Physiological Responses of *Laurus azorica* and *Viburnum rigidum* to Drought Stress: Osmotic Adjustment and Tissue Elasticity

By

A. M. González-Rodríguez*), M. S. Jiménez*), D. Morales*),

G. Aschan**) and R. Lösch**)

With 4 Figures

Summary

González-Rodríguez A. M., Jiménez M. S., Morales D., Aschan G. & Lösch R. 1999. Physiological responses of *Laurus azorica* and *Viburnum rigidum* to drought stress: Osmotic adjustment and tissue elasticity. – Phyton (Horn, Austria) 39 (2): 251–263, with 4 figures. – English with German summary.

Osmotic adjustment and pressure-volume parameters were studied in leaf tissue samples of two woody plants of the Canarian laurel forest during one month under drought stress. The osmotic potential at full turgor decreased from –2.04 to –3.52 MPa in *Laurus azorica* (Seub.) Franco and from –1.91 to –2.59 MPa in *Viburnum rigidum* Vent. The osmotic potential at the turgor loss point decreased from –2.9 to –3.9 MPa in *L. azorica* and did not change significantly in *V. rigidum* (–2.9 to –3 MPa). The maximal bulk modulus of elasticity increased during the water stress period from 20 to 91 MPa and from 12 to 31 MPa in *L. azorica* and *V. rigidum*, respectively.

Proline contents of *L. azorica* leaves increased sharply at a water potential of –2.6 MPa amounting to final values of approximately 4 mg g^{-1} DW. In contrast, proline concentration in *V. rigidum* did not change although leaf water potential values of –3.5 MPa were reached. Our results indicate significant osmotic adjustment due to

*) A. M. González-Rodríguez, M. S. Jiménez, D. Morales, Dpto. Biología Vegetal, Universidad de La Laguna, E-38207 La Laguna, Tenerife, Spain.

**) G. Aschan, R. Lösch, Abt. Geobotanik, H.-Heine-Universität Düsseldorf, Universitätsstrasse 1, 26.13, D-40225 Düsseldorf, Germany.

252

drought stress in *L. azorica* but not in *V. rigidum*. The particular physiological strategy of each species could explain their distribution within the laurel forest.

Zusammenfassung

GONZÁLEZ-RODRÍGUEZ A. M., JIMÉNEZ M. S., MORALES D., ASCHAN G. & LÖSCH R. 1999. Die physiologische Antwort von *Laurus azorica* und *Viburnum rigidum* auf Trockenstress: osmotische Anpassung und Gewebeelastizität. – Phyton (Horn, Austria) 39 (2): 251–263, 4 Abbildungen. – Englisch mit deutscher Zusammenfassung.

An Proben von Blattgeweben zweier Holzpflanzen des Kanarenlorbeerwaldes wurde die osmotische Anpassung und die Druck-Volumen-Parameter während eines Monats unter Trockenstress untersucht. Das osmotische Potential bei vollem Turgor sank von –2,04 auf –3,52 MPa in *Laurus azorica* (Seub.) Franco und von –1,91 auf –2,59 MPa in *Viburnum rigidum* Vent. Das osmotische Potential am Turgorverlustpunkt sank von –2,9 auf –3,9 MPa in *L. azorica*, es änderte sich jedoch nicht signifikant in *V. rigidum* (–2,9 auf –3 MPa). Der maximale Elastizitätskoeffizient stieg während der Periode mit Wasserstress von 20 auf 91 MPa und von 12 auf 31 MPa in *L. azorica* bzw. *V. rigidum*.

Der Prolingehalt in Blättern von *L. azorica* stieg ziemlich genau bei einem Wasserpotential von –2,6 MPa an und betrug schließlich ca. 4 mg g^{-1} DW. Hingegen änderte sich die Prolinkonzentration in *V. rigidum* nicht, obwohl das Blattwasserpotential Werte um –3,5 MPa erreichte. Unsere Untersuchungen weisen auf eine signifikante osmotische Anpassung unter Trockenstress bei *L. azorica,* nicht aber bei *V. rigidum* hin. Die besonderen physiologischen Eigenheiten der einzelnen Pflanzen erklären ihre Verbreitung innerhalb des Lorbeerwaldes.

