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Photosynthetic performance and water use efficiency of the fern *Cheilanthes persica*

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Abstract

Morphological, anatomical and physiological frond traits of *Cheilanthes persica* (Bory) Mett. *ex* Kuhn were studied to analyze its adaptive strategy. Mean frond life span is about 340 d. Mature fronds are characterized by 91 g m^{-2} areal dry mass (ADM) and 217 g m^{-2} succulence. The reduction of frond water content in July (dehydration phase) caused a 51 % decrease in frond surface area (SA). Fronds were dry in August (desiccation phase); nevertheless, in September they showed an increased SA (rehydration phase). Chlorophyll (Chl) *a/b* ratio, above 3, and the well developed palisade parenchyma (two layers, total thickness of 103.9 μm) are typical for sun leaves. Chl and carotenoid contents and net photosynthetic rate (P_N) increased during frond development until the highest values in April-May (maturity phase). When mean air temperature reached 31.3 °C, stomatal conductance (g_s) decreased by 34 % and P_N by 33 %. The high pigment contents can dissipate the excess of radiant energy, particularly under unfavourable conditions, when P_N is low. Rather high P_N was found during the rehydration stage. The pronounced decline of mesophyll activity during the declining phase was confirmed by the lowest P_N.

Additional key words: carotenoids; chlorophyll; cuticle; dehydration; desiccation tolerance; mesophyll; palisade and spongy parenchyma; stomatal conductance; succulence.

Introduction

It is important for plants growing in an environment where water is available for only a limited part of the year to be able to make full use of favourable periods for vegetative and reproductive activity, and to survive the unfavourable season. Stress periods differ in length and intensity, and may coincide with different growth phases which vary in their sensitivity to water stress (Beadle *et al.* 1985, Larcher 1995,

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Pereira and Chaves 1995). Poikilohydric plants can tolerate loss of more than 95 % of water (Walter 1955, Schwab and Heber 1984, Gaff 1989). Numerous reports compare gas exchange and drought tolerance among ecologically different species (Larcher 1995, Lösch and Schulze 1995), but less information is available on the water relations of resurrection or poikilohydric plants (Gaff 1989, Beckett 1997). A substantial portion of ferns are desiccation tolerant (Killian 1951, Morello 1954, Oppenheimer and Halevy 1962, Hevly 1963, Nobel 1978, Eickmeier 1980, Gaff 1989).

The main objective of this paper was to evaluate drought resistance of the fern *C. persica*, comparing its morphological, anatomical, and physiological frond traits. Maximizing carbon gain relative to water use is a whole-plant phenomenon involving a complex balance between several plant functions, and differences in water use are expressed in all plant forms and functions (Ball and Passioura 1995). Water use efficiency (WUE) was monitored during the year to clarify water economy and adaptive strategies of the fern.

Materials and methods

Plants: *C. persica* is a rhizomatose species belonging to xeric ferns (Marsh 1914, Nayar 1963, Knobloch and Volz 1964, Nobel 1978). The genus *Cheilanthes* is represented in Europe by seven species (Tutin *et al.* 1993), and it is distributed from the Mediterranean area to the western Asia (Greuter and Raus 1982). All species of *Cheilanthes* in the Mediterranean Region show a clear tendency to populate arid or semiarid habitats, growing usually on bare rocks or walls (Nardi *et al.* 1978, Pichi Sermolli 1979). Correspondingly, most of these species show morphological adaptations to xeric conditions, such as dense pubescence and small mesophyll cell size (Hevly 1963, Nobel 1978). In Italy, *C. persica* is confined to a restricted area in the Apennines close to Ravenna (Rossi and Bonafede 1995).

