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Phenolics and condensed tannins of high altitude *Pteridium arachnoideum* in relation to sunlight exposure, elevation, and rain regime

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Abstract

Non-adapted plants growing in high altitude such as bracken fern *Pteridium arachnoideum* are exposed to environmental extremes that may induce a chemical adaptive response. Here we show that there is a non-uniform distribution of low (LMP) and high molecular weight (HMP) phenolics in the frond parts of *P. arachnoideum* growing at high elevation. LMP−HMP levels were measured in sun-exposed (E) and self shaded (SS) pinnae between 2100 and 3190 m in the tropical Andes, during dry and rainy seasons. While there was no difference in E vs. SS contents of LMP at 2100 m, E accumulated greater LMP−HMP concentrations relative to SS as altitude increased. This difference was increased during the dry season. Linear correlations between the position of each pinnae relative to the ground level and LMP−HMP occurred along a 2570−3190 m transect. Water restriction in the dry season also caused increase of LMP and HMP. We conclude that excess UV-B radiation and water availability are important modelers of the non-adapted plant acclimation response to stress in tropical high mountain habitats.
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1. Introduction

Phenolics and their oligomeric and polymeric relatives (tannins) continue to receive attention for their regulatory functions in intra-plant, herbivore-plant (e.g. Bernays et al., 1983; Lindroth et al., 1988; Lempa et al., 2004) and pathogen-plant (e.g. McNally et al., 2003) relationships, and also because of their beneficial effects to human and animal health (e.g. Rababah et al., 2004; Skerget et al., 2004; Shahidi, 2004; Veloz-García et al., 2004). The mono- or poly-aromatic character of phenolics imparts a strong filtration power of ultra-violet (UV) light (Caldwell et al., 1983; Krauss et al., 1997). This provides some protection to photosystem II proteins and DNA (Britt, 1996; Stapleton et al., 1997). Thus, phenolics may be of vital importance to plants exposed to excess solar high frequency radiation.

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The biosynthesis of phenolics in plants is activated by enhanced UV-B (280—320 nm) light (e.g. Wellmann, 1974; Vogt et al., 1991; Cuadra et al., 1997; Rozema et al., 1997; Caasi-Lit et al., 1997; Tegelberg and Julkunen-Tiitto, 2001) or whole solar radiation (e.g. Mole et al., 1988; Jaakola et al., 2004), although there are some exceptions (Kyparissis et al., 2001; Levizou and Manetas, 2001). As a result, phenolics accumulate in exposed leaf tissue. The majority of these experiments are based on exposure of potted plants under controlled UV radiation from artificial lamps in laboratory conditions. However, more field studies along elevational gradients for example, would be desirable (e.g. Robberecht and Caldwell, 1978; Mole et al., 1988; Ziska et al., 1992; Chaves et al., 1997; Zidorn and Stuppner, 2001).

The UV light filtering or scattering properties of the lower atmosphere are such that higher the elevation the greater the UV-A/B component of solar radiation (280—380 nm) that reaches the ground. This trend is steepest in tropical mountains (Robberecht et al., 1980) and is modulated by the screening of cloud cover. Hence, it will depend on season.

Recently, we produced an evidence that a typical mountain fern (*Pteridium arachnoideum* Kaulf.) responded to altitude during the rainy season by increasing its content of high molecular weight phenolics (HMP) only at elevations greater than 2600 m above sea level (Alonso-Amelot et al., 2004). In a continued effort to understand the various biological and chemical traits that make *Pteridium* ferns among the most dominant plants in the world (Alonso-Amelot et al., 2001; Alonso-Amelot, 2002), we pursued further dynamics of their LMP—HMP load in order to evaluate the effects of season (rain regime and cloudiness), altitude in exposed and self shaded pinnae, and the position of each pinnae relative to the axis of the blade (rachis) in dense high altitude thickets of tropical *P. arachnoideum*, under field conditions.

