Mugnozza, J. R. Ehleringer

Functional Ecology, Volume 6, Issue 6 (1992), 627-631.

Functional Ecology 1992
6, 627–631

Hydrogen and carbon isotope ratios of selected species of a mediterranean macchia ecosystem

R. VALENTINI, G. E. SCARASCIA MUGNOZZA and J. R. EHLERINGER*

*Department of Forest Science and Environment (DISAFRI), University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy and *Department of Biology and Stable Isotope Ratio Facility for Environmental Research, University of Utah, Salt Lake City, Utah 84112, USA*

Summary

1. Various woody species belonging to the mediterranean macchia ecosystem have been studied in respect of their strategies of water resources acquisition and water-use efficiencies, using stable isotopes analysis.
2. Evergreen species seem to depend more on rain-water utilization than the deciduous ones, which utilize ground-water almost exclusively.
3. This pattern is paralleled by the distribution of $\delta^{13}C$ values which show a greater water-use efficiency for the evergreen species *vs* the deciduous ones.

Key-words: Resources acquisition, stable isotopes, water-use efficiency

Functional Ecology (1992) **6**, 627–631

Introduction

Recent plant ecophysiological studies in mediterranean ecosystems have focused on repeated observations of leaf-level gas exchange over extended periods (Turner, Schulze & Gollan 1984; Tenhunen *et al.* 1987), pointing out the need for long-term integrated responses to understand physiological adaptations and species performance in specific environments. An equivalent understanding of the role of below-ground resources acquisition is also necessary to understand changes in shoot gas exchange, especially in environments subjected to extensive periods of water deficit. Stable isotope analyses at natural abundance levels can serve both the need for integrated aspects of gas exchange and also for improving our understanding of below-ground resource acquisition (Rundel, Ehleringer & Nagy 1988). The relative abundance of ^{13}C *vs* ^{12}C (expressed as $\delta^{13}C$, carbon isotope ratio, in delta notation) in plant tissues provides information on relative water-use efficiency over extended periods (Farquhar, Ehleringer & Hubick 1989). At the same time, the relative abundance of D *vs* H (δD, hydrogen isotope ratio) of xylem sap is particularly valuable for determining utilization of specific water resources (White *et al.* 1985; Flanagan & Ehleringer 1991). When the hydrogen isotope composition of different waters (e.g. rain- or ground-water) is known, it is possible to determine the extent to which the different sources of water that are used by different species (Sternberg & Swart 1987; Dawson & Ehleringer 1991; Flanagan & Ehleringer 1991).

The macchia ecosystem represents a widely spaced, diffuse plant community along the coast of the Italian peninsula. The aim of the present study was to conduct a parallel analysis of the leaf $\delta^{13}C$ (to determine relative water-use efficiencies) and xylem sap δD (to determine water source) of various species belonging to this ecosystem, in order to provide a better understanding of the relationship between water source utilization and water-use efficiency.

Materials and methods

The study was conducted at the ENEL (National Agency of Electric Energy) reservation near Montalto di Castro, Italy (latitude 42°22′N, longitude 11°32′E). The site is situated on the Tirrenian coast on a flat plain limited at the shoreline by sand-dunes. The vegetation is a mediterranean 'macchia' ecosystem, made up of evergreen shrubs and trees. The woody species used in the present investigation are *Quercus ilex* L., *Juniperus oxycedrus* L. var. *macrocarpa* S. et S., *Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Q. cerris* L. and *Q. pubescens* L.