25 plants of *C. persica* were collected in May 1994 at Mount Mauro, locality "Cassano" (44°14'5"N, 11°42'30"E, Italy), where the species grows from 100 to 510 m a.s.l. on chalky substrate, mostly in rocky N-E exposed habitats (Rossi and Bonafede 1995). The transplanted plants were grown in the Botanical Garden of the University of Rome "La Sapienza" (Italy), and cultivated in containers with their own substrate, under local environment. The mean air temperature measured in Rome during January 1995-January 1996 was 15.7 °C, the mean minimum air temperature of the coldest month (January) was 4.8 °C, and the mean maximum air temperature of the hottest month (July) was 31.3 °C. Total annual rainfall was 650 mm, the lowest (15 %) level being in summer (values by the Meteorological Station of the Collegio Romano).

Frond morphology was recorded from February 1995 to January 1996. The frond life span was divided into five developmental phases: (*1*) exponential phase, defined as the time from the visible appearance of fronds to the 90-95 % of full lamina expansion, according to Diemer and Körner (1996); (*2*) frond maturity phase, corresponding to the full lamina expansion; (*3*) dehydration phase; (*4*) desiccation phase; (*5*) rehydration phase; and (*6*) declining phase.

Leaf SA was measured during the year by the *Image Analysis System* (*Delta-T Devices,* England). Fresh mass and dry mass (after oven-drying at 90 °C to constant value) were measured at full lamina expansion, at the end of July, and in the middle of September. From these primary values for mature fronds, ADM and frond succulence (frond water content per leaf FSA, Körner *et al.* 1989) were calculated. On each sampling 9-10 leaves were used for measurements.

Frond anatomy: Sections were taken from mature fronds at the end of May 1995. The pinnulae sections cut by microtome were coloured with carmine red. Sections were stored in 90 % ethanol according to Bolhàr-Nordenkampf and Draxler (1993), and analysed by light microscopy. Lamina thickness, palisade and spongy layer thickness, thickness of upper and lower epidermis, and thickness of dorsal and ventral cuticle were determined.

Chl and carotenoid contents were determined from February 1995 to January 1996. Immediately after collection, fronds were kept cool in the dark, and within 3 h Chls and carotenoids were determined in triplicate by grinding fronds in acetone. The homogenates were centrifuged in the *A.L.C. 4237R* Refrigerated Centrifuge (Milano, Italy). Absorbance of the supernatant was measured by a *Jasco* model *7800 LCD* (Japan) spectrophotometer. Chl content per fresh mass was calculated according to Maclachlan and Zalik (1963), and the content of carotenoids according to Holm (1954).

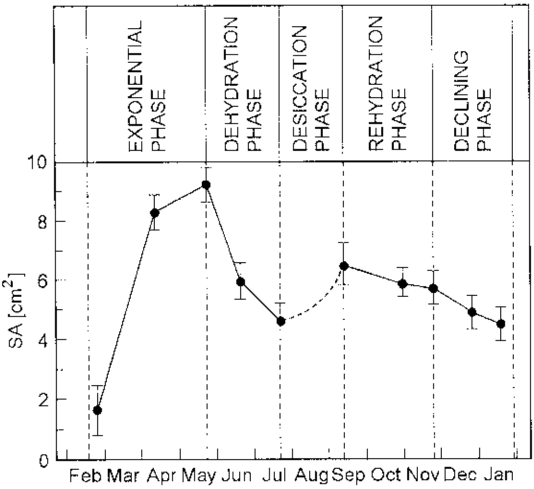

Fig. 1. Frond surface area (SA) of *Cheilanthes persica* from the exponential phase to the declining phase. Standard error is shown. ● = frond maturity.

Frond gas exchange measurements of single leaves (6 to 10) were measured from February 1995 to January 1996 on clear days, from 09:00 to 14:00 h. PAR, P_N, transpiration rate (E), and stomatal conductance to water vapour diffusion (g_s) were monitored by an infrared gas analyser, *ADC LCA3* Open System (Hoddesdon, UK), equipped with a leaf chamber (type *PLC-3*, *ADC*, Hoddesdon, UK). WUE ($P_N : E$) was calculated according to Larcher (1995).