Introduction

Plant tissue responses to water stress include a decline in osmotic potential, due to either the passive concentration of solutes arising from dehydration, or to a net accumulation of solutes in the cell. The latter process has been defined as osmotic adjustment (TURNER 1986). Osmotic adjustment is a widespread response to water deficits in herbaceous and woody plant species (for review MORGAN 1984, LUDLOW 1989, NOBEL 1991, KRAMER & BOYER 1995). The accumulating solutes are, in general, inorganic ions (in vacuoles), sugars, organic acids, amino acids and quaternary ammonium compounds, by preference in the cytoplasmatic compartment (JONES & al. 1981, HANSON & HITZ 1982, McCUE & HANSON 1990, INGRAM & BARTELS 1996). In particular, it has been shown that the amino acid proline accumulates as a compatible solute in the cytoplasm keeping the osmotic balance stable within the cell during the course of osmoregulation (ASPINALL & PALEG 1981, BHASKARAN & al. 1985, RAGGI 1994, HARE & CRESS 1997).

The Canarian laurel forest is an evergreen forest, considered as a relic of the Tertiary Mediterranean flora. The majority of the nearly twenty tree species belonging to different families which make up this formation, are endemic to the Canarian or Macaronesian region (SANTOS 1990). This forest

evolved and still exists under a relatively uniform climate with small temperature oscillations and high moisture (HUETZ DE LEMPS 1969, CEBALLOS & ORTUÑO 1976) although some dry periods can occur in summer.

Physiological studies about this forest indicate that its species are not very prone to endure strong environmental stress (LÖSCH 1993) and field studies on stomatal behaviour and transpiration of laurel forest trees (ZOHLEN & al. 1995, JIMÉNEZ & al. 1996, GONZÁLEZ-RODRÍGUEZ 1998) showed a weak ability of these trees for physiological regulation of water loss.

Nothing is known up to date about the osmotic adjustment characteristics of laurel forest plants. We have therefore carried out a study on the water relations of two woody laurel forest species, *L. azorica* and *V. rigidum,* when affected by increasing drought stress. *L. azorica* is a main tree species in these forests and *V. rigidum* is a shrub growing at the edges of the forest. The objectives were: 1) to determine whether osmotic adjustment and changes in leaf tissue elasticity can be found by analysis of leaf water relations and 2) to characterize the amount of changes in leaf tissue proline content that might be contributing to osmotic adjustments in these two species.

Material and Methods

Small trees (7 or 8 years old) of *L. azorica* and *V. rigidum* were grown in pots (50 l soil volume) under controlled conditions (well-watered) in a greenhouse during winter and in an open nursery in summer. From the start of the experiment, the plants were subjected to the summer drought conditions and water deficit was applied by withholding water. Matric soil water potential was followed by means of tensiometric measurements (Irrometer, USA) with the tensiometer cup at a depth of 20 cm.

Sampling of leaves for experimental investigations was done several times during the course of the experiment. Each time four lots of samples were taken before midday on typical, clear days in July. They consisted of leaves exposed to sunlight. One lot of samples was rehydrated in distilled water for approximately 18 h to make pressure volume (P-V) curves. A second lot was prepared without previous resaturation to analyze the actual osmotic potential by cryoscopical methods, in the third one the current water potential was measured, and the forth, after being weighed, was plunged into liquid nitrogen and stored for proline content determination.

Water potential determination

P-V curves of *L. azorica* and *V. rigidum* leaf tissue were constructed with data from fully resaturated leaves using consecutive determinations of relative water contents and water potentials when leaf disks were left to dry. Three parallel measurements were carried out with samples from each species. Water potential was determined psychrometrically using a WesCor HR 33 microvoltmeter (Wescor, Logan, USA) connected consecutively to six C52 sample chambers with humidity determinations in the dew point mode.

254

Relative water content (RWC) was calculated on the same leaf disks where the water potential was determined and expressed as 100 times the ratio of the actual water content and the water content at saturation. Actual water content was calculated by estimating the fresh weight (FW) of the leaf disks at each step in the desiccation curve and the dry weight (DW) at the end, after oven drying at 105 °C for at least 24 h. Water content at saturation was calculated by estimating the saturated fresh weight (SW) after a previous rehydration for 18 h.

P-V data (osmotic potential at full turgor, π_{sat}, maximal bulk modulus of elasticity, ε_{max}, relative water content and osmotic potential at turgor loss point, RWC_0 and π_0 respectively) were analyzed utilizing non-linear evaluation software (PROC NLIN, SAS, 1988) according to ANDERSEN & al. 1991.

Osmotic potential determination by cryoscopy

After weighing, the samples were dried in an oven at 60 °C and dry weight and water content determined. The dried material was ground in a ball mill (Dangoumill 300/Prolabo, D), double distilled water added, left shaking overnight and centrifuged at 4000 rpm and 20 °C for 10 min. 50 μl of the supernatant were taken, put into the sample holder of the osmometer (Osmomat, GONOTEC, Berlin, D), and the freezing point depression was measured. From this, the osmotic potential was calculated taking into account the dilution with respect to the original water content by the resuspension of the ground sample material.