Leaf area index of the stand was on average 3·5. Samples were collected following the natural distribution of the different species along a transect from the sea inland (Fig. 1). On 5 August 1990, four plants of each species were selected and two samples were collected from each plant for determination of water source through xylem sap analysis. Plant source water was sampled by cutting a section of stem wall away (at least 50 cm) from transpiring leaf surfaces in order to prevent possible enrichment fractionation. Stem segments were immediately inserted in glass tubes, sealed with a rubber stopper, and taken to the laboratory. Samples were kept frozen until water was extracted. To determine the

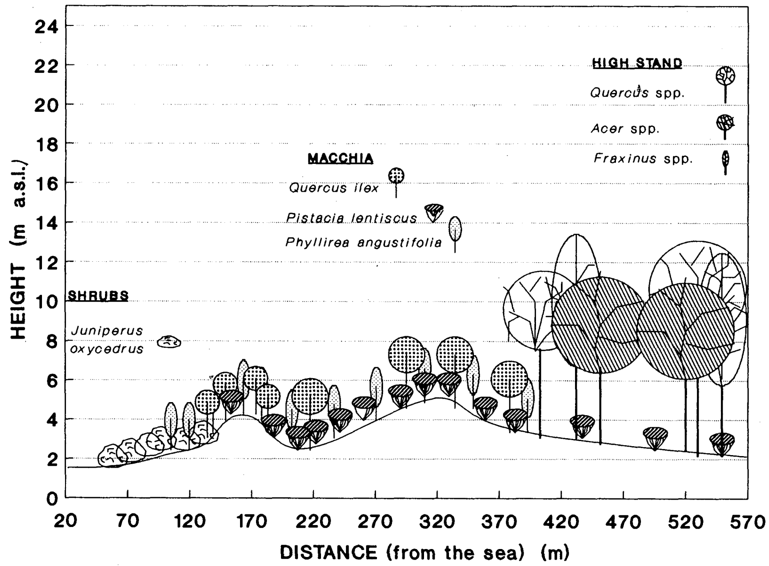

Fig. 1. Vegetation distribution in the experimental site along a sea inland gradient.

possible water source used by these plants, sampling occurred following a rain event in mid-summer. Precipitation was collected and stored in a glass vial. Soils were extracted at depths ranging from 10 to 100 cm. Well-water was sampled as an indicator of ground-water isotopic composition. Again these samples were stored in glass vials until isotopic analyses were conducted. Water from stems was cryogenically extracted under vacuum (Dawson & Ehleringer 1991) and hydrogen in water was reduced to its diatomic form using zinc (Coleman *et al.* 1982). The procedure was modified slightly in that combustion occurred at 500 °C. This modification to a higher temperature of combustion resulted in greater precision than observed in the original study of Coleman *et al.* (1982). The deuterium content was measured on a Finnigan MAT, model delta S, isotope ratio mass spectrometer. Deuterium content is expressed in delta notation as δD (‰) relative to the standard mean ocean water (SMOW) standard:

$$\delta D = (R_{sample}/R_{standard}{}^{-1}) \times 1000‰$$

where R_{sample} and $R_{standard}$ represent the molar deuterium/hydrogen ratios of sample and standard, respectively. The overall precision of preparation and analysis is ±1·0‰.

For carbon isotope determination, five to 10 subunit leaves of the study species were sampled randomly in the upper part of the crown (on the same trees as for xylem sap hydrogen isotope ratio), dried and finely ground before the combustion process. Carbon isotope ratio ($\delta^{13}C$), relative to the PeeDee belemite (PDB) standard (using equivalent equation as above), was determined by combusting samples in an elemental analyser coupled to the same isotope ratio mass spectrometer. Overall precision of the ^{13}C analysis was ±0·11‰.

The salinity content of xylem sap was estimated by means of the analysis of conductivity of effusate of stem segments placed in distilled and deionized water for 24 h at room temperature.

Results and discussion

The study area has a typical mediterranean climate with an annual precipitation of 860 mm. Sampling occurred 3 days following a rain event of 6·5 mm. This was the first rain following a 25-day drought; the total rainfall for the period from the beginning of June to the date of sampling was 44 mm. The rain-water had a δD value of −24·7±1·7‰, while the ground-water was −43·1±1·8‰ (Table 1). The isotopic composition of rain-water varies seasonally (Dansgaard 1964) and the observed value was typical for this part of Italy during the summer (Yurtsever & Gat 1981; IAEA 1986). The well-water value was close to the long-term mean precipitation input value for Genoa (380 km distant), the closest long-term IAEA monitoring station (Yurtsever & Gat 1981). Ground-water δD values are relatively stable throughout the year, representing a long-term average of the isotopic composition of annual precipitation that falls in the area.