2. Materials and methods

2.1. Environmental measurements

At each of four selected locations, air temperatures were recorded using field loggers (Onset Computer Corp., Massachussetts) placed at 1 m above the ground in the shade, during 1999, 2001 and 2003. Recordings are in degree Celsius. At the lowest station (LH, 2200 m elevation) visible sunlight (400—700 nm) was recorded ($lm\ m^{-2}$) with the aid of light meters using a semispherical optical diffuser, coupled to a data logging module (Extech Instruments, Waltham, Massachussetts). Meters were placed for 30 day periods on the ground surface, at 40 cm and above the canopy in a high density bracken sward (1.80 m) during the months of April 2001, when the sun hits vertically at the 8° latitude at which the sampling sites were located, and June 2001 around the northern summer solstice. These dates correspond to the end of the dry season and the first peak of the bimodal rain profile of the area.

2.2. Sampling of fronds

Four sun-exposed sites located between 2200 and 3190 m elevations with dense bracken growth (>5 fronds m^{-2}) were selected. Location specifics are indicated in Table 1. Sites Baho (BH), Frailes (FR), and Corcovada (CV) were situated along a 5 km transect of the upper Santo Domingo river valley, Mérida State, Venezuela. Site La Hechicera (LH) was a slope of the vicinal Chama river basin, 49 km to the west. In this location a subset of samples were collected in April 2005 at the end of the dry season to study the influence of sunlight at mid altitude on LMP and HMP

Table 1
Location and description of sampling sites of *Pteridium arachnoideum* in this study

Name of locality	Site acronym	Coordinates	Elevation (m)	Habitat type
La Hechicera	LH	8°31′ N-71°13′ W	2100	Exposed slope, seasonal humid, T (°C): 16.0; 10.4; 25.8
Baho	BH	8°50′ N-71°44′ W	2570	Steep valley slope, humid, T (°C): 10.2; 2.9; 21.4
Frailes	FR	8°49′ N-71°47′ W	2930	Exposed *páramo** slope, seasonal humid, subalpine, T (°C): 8.8; 1.1; 20.6
Corcovada	CV	8°50′ N-71°48′ W	3190	Exposed *páramo* slope, seasonal humid, subalpine, T (°C): 8.3; −1.7; 19.3

Temperatures are yearly average, minima and maxima, as measured during 1999, 2001 and 2003.
Páramo is the accepted term for tropical ecosystems higher than 2900 m above the sea level.

contents. This subset was composed by 12 fully grown fronds growing in the shade of a riparian forest along La Hechicera creek, and 12 additional fronds from a nearby open grassland.

The fern species was determined according to existing taxonomic keys and voucher accessions were deposited in the New South Wales Botanical Gardens, Sydney, Australia, under the care of Dr. John Thomson, and numbered NSW 505771. Frond collections were carried out in June−July for the rainy period and February−April for the dry season, in 1998, 2001, and 2005. In a first series of samples, frond parts were classified as sun-exposed (E) and self shaded (SS) according to their relative position within the bracken sward and exposure to sun radiation (see below). The E group was composed by the second pair of pinnae of fronds growing in the open without shading from other fronds in the sward. SS samples were the first pair of pinnae stemming from the end of the stype in fronds with shading from vicinal ramets and pinnae above. Twelve samples per site, season and year in each of four sites (LH, BH, FR, CV) were recovered.

For the second series of experiments in which it was of interest to relate the phenolics content with the position of the pinnae relative to the rachis in high altitude fern populations during the rainy season, fully developed fronds from sites BH, FR and CV were cut at ground level before 10:00 AM. Pinnae were excised in pairs from the rachis in the field, stored at 10−15 °C in clip bags in a cold box and carried to the laboratory for analysis within 4 h. Pinnae along the rachis were numbered P1−P4 from the ground up. P4 included not only the fourth pair of pinnae but also the 2−6 additional small ones that form the tip of the blade. All samples had soft tips, showing recent maturation. The number of samples was the same as above.