Current water potential

Current water potential was measured during the water stress treatment immediately after leaf sampling. The Wescor HR 33 microvoltmeter (Wescor, Logan, USA) was used in connection with C52 sample chambers for water potential determinations in the dew point mode as described before.

Proline determination

The deep-frozen leaves were thawed and ground with mortar and pestle under addition of 5 ml H_2O. Proline content of these samples was determined following the procedure of TROLL & LINDSLEY 1955 with some modifications. The homogenous, water-diluted plant material was extracted in boiling water for ten minutes. Thereafter, it was mixed with a foline-type ion exchange resin (Röhm & Haas, D) to make the analysis specific for proline. After centrifugation equal aliquots (2.5 ml) of the water-diluted supernatant, of ninhydrin reagent (2.5 g ninhydrine + 40 ml phosphoric acid 85 %, concentration 6 mol l^{-1} + 60 ml water-free acetic acid) and of water-free acetic acid were mixed in test tubes and boiled for one hour at 100 °C. When cooled to room temperature, 4 ml xylene were emulsified in this solution. While stored overnight in a refrigerator (7 °C) the red-colored proline-xylene complex accumulated in the unpolar xylene phase that separates above the aqueous phase. The extinction of 510 nm light by the xylene phase was measured thereafter spectroscopically, and the proportional proline concentration was determined using calibration data derived from measurements of a series of pure proline solutions. Sample water contents and dry weights were calculated from weight determinations with oven-dried parallel samples.

Results

Climatic conditions during the treatment were characterized by a summer drought period without precipitation except for a strong thunderstorm on day 17. Tensiometrically determined soil water potentials decreased in all pots from −0.01 MPa at the beginning to −0.08 MPa at the end of the nearly one month drought period. The decrease occurred steadily, interrupted by a transient improvement from −0.07 to −0.04 MPa on day 17.

Current leaf water potential measured by dew point hygrometry during the drought treatment decreased from −1.95 to −4.62 MPa in *V. rigidum* and from −2.55 to −4.00 MPa in *L. azorica*. Mean values of the osmotic potential measured by cryoscopy, at the beginning of the desiccation course, were −2.58 and −2.00 MPa in *V. rigidum* and *L. azorica* respectively, decreasing to −5.25 and −3.96 MPa just after a fortnight of withholding water (Figs. 1A, B). The cryoscopically determined osmotic potentials were similar to those estimated for the respective actual water contents from the P-V data.

The water content/dry weight ratio values decreased within the four weeks of drought from 1.42 to 0.73 g g^{-1} in *V. rigidum* and from 1.31 to 0.80 g g^{-1} in *L. azorica* (52 % and 62 % of the water content at saturation, respectively) (Figs. 1C, D). Due to the summer storm a slight recuperation of the values could be seen on day 17.

We could not calculate the turgor potential as [water potential minus osmotic potential] from the direct measurements, because both values were measured with different methods and each one presents systematic errors. But the distance between the curves of water potential and osmotic potential may be compared (Figs. 1A, B). Then it is obvious − most clearly during the first fortnight of the drying cycle − that *V. rigidum* roughly keeps the distance between the curves with the osmotic potential always being more negative than the water potential (by 0.5 MPa if values are derived from P-V-analysis, by 1.0 MPa in the case of cryoscopically determined values), but *L. azorica* does not; on the contrary the values are all quite similar indicating rather small turgor potentials even during the first two weeks of the drought course. This indicates that the water loss rate per change of tissue water relations was more stable in *V. rigidum* than in *L. azorica*.