Table 1. Values of δD from soil samples collected at various soil depths

Soil depth (cm)	δD (‰)
10	−26·6
30	−27·9
60	−30·7
100	−31·1
Ground-water (12 m)	−41·1

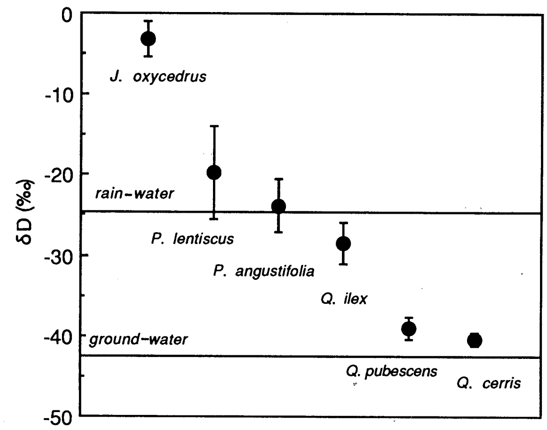

Fig. 2. Distribution of δD values (±1 SE) among the study species. Rain-water and ground-water levels are indicated by solid lines.

The upper soil layers (10 and 30 cm) had δD values similar to those of the recent precipitation events (Table 1), consistent with the high infiltration capacity known for these soils. The deepest soil layers showed more negative δD values, consistent with precipitation values expected during the cooler, winter recharge period (Dansgaard 1964; Yurtsever & Gat 1981; IAEA 1986).

Differences in the isotopic composition of ground-water and rain-water were large enough to allow evaluation of the relative uptake rates of surface and deep-water sources by analysis of the isotopic composition of stem xylem sap water. As shown in Fig. 2, the variation of δD among species was quite large, ranging from −3·2‰ to −40·4‰.

Juniperus oxycedrus var. *macrocarpa* showed the highest δD values (−3·2±2·3‰), which were more positive than the observed rain-water values. Sea-water isotopic composition was not measured in the present work, but to interpret these data, it should be noted that the 0‰ reference value is the SMOW and the actual sea-water composition at the experimental site should not be very different from this value. The most likely explanation of the observed high δD values is that *J. oxycedrus* was using a different water source combination other than rain-water and ground-water. A likely explanation is that *J. oxycedrus* was using a mixture of sea-water and recent precipitation. *Juniperus oxycedrus* is the first woody species to appear on the dunes; it occupies sites very close to the seashore. The soils on the front of the dunes are frequently infiltrated by sea-water, and pools of a saline and freshwater mixture commonly occur in this coastal environment. Berger & Heurteaux (1985) have observed freshwater lenses beneath the dunes in hydrodynamic equilibrium upon the saline aquifer on the sandy dunes in the Camargue (France); these lenses are thought to sustain the growth of *J. phoenicea*. While Berger & Heurteaux (1985) excluded the possible utilization of saline water by *Juniperus*, our data indicated the uptake of a mixture of fresh and saline water by *J. oxycedrus*.

Similar situations in which a mixture of fresh and saline water are utilized by plants in coastal areas have been observed by Sternberg & Swart (1987) in the Florida Keys. In addition to mangroves, for which the direct utilization of saline water is well known, various species at the mangrove margin were found to utilize a mixture of fresh and saline water.