2.3. Extraction and analysis

From each pair of pinnae all pinnulae were cut off from the rachis, weighed and dried under high vacuum (<1 mm Hg) for 48 h. This method has been shown recently to prevent the decomposition of phenolics and makes extractability more efficient (Ferreira et al., 2004; Alonso-Amelot et al., 2005a). The dried pinnae were thoroughly mixed by hand and about 50% of the sample (2−4 g) was blended at high speed and extracted with 70% aqueous acetone (70 ml, 0.01 M ascorbic acid (Merck) as antioxidant), with turbulent mixing for 30 min at room temperature, followed by sonication (Elma Transsonic-310, Germany) for an additional 10 min at 4 °C. Solvents were separated by centrifugation and the residual pellet was re-extracted with a second portion of solvent as above (15−20% additional recovery, a third extraction contained <1% of the total phenolic load). Acetone was evaporated under vacuum (<30 °C) from the combined extracts and the volume of the water solution was corrected to 100.0 ml. A 2.0 ml aliquot from this solution was passed through a column of lypophilic Sephadex LH-20 (Sigma). Fractions eluted with 90% methanol (Riedel de Häen AG) (90 ml) gave the LMP fraction, and additional elution with aqueous acetone (70%, 90 ml) yielded the HMP fraction. Volatile solvents were removed from each fraction and their volume was corrected to 100.0 ml. Aliquots (1 ml) were destined for analysis using the reported method (Alonso-Amelot et al., 2004) and employing Sephadex LH-20-purified quebracho (Schinopsis quebracho-colorado (Schltdl.) F.A. Barkley and Mey) tannin or recrystallized salicylic acid (Sigma) as standards for HMP and LMP, respectively. Therefore, contents of HMP are reported as mg equivalents of quebracho tannin (QT) g^{-1} dry weight (dw) of plant and LMP as mg equivalents of salicylic acid (SA) g^{-1} dw.

3. Results

3.1. Relative exposure of pinnae to sun radiation

Within dense bracken swards (>5 blades/m^2), fronds expand horizontally into the space occupied by neighbouring blades, thus causing shading to other pinnae. The first two pairs of pinnae (P1, P2) from the ground up are generally more shaded than the upper ones, which may reach 1.2−1.8 m above the ground in P. arachnoideum. Total sunlight (400 and 700 nm) reaching the upper, middle and bottom sections of the sward was recorded at regular intervals for several months. The middle sector comprising pinnae P1 received an average of 26.9% of the full solar radiation during the circadian cycle, in consonance with values observed under tree canopies (Kotzen, 2003). Solar flecks entering the sward through gaps of the upper canopy were also evident in the recordings as radiation spikes and allowed an average of 50.2% of the total solar radiation to pass through the lower levels 2 h before and after the sun's zenith. By contrast, small light gaps falling on ferns growing in the lower stratum of the riparian forest in site LH were

much less numerous and the total solar radiation reaching the fronds averaged 18.2% of the total sunlight falling on open grassland.

3.2. LMP−HMP and relative sun radiation exposure

First we determined the general influence of sunlight exposure on LMP−HMP synthesis and accumulation on bracken fronds and then addressed the question of self shading in exposed swards. Bracken fronds growing at 2100 m asl under the tree canopy of a dense riparian forest at site LH in the dry season, when the greatest phenolics content is recorded, showed a statistically significant ($p < 0.01$) lower concentration of phenolics relative to those on a nearby sun-exposed meadow, as expected. LMP amounted to 18.9 ± 2.3 mg SA eq g^{-1} dw, whereas exposed fronds accumulated 47.7 ± 4.9 mg g^{-1}. HMP of forest fronds reached 5.7 ± 0.9 mg QT eq g^{-1} dw while exposed fronds possessed 12.7 ± 1.8 mg g^{-1}.

The effect of the self shading on LMP−HMP was measured in plants from various locations during the rainy season (Table 2). Paired samples of E and SS pinnae (data not shown) at lower elevations gave consistently comparable amounts of LMP, but plants at elevations higher than 2400 m altitude tended to show increasing differences favoring the E pinnae. The paired samples invariably yielded a greater HMP content for the E part of the frond at all elevations. The ratio of phenolics contained in E vs. SS pinnae (Table 2) was around one in fronds at the lower end of the altitudinal transect (e.g. LH) during the rainy season, but increased up to 3.51 in the highest site (CV). This location lies well within the *páramo* heath near the night freezing isotherm around 3100 m and where the UV-B component of sunlight is strongest in the bracken populations examined. By contrast, while the content of HMP in E and SS pinnae increased with elevation in the wet season, the E/SS ratio tended to remain around 1.6 ± 0.4 at all locations.