P-V curve analysis during the drought treatment showed significant differences in the two species studied. The evaluation of the untransformed values by non-linear analysis showed that the turgor loss point of *L. azorica* leaf tissue shifted to more negative water potentials (π_0 from −2.9 to −3.9 MPa) and to values of RWC_0 by 9 % higher than at the start of treatment. No significant differences between the P-V-curve shapes were found during the water stress period in *V. rigidum*, turgor loss point remained almost at the same position throughout the treatment (Table 1).
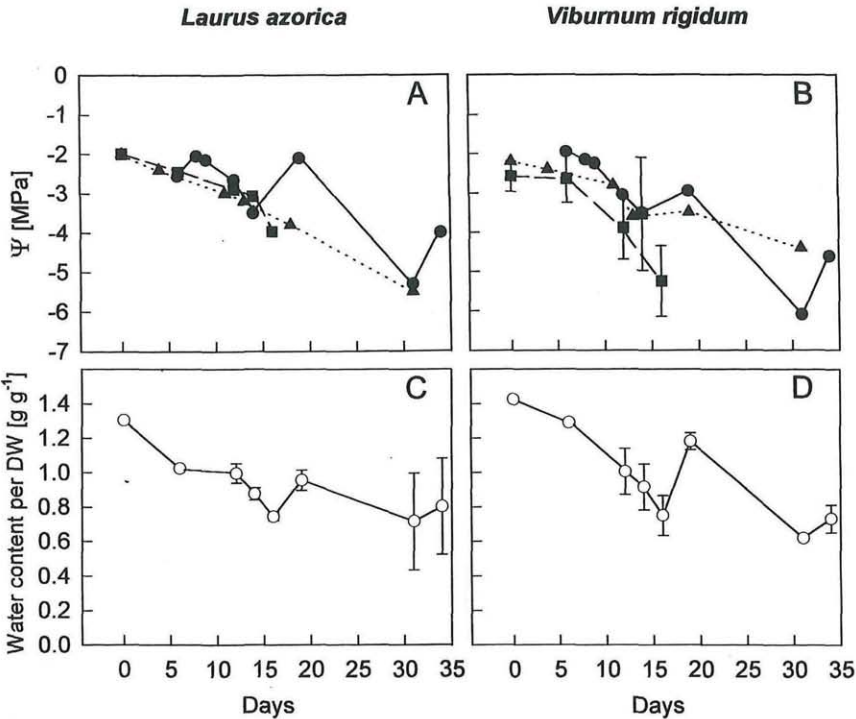
256

Fig. 1. Changes in the current water potential determined by dew point hygrometry (●), osmotic potential measured by cryoscopy (■) and derived from pressure volume curves for the appropriate water content values (▲) (A, B); and water content per dry weight ratios (○) during the drought treatment (C, D), in *Laurus azorica* and *Viburnum rigidum* respectively (mean values with standard deviation).

Table 1.

Parameters of leaf water relations derived from pressure volume curves: Relative water content (RWC_0) and osmotic potential (π_0) at turgor loss point in *Laurus azorica* and *Viburnum rigidum* during the experimental period of increasing water stress (mean values with standard deviations).

Laurus azorica	0 days	4 days	11 days	13 days	17 days	30 days
RWC_0 [%]	80 ± 0.03	91 ± 0.0	86 ± 0.02	89 ± 0.03	86 ± 0.03	91 ± 0.03
π_0 [MPa]	-2.9 ± 0.24	-2.3 ± 0.19	-2.7 ± 0.06	-2.6 ± 0.13	-3.1 ± 0.11	-3.9 ± 0.42
Viburnum rigidum						
RWC_0 [%]	76 ± 0.01	80 ± 0.02	81 ± 0.02	79 ± 0.0	81 ± 0.0	84 ± 0.02
π_0 [MPa]	-2.9 ± 0.08	-2.8 ± 0.13	-2.3 ± 0.35	-2.9 ± 0.14	-2.6 ± 0.1	-3.0 ± 0.12

Values of π_{sat} ranged from -2.04 MPa to -3.50 MPa in *L. azorica* and from -1.90 to -2.60 MPa in *V. rigidum* during the drought treatment (Fig. 2). While numerical values of π_{sat} at the end of the experiment were 1.72 and
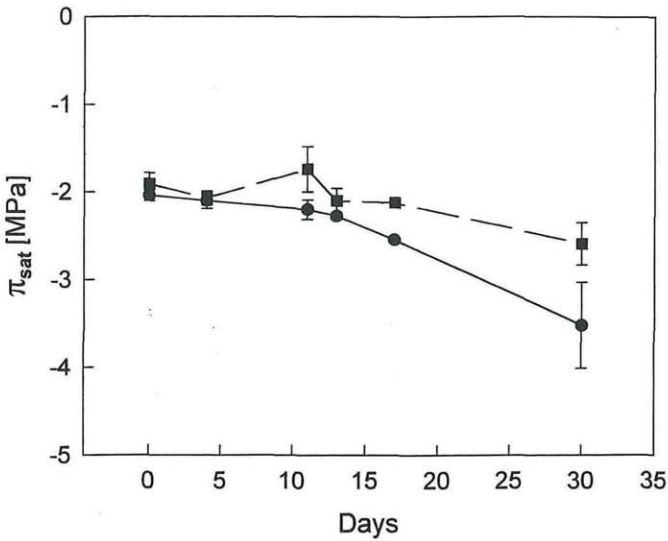
Fig. 2. Osmotic potential at full turgor (π_{sat}) of *Laurus azorica* (●) and *Viburnum rigidum* (■) during the drought stress treatment (mean values with standard deviation).
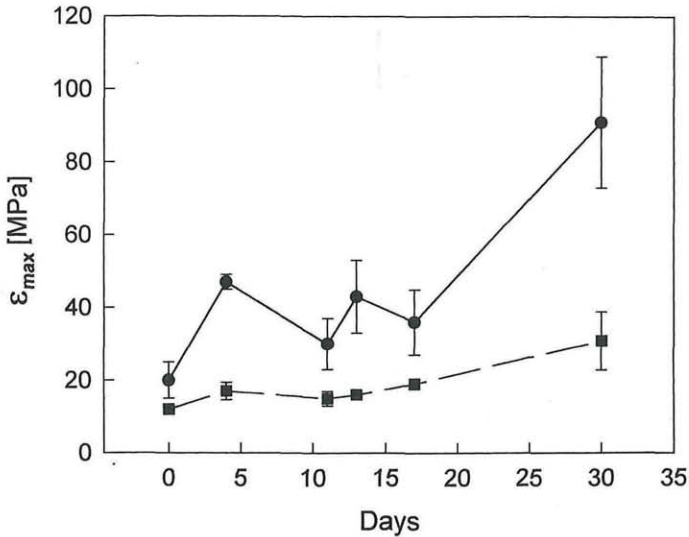