The evergreen broad-leaved species *Pistacia lentiscus*, *Phyllirea angustifolia* and *Q. ilex* showed δD values of −19·7±5·8‰, −23·8±3·3‰, and −28·4±2·6‰, respectively, indicating utilization of a mixture of both rain-water and ground-water. In particular, *Pistacia lentiscus* seemed to show slightly higher δD values, which can result, partially, from a utilization of the mixture of fresh and saline water in lenses beneath the dune. Indeed *Pistacia lentiscus* root system penetrates quite deep in the soil. Spiegel-Roy, Mazigh & Evenari (1977) observed on clones of *Pistacia vera* and *Pistacia altantica* planted in Negev highlands of Israel, a root distribution up to 210 cm in depth.

In a comparative study of water relations on a number of mediterranean shrubs, Rhizopoulou & Mitrakos (1990) observed that *Phyllirea latifolia* and *Q. ilex* showed lower water potentials than *Pistacia lentiscus* during the summer drought and therefore concluded that these species can withdraw water from very dry soils. In particular *Phyllirea latifolia* seemed to reach the lowest water potential values and have the most hardy turgor loss point among the shrubs of the macchia ecosystem. Perhaps this result is now not surprising, since it appears that these species do not have access to a stable water source (such as ground-water) during the summer drought periods.

The two deciduous oaks, *Q. pubescens* and *Q. cerris* showed δD values of −39·0±1·33‰ and −40·4±0·8‰, respectively, indicating an almost exclusive utilization of ground-water during the summer drought. These two species did not switch to utilizing surface moisture following rain events as noted for pine trees by White *et al.* (1985). Instead they follow a pattern noted by Ehleringer *et al.* (1991), where many long-lived perennials relied on only deep-soil-water even after substantial summer rain. These two oak species usually colonize the bottom parts of the dune, where water conditions are more favourable, due to the emerging water table and the soil texture, which becomes richer in clay.

The difference in water utilization between evergreen and deciduous species is particularly evident. It is reasonable to hypothesize that deciduous species, due to their relative short period of growth, which essentially coincides with the driest months, should depend more on constant sources of water, like ground-water, than on scarce and highly unpredict-

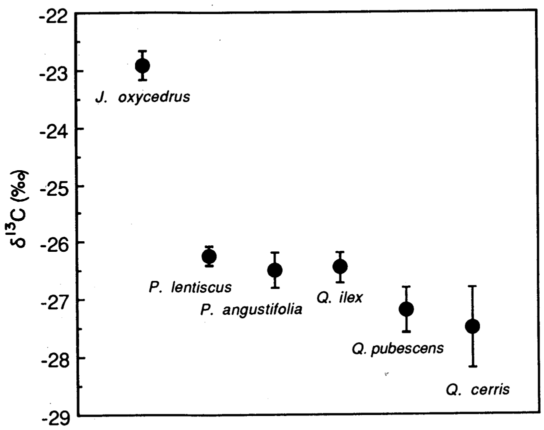

Fig. 3. Distribution of carbon isotopes discrimination values (±1 SE) among the study species.

able precipitation events. This tendency is also supported by the relatively higher occurrence of xylem embolism in deciduous oaks than in evergreen species (Borghetti *et al.* 1992), during periods of water shortage. The evergreen species can modulate their growth patterns throughout the year in accordance with periods of maximum soil-water availability.

The observed range of $\delta^{13}C$ values of leaves of different species indicated that all species were C_3 plants (Farquhar *et al.* 1988) (Fig. 3). However, there was an unusually large variation in isotope values. Particularly evident was the relatively high value of $\delta^{13}C$ for *J. oxycedrus* (−22·9±0·2‰), indicating a rather high carbon discrimination. *Pistacia lentiscus*, *Q. ilex* and *Phillyrea angustifolia* showed almost equivalent $\delta^{13}C$ values (−26·24±0·18‰, −26·44±0·27‰ and −26·49±0·32‰, respectively), while the two deciduous oaks, *Q. pubescens* and *Q. cerris* had lower $\delta^{13}C$ values (−27·19±0·39‰ and −27·5±0·7‰, respectively).