The same four sites were studied during the dry season as well. The dry season with its typical clear skies brought a considerable increase in total LMP and HMP of both E and SS pinnae, but the E/SS ratio remained comparable between sites (Table 3). The contrasting trends of LMP−HMP accumulation in wet and dry seasons along the BH − FR − CV transect are illustrated in Fig. 1.

3.3. LMP−HMP and position of pinnae along the rachis

Generally, mature bracken fronds are composed of 8−10 pinnae which stem from the rachis at ever shorter inter-axillary distances as they unfold towards the top end. The majority of the leaf area is contributed by the three lower pinnae, as can be appreciated by the biomass distribution of each one (Table 4). At the higher three collection sites (2500−3200 m) this distribution did not vary substantially with altitude suggesting that all are similar phenotypes. It was of interest to determine the variation of LMP−HMP contents in the individual pinnae growing along the rachis of the frond, as the accumulation of these compounds depends on two opposing factors: the degree of exposure to sun radiation and frond ontogeny, since we had observed earlier (Alonso-Amelot et al., 2004) a positive correlation between frond development stage and LMP−HMP accumulation. Upper pinnae typically are 3−4 weeks younger than lower pinnae under the low temperature conditions of high elevation and would be expected to contain less LMP−HMP if their accumulation was dominated by ontogeny alone.

To shed light on this dilemma, excised pinnae P1−P4, numbered from the ground up, were analyzed individually for LMP−HMP contents. The three highest elevation locations were selected since there the plants showed the most

Table 2
Content of phenolics in sun-exposed (E) and self shaded (SS) pinnae of fronds of *P. arachnoideum* at various sites in the Venezuelan Andean range during the rainy season

Location	Elevation (m)	LMP			HMP		
		E	SS	E/SS	E	SS	E/SS
LH	2100	23.7 ± 2.3	24.6 ± 1.6	1.0 ± 0.1	20.4 ± 1.3	11.7 ± 1.0	1.7 ± 0.2
BH	2570	34.8 ± 5.5	20.6 ± 5.4	1.7 ± 0.4	30.7 ± 5.0	18.0 ± 3.9	1.7 ± 0.3
FR	2930	39.4 ± 8.4	25.3 ± 4.8	1.6 ± 0.3	46.6 ± 5.9	22.5 ± 4.1	2.1 ± 0.4
CV	3190	82.2 ± 11.4	23.4 ± 6.7	3.5 ± 0.7	78.1 ± 6.6	48.5 ± 5.5	1.6 ± 0.2

LMPs are expressed as mg eq SA g^{-1} dw of pinna, and HMPs as mg eq QT g^{-1} dw of pinna. Incertitudes are standard error of the means, $N = 36$ per site.

Table 3
LMPs and HMPs of exposed (E) and self shaded (SS) *P. arachnoideum* fronds in selected sites of the Venezuelan Andes three months after the onset of the dry season

Location	Elevation (m)	LMP			HMP		
		E	SS	E/SS	E	SS	E/SS
LH	2100	47.7 ± 4.9	46.8 ± 8.5	1.15 ± 0.1	12.7 ± 1.8	15.1 ± 2.7	1.00 ± 0.2
BH	2570	143.4 ± 13.4	49.4 ± 16.6	2.9 ± 1.0	120.1 ± 13.8	85.2 ± 18.4	1.4 ± 0.4
FR	2930	141.4 ± 12.7	84.4 ± 0.3	1.7 ± 0.8	107.4 ± 6.8	74.0 ± 10.9	1.5 ± 0.2
CV	3190	183.0 ± 35.9	95.9 ± 8.6	1.9 ± 0.5	111.5 ± 18.4	68.2 ± 21.0	1.6 ± 0.4