Fig. 3. Mean values of maximal bulk modulus of elasticity (ε_{max}) of *Laurus azorica* (●) and *Viburnum rigidum* (■) during the drought stress treatment. Vertical bars represent standard deviation.
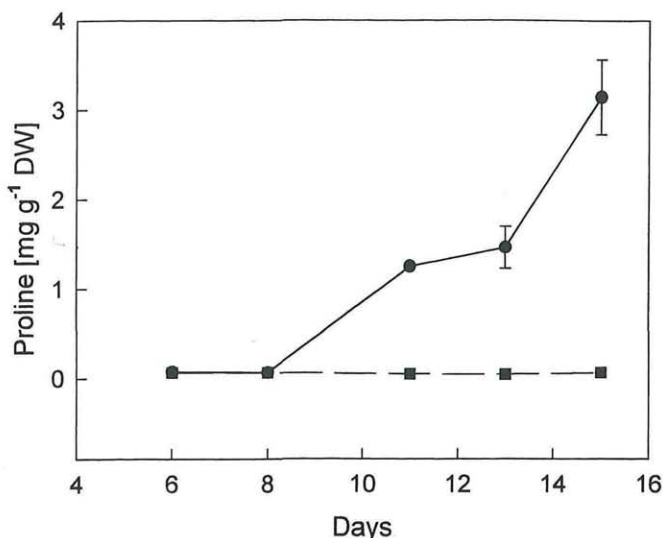
258

Fig. 4. Mean values of proline content during the drought stress treatment in *Laurus azorica* (●) and *Viburnum rigidum* (■). Vertical bars represent standard deviation.

1.36 times those at the beginning in *L. azorica* and *V. rigidum* respectively, values of modulus of elasticity increased 4.6 and 2.6 times in the same order of the species (Fig. 3). Mean values of ε_{max} ranged from 20 to 91 MPa in *L. azorica* and from 12 to 31 MPa in *V. rigidum* respectively, indicating a drastic decrease in tissue elasticity during the course of desiccation in *L. azorica*.

During the drought period, proline levels in *L. azorica* leaves increased enormously. *V. rigidum* showed no changes of proline content during the water stress treatment (Fig. 4). The increase of proline started one week after withholding water from the *L. azorica* plants, approximately at the same time when π_{sat} commenced decreasing according to the P-V-data. During the second week, the proline level increased steeply and two weeks later it had reached the order of magnitude of 4 mg g^{-1} DW. (Due to technical problems the last determinations were not as exact as the previous measurements and are not therefore included in Fig. 4).

Discussion

Ontogenetic and stress-dependent changes of tissue water relations include osmotic and cell wall elasticity adjustments that can effectively moderate the water balance of plants in response to dry conditions (TURNER 1986, SCHULZE & al. 1987, RICHTER & KIKUTA 1989). A decrease in osmotic potential can be brought about by an increase in the number of

osmoles of solutes in the symplasm or by a decrease in the weight of water in the symplasm (TYREE & JARVIS 1982).

In our experiment osmotic potentials decreased with time in the two studied species, with the only exception of the 17th day when the summer storm took place. Discrepancies between cryoscopically determined osmotic potentials and those derived from P-V-curves at the actual water contents were not higher than 0.3 MPa in *V. rigidum* and 0.1 MPa in *L. azorica*. This indicates that the dilution of the cellular solutes by apoplastic water that often occurs by the cryoscopic method (TYREE 1976) was not important in these species and therefore the symplastic water made up the majority of the leaf water content in *V. rigidum* and particularly in *L. azorica*. Therefore the cell wall material of the stiffened-malacophyllous *V. rigidum* and the coriaceous *L. azorica* leaves was not imbibed with much water. This might be an explanation for the rather rigid structure of these (and other) laurel forest tree leaves.