Results

Frond growth: The arrangement of the leaves on the stem was radial. Mature fronds were subdivided into distinct pinnae and small pinnulae. Buds opened in the middle of February, and the full lamina expansion, attesting frond maturity, was achieved at the end of May. Mature fronds were characterized by SA of 9.3 cm^2, ADM of 91±9 g m^{-2}, succulence of 217±17 g m^{-2}, and water content of 70.4 %. The reduction of frond water content in July caused a 51 % decrease of SA (Fig. 1). This partial dehydration phase determined the typical rolling up of fronds manifested during dry periods. Leaf rolling in summer reduced the transpirative SA, and it could be a useful indicator of plant water status. Fronds appeared dry in August, nevertheless, the same fronds showed in September an increased area (41 %) attesting the rehydration phase. Frond SA diminished during the declining phase until the frond death (end of January). The mean frond life span, from bud break to frond death, was about 340 d.

Table 1. Chlorophyll (Chl) *a* and *b* and carotenoid (Car) contents [g kg^{-1}(f.m.)] and Chl *a/b* ratio of *Cheilanthes persica* during the year. Standard error is shown.

	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a/b*	Car
Feb	0.79±0.05	0.22±0.02	1.01	3.6	0.20±0.01
Mar	0.87±0.06	0.24±0.04	1.11	3.6	0.24±0.02
Apr	1.10±0.06	0.30±0.03	1.40	3.7	0.26±0.03
May	1.30±0.08	0.36±0.03	1.66	3.6	0.30±0.04
Jun	1.20±0.07	0.35±0.03	1.55	3.4	0.30±0.04
Jul	1.15±0.07	0.34±0.04	1.49	3.4	0.30±0.05
Sep	1.55±0.09	0.53±0.04	2.08	2.9	0.33±0.03
Oct	1.61±0.09	0.55±0.05	2.16	2.9	0.30±0.04
Nov	1.53±0.08	0.52±0.05	2.05	2.9	0.26±0.02
Dec	0.98±0.06	0.34±0.03	1.32	2.9	0.27±0.02
Jan	0.70±0.05	0.20±0.02	0.90	3.5	0.21±0.02

Leaf anatomy showed that the margin of epidermis was incurved to form a flap (pseudoindusium; Fig. 2). The mesophyll was dorsiventral. The cells of the upper side epidermis, characterized by sinuous border, were 7.2 % larger than those of the lower side. Stomata were localizated on the lower epidermis, protected by the frond margin alone. Differences in cuticle thickness between the upper and the lower epidermis (35.5 %) were observed. Bulliform cells were localized in the epidermis and participated in the involution and rolling up of frond blade during the dehydration stages. Blade thickness was 225.1 μm, decreasing toward the edge. The palisade tissue was well developed (103.9±11.7 μm), comprising two layers.