E = Exposed pinnae, SS = self shaded pinnae. Incertitudes are standard error of the means, $N = 36$ per site.

pronounced response to environmental stress in terms of tannin accumulation in E vs. SS leaves (previous section). The results of Fig. 2 indicate that, for the wet season sampling, LMP–HMP accumulations increase with distance of pinnae from the ground in a linear fashion and are enhanced at the highest elevation (CV). Also, the content of simple phenolics correlates well with altitude varying from $16 ± 4$ mg SA eq g^{-1} dw in P1 in location BH (2570 m) to $67 ± 8$ mg SA eq g^{-1} dw in P4 growing in *páramo* site CV (3190 m). HMPs vary similarly from $10 ± 1$ in P1 of BH to $80 ± 6$ mg QT eq g^{-1} dw in P4 of CV.
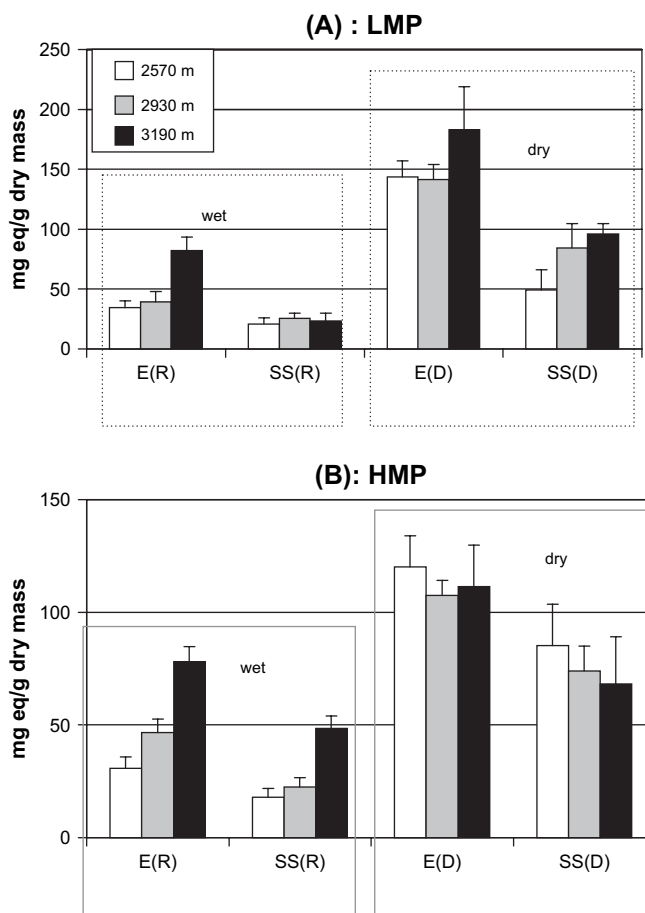

Fig. 1. Variation in the content of (A) LMP and (B) HMP of exposed (E) and self shaded (SS) pinnae of *P. arachnoideum* fronds growing at high altitude in the Venezuelan Andes. Quantities are in mg eq SA g^{-1} dw and mg eq QT g^{-1} dw of plant, respectively. Bars are standard error of the means, $N = 12$ per site and season.

Table 4
Moisture and biomass distribution of pinnae along the rachis in fronds of *P. arachnoideum* collected at sites BH, FR and CV, at the upper end of the altitudinal range of this fern in the neotropics

Location	Pinna #	% Moisture ± SD	Biomass (g) ± SD	%
BH (2570 m)	P1	59.9 ± 1.7	3.2 ± 0.9	30.2
	P2	58.6 ± 4.1	2.6 ± 0.4	25.3
	P3	61.9 ± 3.4	2.0 ± 0.6	18.8
	P4	63.6 ± 2.7	2.8 ± 1.1	25.7
FR (2930 m)	P1	62.0 ± 2.2	3.2 ± 0.8	28.5
	P2	63.1 ± 5.2	3.6 ± 1.7	30.6
	P3	65.8 ± 5.5	2.1 ± 0.5	19.2
	P4	65.6 ± 3.7	2.4 ± 0.7	21.7
CV (3190 m)	P1	64.6 ± 1.8	2.2 ± 0.7	27.3
	P2	65.4 ± 3.3	2.0 ± 0.6	24.1
	P3	65.5 ± 2.8	1.7 ± 0.5	21.4
	P4	66.6 ± 3.3	2.2 ± 0.6	27.3

While P1–P3 are individual pinnae, P4 comprises from the fourth pinna to the frond tip.
Incertitudes are SE ($N = 36$).