Water stress has been shown to alter the elastic properties of tissues but the effects are not always the same. Increases of leaf elasticity have been reported in some species (DAVIES & LAKSO 1979, CASTRO-JIMÉNEZ & al. 1989) and decreases observed in others (TYREE & al. 1978, MEIER & al. 1992). Increased tissue elasticity has been proposed as a mechanism for turgor maintenance (WEATHERLEY 1970, JONES & al. 1981, PAVLIK 1984) and as an additional one for decreasing the osmotic potential at turgor loss point, providing the capacity to maintain growth (WEATHERLEY 1970, HINCKLEY & al. 1980, PAVLIK 1984). On the other hand, BOWMAN & ROBERTS 1985 suggest that decreases in tissue elasticity over the long term result in lower turgor and thus lower tissue water potential, a condition that could enhance water uptake from dry soils. This could be the case in *L. azorica* leaves.

L. azorica displayed a large increase of ε_{max} during the experiment, indicating a decrease in tissue elasticity with increasing drought stress. This drastic change of ε_{max} was also accompanied by a 9 % change of RWC_0 that occurred at the high level of 82 to 91 %. At the same time π_{sat} dropped by 1.5 MPa and the osmotic potential at zero turgor by approximately 1 MPa. Both changes together brought about a functionally efficient osmotic adjustment which being accompanied by tissue elasticity changes, the overall water uptake became enhanced by lower water potentials at higher RWC levels. On the other hand *V. rigidum* showed lower values of ε_{max} in general and they increased very little during most of the treatment period. Only the samples of the last day with very dry leaves showed a higher increase. RWC_0 values increased during the experimental period by approximately 7 % so the RWC_0 range of turgescence became narrowed, π_{sat} decreased during this time course by 0.7 MPa and the osmotic potential at RWC_0 did not change significantly. As a result, a substantial

260

improvement of the capability of *V. rigidum* leaf tissues to gain water under a restricted water supply was not achieved.

Concentration changes of compatible solutes with water stress are important to maintain osmotic equilibrium between the cytoplasm and the vacuole during an osmoregulatory process (ASPINALL & PALEG 1981, HARE & CRESS 1997). Differences between the osmoregulatory efficiency of the two laurel forest species became obvious from the tissue concentration changes of the compatible solute proline during progress of drought (Fig. 4). While the proline levels in *V. rigidum* leaves were always low, those of *L. azorica* leaves strongly increased within two weeks. This increase started at a leaf water saturation deficit of approximately 25 % (RWC of 75 %) a value lower than RWC_0, but only half of the saturation deficit that brings about vitality loss of the *L. azorica* leaves (LÖSCH 1993). MAESTRI & al. 1995 showed that the concentrations of proline and betaine in coffee plant leaves correlated very well with the osmotic potential at zero turgor and that the accumulation of these compatible solutes in the cytoplasm followed closely the accumulation of solutes in the vacuoles. In *L. azorica* the increase of proline is more than sufficient to account for the decrease of π_{sat} during the observed course of osmotic adjustment, provided it accumulates in the cytoplasm exclusively. If the cytoplasmic volume is taken as 1/20 of the total volume of the symplasm (SITTE 1991) and the leaf water and proline contents on day 15 of the treatment are 0,75 g H_2O g^{-1} DW and 3.3 mg proline g^{-1} DW respectively, the cytoplasmic proline concentration will be 0.77 mol l^{-1}. This corresponds roughly to an osmotic potential of −1.8 MPa assuming that the osmotic coefficient is 1.0. This is more than twice as much as required for the π_{sat} depression determined by P-V analysis on this day in relation to the value when the experiment started, so part of this proline may function as an osmotically inactive protectant stabilizing the cytoplasmic macromolecular structures (HARE & CRESS 1997). Our determination of the π_{sat} depression by P-V analysis on day 30 showed a decrease of 1.46 MPa, on this day the proline content was of approximately 4 mg g^{-1} DW which is more than sufficient for this lowering. At this stage of the experiment the leaf water content showed 55 % of the saturation value which is very close to the lethal value of this species (LÖSCH 1993).