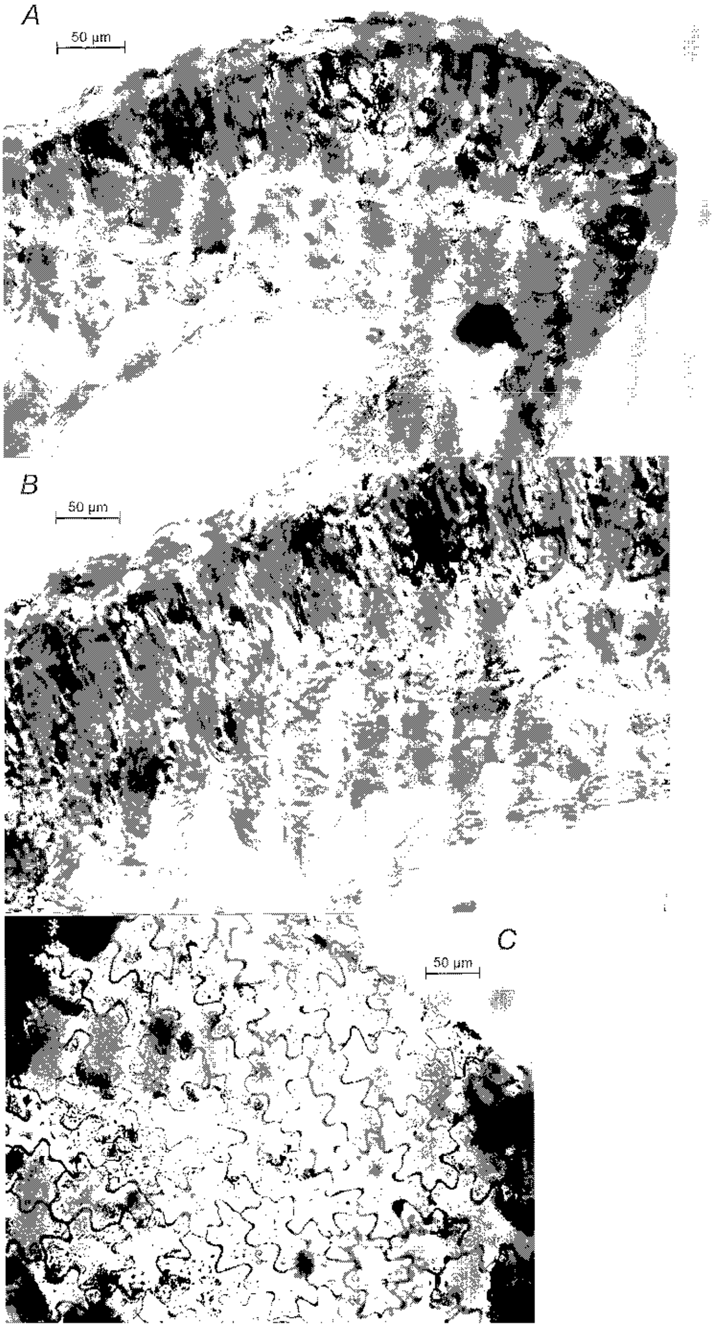

Fig. 2. Cross sections of *Cheilanthes persica* frond at full lamina expansion (*A, B*) and cells of the upper side epidermis (*C*).

The sponge parenchyma thickness (84.3±10.0 μm) was 44.8 % of the total mesophyll thickness. Well-developed intercellular spaces were observed. Mechanical tissues were absent. The ratio of mesophyll thickness/total leaf thickness was 0.84. Upper cuticle thickness was 3.1±1.0 μm, upper epidermis thickness 16.5±2.1 μm, lower epidermis thickness 15.3±1.0 μm, and lower cuticle thickeness 2.0±0.8 μm.

Pigment contents: There was a general increase in Chl (*a*+*b*) content during the frond expansion until the peak at maturity (1.66 g kg^{-1}; Table 1). A slow decrease in Chl content (by 8.4 %) followed in June-July, during the dehydration phase. The maximum value was observed in autumn (2.16 g kg^{-1}) during the rehydration phase, followed by a rapid falling during the declining phase. Chl *a* content showed more pronounced peaks and higher decrease during summer than the Chl *b* content. Contents of carotenoids showed a similar trend; nevertheless, the summer decrease did not occur and the maximum was reached in September. Mean annual Chl *a/b* ratio was above 3.

Frond gas exchange: There was a diurnal trend in P_N with a maximum between 10:45 and 12:00 h (Fig. 3). During the year, maximum daily P_N ranged from 2.2 to 9.7 μmol m^{-2} s^{-1}. Rapid increases were determined over the exponential phase, and the highest rates were reached in April, during the frond maturity phase. Maximum daily g_s ranged from 0.12 to 0.48 mol m^{-2} s^{-1}. From February to April, g_s increased by 60 % and it sustained the 59 % increase in P_N. In July, when mean maximum air temperature peaked at 31.3 °C, g_s decreased by 34 % and P_N by 33 %. During the rehydration phase (September) fairly high rates of g_s and P_N were found, both decreasing during the end of autumn and reaching the minimum in winter. The optimum air temperatures which allowed high P_N shifted from 11 to 23 °C.