4. Discussion

Phenolics and tannins are synthesized in plants not only under genetic determinants, physiological demands and evolution-controlled defense needs (Cole et al., 1988; Bryant et al., 1991), but also by the influence of environmental stress such as disturbance, drought, and electromagnetic radiation (Woodhead, 1981; Waterman and Mole, 1989; Lovelock et al., 1992). Other factors being equal, four fundamental components mitigate solar UV-B flux density reaching plants: stratospheric ozone, atmospheric thickness and aerosols leading to light scattering, cloudiness, and reflection-filtration through the upper components of the plant canopy (Grant, 1997). The first two account for most of the short wave absorption of the solar spectrum reaching the lower atmosphere. The second tropospheric contributors are associated with elevation and latitude (Caldwell et al., 1980), causing steep radiation gradients. Cloud cover also exerts considerable influence in the short wave photon flux (e.g. Díaz et al., 2000; Krzyscin et al., 2003). The short wave radiation reaching the sampling sites here studied is the combined result of these factors.

4.1. Exposed vs. self shaded pinnae and elevation

The synthesis and accumulation of phenolic derivatives in bracken fronds is regulated by the total solar radiation reaching the plant as indicated by the lower amounts of both LMP and HMP recorded in the forest plants relative to thickets in the open areas (40% and 45%, respectively) at mid elevation during the dry season (Section 3.2). This phenomenon has been observed in other plants of tropical forested environments at low elevation (e.g. Mole et al., 1988).

While bracken ferns do not possess typical traits of high mountain plants, such as low stature, waxy cuticles or abundant trichomes, grayish light reflecting surfaces, low leaf-stem angle and resistance to frost (Vareschi, 1970), *P. arachnoideum* is capable of growing successfully in high regions such as the *páramo* zone of tropical mountains up to the night freezing isotherm and way above the natural treeline (2800 m asl). There, among other sources of climatic stress, naturally higher solar UV radiation attains potentially harmful effects on unprotected plants, particularly under the clear skies of the 3–4 months long dry season (UV-A: 320–380 nm; 0.52 mW cm^{-2} under heavy clouds vs. 3.02 mW cm^{-2} under clear skies measured at 3100 m asl in two contrasting days of August 2002).