Considering the water balance, it is possible to distinguish two contrasting strategies, hydrostable and hydrolabile (LARCHER 1995). It is known that the Mediterranean *L. nobilis* has a hydrostable water balance, buffering the water status by sensitive adjustments of transpiration. It has a "water saving" strategy that is combined, however, with the capability of recovering even minimal water losses by dropping leaf water potential drastically (LO GULLO & SALLEO 1988). Previous studies of *L. azorica* showed a weak ability for physiological regulation of leaf water relations

under water-supplied conditions (ZOHLEN & al. 1995, GONZÁLEZ-RO-GRÍGUEZ 1998) and a relatively high transpiration during the year (JIMÉNEZ & al. 1996). These studies were made in the field when water supply was not limited by the climatic situation, it has still to be analyzed whether the sensitivity of L. *azorica* stomata increases under water stress conditions, but its efficient osmotic adjustment under drought conditions and the large water potential fluctuations make us think that it follows the hydrolabile rather than the hydrostable strategy. The latter term could be used to characterize V. *rigidum*, in which relatively moderate changes in tissue elasticity under desiccation stress are not accompanied by solute increases in its cells. Thus it has poor osmotic adjustment, and according to un-published preliminary findings it probably has a higher stomatal control. This species is a shrub growing at the edges of the forest and under natural conditions it might often experience short-term reductions of ambient humidity. V. *lantana,* a related shrub from eastern Austria did not open its stomata during a drought period of 19 days (HINCKLEY & al. 1980) clearly showing a hydrostable strategy, while V. *tinus*, a Mediterranean macchia shrub showed an intermediate behaviour (LÖSCH 1993).

Species living in the same environment can present different water balance variants and representatives of hydrostable as well as hydrolabile functional types have also been found among the Mediterranean scler-ophyll shrubs (LARCHER 1995). Gradients in species composition of the laurel forests from the edges to the interior and in the altitudinal distribution of the taxa on the mountain slopes (CEBALLOS & ORTUÑO 1976) could possibly be related to the particular physiological strategy of each individual species.

References

ANDERSEN M. N., JENSEN C. R. & LÖSCH R. 1991. Derivation of pressure-volume curves by a non-linear regression procedure and determination of apoplastic water. – J. Exp. Bot. 42: 159–65.

ASPINALL D. & PALEG L. G. 1981. Proline accumulation: Physiological aspects. – In: PALEG L. G. & ASPINALL D. (Eds.), The physiology and biochemistry of drought resistance in plants, pp. 206–241. – Academic Press, New York.

BHASKARAN S., SMITH R. H. & NEWTON R. J. 1985. Physiological changes in cultured sorghum cells in response to induced water stress. I. Proline. – Plant Physiol. 79: 266–269.

BOWMAN D. W. & ROBERTS S. W. 1985. Seasonal changes in tissue elasticity in chaparral shrubs. – Physiol. Plant. 65: 233–236.

CASTRO-JIMÉNEZ Y., NEWTON R. J., PRICE H. J. & HALLIWELL R. S. 1989. Responses to drought stress in *Microseris* species differing in nuclear DNA content. – Amer. J. Bot. 76: 789–795.

CEBALLOS L. & ORTUÑO F. 1976. Vegetación y Flora Forestal de las Canarias Occidentales. – Cabildo Insular de Santa Cruz de Tenerife.

DAVIES F. S. & LAKSO A. N. 1979. Diurnal and seasonal changes in leaf water potential components and elastic properties in response to water stress in apple trees. – Physiol. Plant. 46: 109–114.

GONZÁLEZ-RODRÍGUEZ A. M. 1998. Caracterización fotosintética de árboles de la laurisilva canaria (*Laurus azorica, Persea indica y Myrica faya*). Thesis. Univ. La Laguna, Spain.

HANSON A. D. & HITZ W. D. 1982. Metabolic responses of mesophytes to plant water deficits. – Ann. Rev. Plant Physiol. 33: 163–203.

HARE P. D. & CRESS W. A. 1997. Metabolic implications of stress-induced proline accumulation in plants. – Plant Growth Regulation 21: 79–102.

HINCKLEY T. M., DUHME F., HINCKLEY A. R. & RICHTER H. 1980. Water relations of drought hardy shrubs: Osmotic potential and stomatal reactivity. – Plant, Cell & Envir. 3: 131–140.

HUETZ DE LEMPS A. 1969. Le climat des Iles Canaries. – CNRS, SEDES, Paris.

INGRAM J. & BARTELS D. 1996. The molecular basis of dehydration tolerance in plants. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 377–403.

JONES M. M., TURNER N. C. & OSMOND C. B. 1981. Mechanisms of drought resistance. – In: PALEG L. & ASPINALL D. (Eds.), Physiology and biochemistry of drought resistance in plants, pp. 15–37. – Academic Press, New York.