WUE was rather low over most of the year (Fig. 4); its peak in March was followed by a considerable decrease in April and May to minima in June and July, during the partial dehydration phase, and in January during the declining phase. The low WUE associated with a fairly high g_s (0.33 mol m^{-2} s^{-1}) in summer sustained the hypothesis of a low stomatal control.

Discussion

The results suggest the adaptive strategy of *Cheilanthes*. From morphological and anatomical points of view, ADM of 91 g m^{-2} is indicative of compact leaves (Christodoulakis 1984) and it is similar to that in *Notholaena parryi* (D.C.) Eat, a xeric fern growing in the western Colorado desert (Nobel 1978). ADM reflects leaf compactness of conductive and mechanical tissues (Christodoulakis 1984, Witkowski and Lamont 1991), also in response to water stress (Rascio *et al.* 1990). The well developed palisade parenchyma, the ratio palisade/sponge parenchyma (1.2), and the great elongation of individual palisade cells are typical for sun leaves (Dengler 1980). The ratio of palisade parenchyma thickness to mesophyll thickness reveals xeromorphic habitus, according to Christodoulakis and Mitrakos (1987).

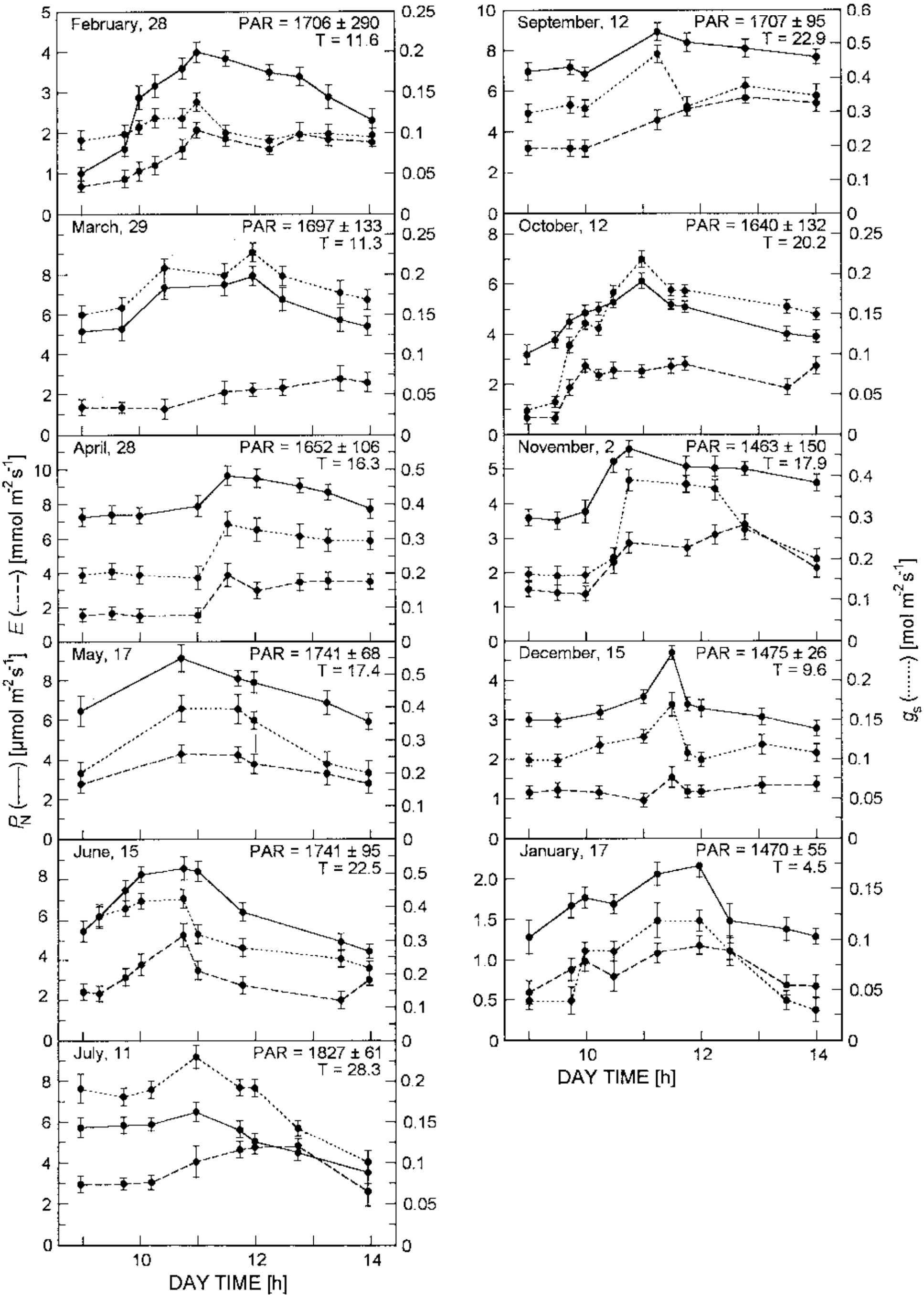

Fig. 3. Diurnal courses of net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s), from 09:00 to 14:00 h during the year. Mean photosynthetically active radiation (PAR) and mean air temperature (T) are also given. Means ± standard errors.

Despite the highest P_N in comparison with other fern species (Maeda 1970, Magomedov 1988), *C. persica* showed high g_s and low WUE during summer, *i.e.*, in the period of highest evaporative demand. Chl *a*/*b* above 3 is typical for sun leaves (Šesták 1971, Alberte *et al.