Some adapted plant species of these altitudes have developed physical traits for partitioning incident solar radiation between reflection scattering, using adaxial cuticular surfaces and hairs that felt the entire leaf and stem surfaces, and absorption in the cuticle and mesophyll of the transmitted light (Nybakken et al., 2004; Vigneron et al., 2005). Such physical traits are common (Mulroy, 1979). A survey of 100 plant species (Robberecht and Caldwell, 1978) showed that essentially all UV radiation is deflected and/or absorbed in the leaf, while other studies show low UV transmittances by isolated cuticles at wavelengths under 400 nm (Krauss et al., 1997). This implies that much less direct UV radiation will be passing through the level of the upper pinnae forming the canopy to the lower levels of the thickets.
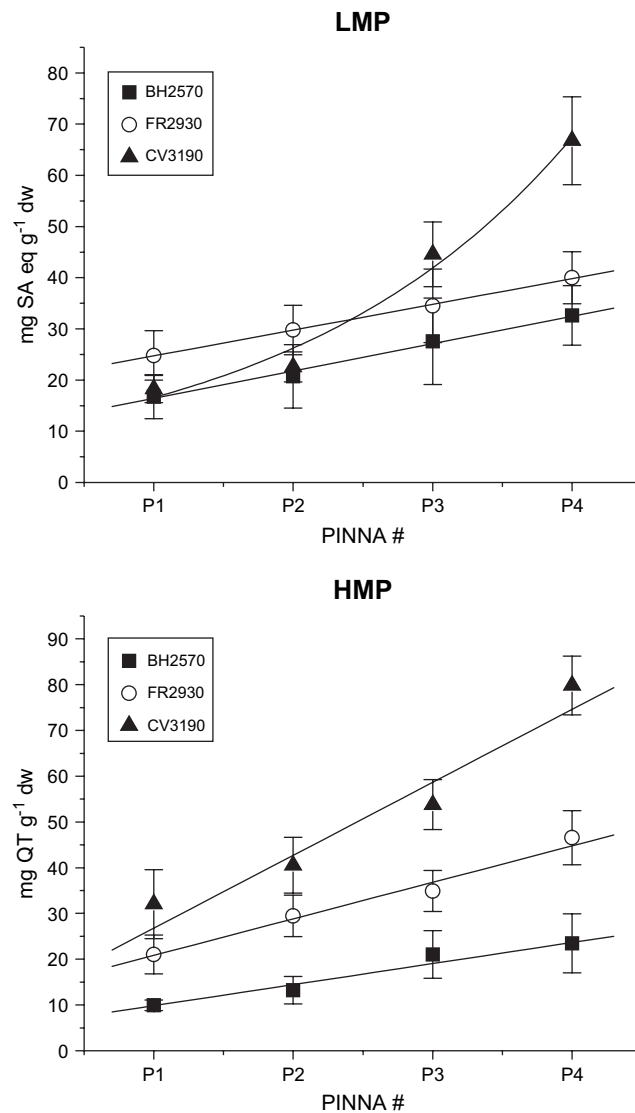
Fig. 2. Variation in the contents of LMP and HMP with the position of pinnae along the frond rachis, and elevation in sites BH, FR and CV at the upper end of the altitudinal distribution of *P. arachnoideum* for the rainy season. Ordinate units are mg eq SA g^{-1} dw for LMP and mg eq QT g^{-1} dw for HMP. Error bars indicate the standard error of the means ($N = 12$). All correlation coefficients are >0.97 and $p < 0.04$.

Only diffuse and reflected radiation will reach the interior of the bracken sward, except for solar flecks entering through gaps in the canopy.

As a result much less UV-B filtering power will be needed by lower pinnae in bracken, which grow well within the light shielding effect of this thicket. However, this capacity appears to be divided between LMP and HMP to a different extent depending on elevation. As Table 2 shows, LMP content does not vary from E to SS pinnae at 2100 m elevation in the wet season, whereas the E/SS ratio of HMP in these plants is 1.7 ± 0.2, thus suggesting that proanthocyanidins in bracken offer added protection against excess solar radiation.

Elevation strongly influences the synthesis and accumulation of both types of phenolics in *P. arachnoideum* (Table 3) likely through PAL activity regulation. The fact that elevation appears well correlated with the LMP—HMP content of exposed sections of the pinnae and also the E/SS ratio suggests that the increment in the UV-B component of solar radiation at higher altitudes is responsible for this effect. This is in accordance with earlier observations in other plant species (Reynolds and Crossley, 1997; Cuadra et al., 1997; Caasi-Lit et al., 1997) exposed to artificial UV irradiance treatments.