JIMÉNEZ M. S., CERMÁK J., KUCERA J. & MORALES D. 1996. Laurel forests in Tenerife, Canary Islands: The annual course of sap flow in Laurus trees and stand. – J. Hydrol. 183: 307–321.

KRAMER P. J. & BOYER J. S. 1995. Water relations of plants and soils. – Academic Press. San Diego.

LARCHER W. 1995. Physiological plant ecology. – Springer. Berlin.

LÖSCH R. 1993. Water relations of Canarian laurel forest trees. – In: BORGHETTI M., GRACE J. & RASCHI A. (Eds.), Water transport in plants under climatic stress, pp. 243–246. – Cambridge Univ. Press, Cambridge.

LO GULLO M. A. & SALLEO S. 1988. Different strategies of drought resistance in three Mediterranean sclerophyllous trees growing in the same environmental conditions. – New Phytol. 108: 267–276.

LUDLOW M. M. 1989. Strategies of response to water stress. – In: KREEB K. H., RICHTER H. & HINCKLEY T. M. (Eds.), Structural and functional responses to environmental stresses, pp. 269–281. – SPB Academic Publishing bv. The Hague, The Netherlands.

MAESTRI M., DA MATTA F. M., REGAZZI A. J. & BARROS R. S. 1995. Accumulation of proline and quaternary ammonium compounds in mature leaves of water stressed coffee plants (*Coffea arabica* and *C. canephora*). – J. Hort. Sci. 70: 229–233.

McCUE K. F. & HANSON A. D. 1990. Drought and salt tolerance: towards understanding and application. – Trends Biotech. 8: 358–362.

MEIER C. E., NEWTON R. J., PURYEAR J. D. & SEN S. 1992. Physiological responses of loblolly pine (*Pinus taeda L.*) seedlings to drought stress: Osmotic adjustment and tissue elasticity. – J. Plant Physiol. 140: 754–760.

MORGAN J. M. 1984. Osmoregulation and water stress in higher plants. – Ann. Rev. Plant Physiol. 35: 299–319.

NOBEL P. S. 1991. Physicochemical and environmental plant physiology. – Academic Press, San Diego, California.

PAVLIK B. M. 1984. Seasonal changes of osmotic pressure, symplastic water content and tissue elasticity in the blade of dune grasses growing in situ along the coast of Oregon. – Plant Cell & Envir. 7: 531–539.

RAGGI V. 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. – Physiol. Plant. 91: 427–434.

RICHTER H. & KIKUTA S. B. 1989. Osmotic and elastic components of turgor adjustments in leaves under stress. – In: KREEB K. H., RICHTER H. & HINCKLEY T. M. (Eds.), Structural and functional responses to environmental stresses, pp. 129–137. – Academic, The Hague.

SANTOS A. 1990. Evergreen forests in the Macaronesian region. – Council of Europe, Strassbourg.

SCHULZE E. D., ROBICHAUX R. H., GRACE J., RUNDEL P. W. & EHLERINGER J. R. 1987. Plant water balance. – BioScience 73: 30–37.

SITTE P. 1991. Einleitung und Morphologie. – In: STRASBURGER E., Lehrbuch der Botanik, 33. Aufl. – Fischer-Verlag, Stuttgart, New York.

TROLL W. & LINDSLEY J. 1955. A photometric method for the determination of proline. – J. Biol. Chem. 215: 655–660.

TURNER N. C. 1986. Adaptation to water deficits: A changing perspective. – Aust. J. Plant Physiol. 13: 175–190.

TYREE M. T. 1976. Negative turgor pressure in plant cells: Fact or fallacy? – Canadian Journal of Botany 54: 2738–2746.

—   & JARVIS P. G. 1982. Water in tissues and cells. – In: LANGE O. L., NOBEL P. S., OSMOND C. B. & ZIEGLER H. (Eds.), Physiological plant ecology II, Encyclopedia of plant physiology, New series, Vol. 12 B, pp. 35–77. – Springer Verlag, Berlin, Heidelberg, New York.

—   , CHEUNG Y. N. S., MACGREGOR M. E. & TALBOT A. J. B. 1978. The characteristics of seasonal and ontogenetic changes in the tissue water relations of *Acer, Populus, Tsuga* and *Picea.* – Can. J. Bot. 56: 635–647.

WEATHERLEY P. E. 1970. Some aspects of water relations. – Advances in Botanical Research 3: 171–206.

ZOHLEN A., GONZÁLEZ-RODRÍGUEZ A. M., JIMÉNEZ M. S., LÖSCH R. & MORALES D. 1995. Transpiración y regulación estomática en árboles de la laurisilva canaria medidos en primavera. – Vieraea 24: 91–104.