The $\delta^{13}C$ value paralleled the salinity content of stem water effusates of the study species (Table 2). The higher $\delta^{13}C$ values, especially marked for *J. oxycedrus*, suggest that water-use efficiency and exposure to sea-water were positively correlated. Other studies have documented an increase in $\delta^{13}C$ values in response to increasing salinity in the environment (Guy, Reid & Krause 1980; Neales, Fraser & Roksandic 1983; Seemann & Critchley 1985; Flanagan & Jeffries 1989). Therefore, the

Table 2. Conductivity values of stem water effusates for the study species, expressed on a dry weight basis

Species	C (μmhos mg^{-1})
Juniperus oxycedrus	750±41
Pistacia lentiscus	410±32
Phillyrea angustifolia	360±18
Quercus ilex	250±28
Q. pubescens	120±15
Q. cerris	140±30

utilization of a mixture of saline and freshwater by *J. oxycedrus*, as indicated by δD values, was associated with a higher water-use efficiency and reflects a certain 'halophytic' tendency by this species, which is the only mediterranean-climate woody species to grow close to the shoreline. Differences in water-use efficiency between *Q. ilex* and *Q. pubescens* seedlings have been observed in gas-exchange studies (Epron & Dreyer 1990). Over a range of pre-dawn water potential between −2·0 and −4·0 MPa, seedlings of *Q. ilex* showed a steeper slope of the assimilation–stomatal conductance relationship, indicating a lower intercellular CO_2 concentration than *Q. pubescens*. Although the difference in $\delta^{13}C$ is rather small, it seems there is a tendency in *Q. pubescens* to be more water conservative than *Q. cerris*, an ecological trait that is quite well known in the mediterranean area. The evergreen species showed $\delta^{13}C$ values significantly more positive ($P<0{\cdot}05$) than the deciduous species. This would suggest that the evergreen species of the macchia ecosystem have a higher water-use efficiency than the deciduous species.

Together these data suggest significant partitioning of water sources at the community level. Dependence on more reliable water sources (e.g. groundwater) was associated with less efficient use of water, whereas utilizing and presumably being dependent on summer moisture input had more conservative water-use efficiencies. This is probably due to differences in water potential for species utilizing the scarcer and more unreliable summer rains, *vs* deep-rooting species, as noted by DeLucia & Schlesinger (1991) and Flanagan, Ehleringer & Marshall (1992), who have observed a negative relationship between plant water-use efficiency and water potential for a variety of perennial species.

Acknowledgements

We wish to thank G. Matteucci for sample collection in the field and C. Cook for his help in isotope analyses.

References

Berger, A. & Heurteaux, P. (1985) Response of *Juniperus phoenicea* on sandy dunes in the Camargue (France) to water and saline constraints in summer. *Vegetatio* **62**, 327–333.

Borghetti, M., De Angelis, P., Raschi, A., Scarascia Mugnozza, G. & Valentini, R. (1992) Concurrent measurement of sap velocity and cavitation in broadleaf trees. *Global Changes and Plant Water Relations* (eds. M. Borghetti, A. Raschi & J. Grace). Cambridge University Press, Cambridge, in press.

Coleman, M.L., Shepherd, T.J., Durham, J.J., Rouse, J.E. & Moore, G.R. (1982) Reduction of water with zinc for hydrogen isotope analysis. *Analytical Chemistry* **54**, 993–995.

Dansgaard, W. (1964) Stable isotopes in precipitation. *Tellus* **16,** 436–468.

Dawson, T.E. & Ehleringer, J.R. (1991) Streamside trees that do not use stream water. *Nature* **350,** 335–337.

DeLucia, E.H. & Schlesinger, W.H. (1991) Resource-use efficiency and drought tolerance in adjacent Great Basin and Sierran plants. *Ecology* **72,** 51–58.

Ehleringer, J.R., Phillips, S.L., Schuster, W.F.S. & Sandquist, D.R. (1991) Differential utilization of summer rains by desert plants: implications for competition and climate change. *Oecologia* **88,** 430–434.