* 1976). Desiccation tolerant species vary greatly in the retention of chloroplast pigments during drying (Gaff 1989). In species which lose Chl on drying, resynthesis of the pigment usually begins after 24 h of rehydration and photosynthesis is correspondingly delayed (Gaff 1989). Chl content of *Cheilanthes* mature leaves (464 mg m^{-2}) is higher than in *N. parryi* (370 mg m^{-2}; Nobel 1978) and it can be partially involved in the dissipation of harmful excess excitation energy. Other xeric-adapted plants from high-irradiance environments also benefit from the dissipation of excess radiant energy in similar ways (Demmig *et al.* 1988, Winter *et al.* 1990). The slight decrease of *C. persica* Chl content during summer enables the rapid functionality after rehydration. Photoinhibition is typical for resurrection plants during dehydration, and the early stage of plant rehydration before full photosynthetic potential has been regained (Lebkuecher and Eickmeier 1991).

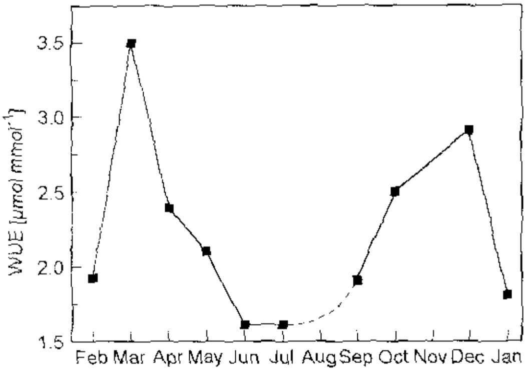

Fig. 4. Time course of water use efficiency (WUE) from February 1995 to January 1996. Maximum daily values were used.

As a concomitant response of the fairly high g_s, *Cheilanthes* reduces its transpirative surface area, according to Eickmeier (1980) for most desiccation tolerant pteridophytes of the Chihuahuan desert. Leaf rolling limits high photoinhibition damages that could occur during rehydration, by reducing radiant energy interception when photosynthetic capacity is low (Lebkuecher and Eickmeier 1991). Therefore the poikilohydry of *Cheilanthes* is a prerequisite for the survival under unfavourable conditions. It is a desiccation tolerant species frond physiology of which correlates with morphological and anatomical frond traits. Frond curling and uncurling that accompany desiccation and rehydration phases maximize WUE during drought thus adjusting the low sensitivity of stomata.

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