4.2. Phenolics content and relative position of pinnae along the rachis

We had observed earlier that a direct correlation exists between total phenolics accumulation in aerial parts of *P. arachnoideum* and frond ontogeny (Alonso-Amelot et al., 2004). Although this might imply a reduced chemical defense at the fragile young stage of the frond, other compensatory mechanisms are present in the growing blade to protect it. The reduced phenolics-based defense capacity is counterbalanced by other xenobiotics in high concentration in the crozier such as prunasin, a cyanogenic glycoside, and illudanes. Their levels wane with frond growth as phenolics contents increases (Alonso-Amelot et al., 2001; Alonso-Amelot and Oliveros-Bastidas, 2005b). The fronds growing at high altitude seem to invert the trend of phenolic accumulation in non herbivore-stressed plants. Since there is a difference of 4−5 weeks between the unfolding of lower and upper pinnae (P1−P4), one might expect a greater amount of these materials in P1 and P2 than the upper frond parts that are just reaching maturity. However, the upper sections are more exposed to direct sunlight than the lower portions which, as we have seen, are subjected to self shading and hence UV light protection. The results of Fig. 2, which show excellent correlations between position along the rachis and LMP−HMP contents clearly suggest that light exposure is a stronger driving force than ontogeny for the synthesis and accumulation of these materials.

Additionally, the slope of these plots at those sites located along the BH − CV high elevation transect was taken to represent the phenolics response to the prevailing conditions, as the pinnae became further distanced from the ground and surrounding vegetation. In as much as the pinnae emerge from the thicket the protection from UV radiation offered by neighbouring pinnae within the sward is reduced and as a result plant tissue becomes more exposed to damaging radiation (Grant, 1997). The slopes of plots in Fig. 2 increase markedly, especially in the proanthocyanidins, as elevation changes from 2570 m to 3190 m. This represents a transition of upper cloud forest and the humid sub-*páramo* to the sun-exposed *páramo* grassland. Again, the need for protection against increasingly stronger UV-B photon flux is likely to be responsible.

4.3. Phenolics, relative exposure and season

Season in the tropics involves two primordial environmental effects: reduced water availability and greater solar irradiance due to less cloud cover. Average temperatures vary only marginally. Both factors may influence the phenolics response in plants. Water availability alone (Levizou and Manetas, 2001; Glynn et al., 2004), or in combination with enhanced UV-B radiation (Kyparissis et al., 2001) may influence the production of phenolics. In addition, as clouds attenuate the UV light reaching the earth surface, as we have attested here, cloud cover should modulate the phenolics response as well. However, its influence on the phytochemical response of alpine plants to our knowledge remains unknown. In our experimental area at high elevation, the rainy months are characterized by frequent storms, dense nimbus clouds and fogs, with total precipitation averaging 900 mm, whereas the dry season in the study area is typified by clear skies alternated with high altitude tenuous cirrostratus and altostratus cloud layers, and an average of less than 20 mm of rain per month (Sarmiento, 1986). A measurable contrast in the amount of UV-A/B solar radiation reaching the high altitude plants during both seasons results.

Non-adapted species such as *P. arachnoideum* respond to these sources of stress by the accumulation of LMP and HMP in exposed laminae. During the rainy period when there is no water constraint, we observed a clear influence of altitude in both LMP and HMP accumulation (Table 1). The higher the elevation the greater the phenolic content, as well as its difference between E and SS pinnae. Therefore, as moisture is not a limiting factor, the observed LMP−HMP dynamics must be chiefly due to UV-A/B solar radiation in spite that it may be attenuated by cloudiness. By contrast, while bracken fronds remain green new growth is halted during the dry season and many other plants around all study locations show clear signs of water deficiency. LMP and HMP in bracken fronds appear in much greater quantity in all sites at this time (Table 3, Fig. 1). At mid altitude, phenolics in both E and SS blades in the dry period increase relative to the wet season, even though SS blades are protected from the more intense solar radiation of the summer clear skies. Therefore, phenolics enhancement is likely due to water stress for the most part. However, above 2500 m altitude a four-fold increase in the accumulation of LMP and HMP takes place. This increment is more noticeable in the exposed parts of the blade in both seasons but more manifest in the dry period. Apparently, increased solar radiation and water stress act in combination. The smooth correlation of phenolics contents and elevation in association with pinnae distance from the ground up (Fig. 2B), the homogeneity of plant response at each site and the

similarity of frond architecture and morphology suggest that acclimation rather than genotype selection is responsible for the observed chemical compositions.

Finally, the survival of the majority of fern blades during the length of the dry season suggests that the amount of LMP and HMP in the plant are adequate to protect them from solar UV-A/B radiation stress.

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