Epron, D. & Dreyer, E. (1990) Limitation d'origine stomatique et non stomatique de la photosynthése de trois espéces de chene soumises á la sécheresse: comparaison de mesures d'echanges gazeux et de fluorescence de la chlorophylle. *Annales des Sciences Forestieres* **47,** 435–450.

Farquhar, G.D., Hubick, K.T., Condon, A.G. & Richards, R.A. (1988) Carbon isotope fractionation and plant water-use efficiency. *Stable Isotopes in Ecological Research* (eds. P.W. Rundel, J.R. Ehleringer & K.A. Nagy), pp. 41–54. Springer-Verlag, Berlin.

Farquhar, G.D., Ehleringer, J.R. & Hubick, K.T. (1989) Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40,** 503–537.

Flanagan, L.B. & Ehleringer, J.R. (1991) Stable isotope composition of stem and leaf water: applications to the study of plant water use. *Functional Ecology* **5,** 270–277.

Flanagan, L.B. & Jeffries, R.L. (1989) Effect of increased salinity on CO_2 assimilation, O_2 evolution and δC^{13} values of leaves of *Plantago maritima* L. developed at low and high NaCl levels. *Planta* **178,** 377–384.

Flanagan, L.B., Ehleringer, J.R. & Marshall, J.D. (1992) Differential uptake of summer precipitation among co-occurring trees and shrubs in a the Southwestern United States. *Plant Cell and Environment* **15,** 831–836.

Guy, R.D., Reid, D.M. & Krause, H.R. (1980) Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions. *Oecologia* **44,** 241–247.

IAEA (1986) *Environmental Isotope Data No. 8: World Survey of Isotope Concentration in Precipitation (1980–1983).* IAEA, Vienna.

Neales, T.F., Fraser, M.S. & Roksandic, Z. (1983) Carbon isotope composition of the halophyte *Disphyma clavellatum* (Haw.) Chinnock (Aizoaceae), as affected by salinity. *Australian Journal of Plant Physiology* **10,** 437–444.

Rhizopoulou, S. & Mitrakos, K. (1990) Water relations of evergreen sclerophylls. I. Seasonal changes in the water relations of eleven species from the same environment. *Annals of Botany* **65,** 171–178.

Rundel, P.W., Ehleringer, J.R. & Nagy, K.A. (1988) *Stable Isotopes in Ecological Research.* Springer-Verlag, Berlin.

Seemann, J.R. & Critchley, C. (1985) Effects of salt stress on growth, ion concentration, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta* **164,** 151–162.

Spiegel-Roy, P., Mazigh, D. & Evenari, M. (1977) Response of *Pistachio* to low moisture conditions. *Journal of the American Society of Horticultural Science* **102,** 470–473.

Sternberg, L.S.L. & Swart, P.K. (1987) Utilization of freshwater and ocean water by coastal plants of southern Florida. *Ecology* **68,** 1898–1905.

Tenhunen, J.D., Catarino, F.M., Lange, O.L. & Oechel, W.C. (1987) *Plant Response to Stress. Functional Analysis in Mediterranean Ecosystems.* NATO ASI series, vol. G 15. Springer-Verlag, Berlin.

Turner, N.C., Schulze, E.-D. & Gollan, T. (1984) The responses of stomata and leaf gas exchange to vapour pressure deficits and soil water content. I. Species comparisons at high soil water contents. *Oecologia* **63,** 338–342.

White, J.W.C., Cook, E.R., Lawrence, J.R. & Broecker, W.S. (1985) The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta* **49,** 237–246.

Yurtsever, Y. & Gat, J.R. (1981) Atmospheric waters. *Stable Isotope Hydrology Deuterium and Oxygen-18 in the Water Cycle* (eds. J.R. Gat & R. Gonfiantini), pp. 103–142. IAEA, Vienna.

Received 26 March 1992; revised 9 July 1992; accepted 29 July 1992