

Leaf anatomy of a secondary montane *Quercus* forest in Costa Rica

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Abstract: The leaf anatomy of 14 secondary and 17 primary tree species was studied in a 30 yr old secondary, montane *Quercus* forest in the Costa Rican Cordillera de Talamanca. On the average, foliar tissues are thicker in secondary species, due to the high proportion of spongy parenchyma for water storage. In secondary species the mean lamina thickness was 249.5 μm and the mean thicknesses of palisade and spongy parenchyma were 85.8 μm and 133.9 μm , respectively. In primary species the mean lamina thickness was 220.7 μm and the mean thicknesses of palisade and spongy parenchyma were 75.7 μm and 107.9 μm , respectively. The non-palisade parenchyma / palisade parenchyma ratio was slightly higher for secondary species. The thickness of palisade parenchyma and spongy parenchyma were significantly correlated for both primary and secondary species. Lamina thickness was significantly correlated with thickness of palisade parenchyma and spongy parenchyma for primary species only. Leaf anatomical values measured for this montane secondary forest fall mainly within the ranges known from montane primary forests in other tropical countries.

Key words: Costa Rica, leaf anatomy, *Quercus*, secondary forest, tropical montane forest.

Recently, several studies have become available about the leaf anatomy of species in mature tropical montane forests (e.g. Malaisse and Colonval-Elenkov 1981, 1982, Tanner and Kapos 1982, Cavellier and Goldstein 1989). Results have shown the xeromorphic, sclerophyllous and pachyphyllous character of many species in these habitats (Grubb 1974, Tanner and Kapos 1982, Sugden 1985). However, still little is known about the foliar anatomy of plant species in montane forests of secondary origin. Roth (1984) and Gómez (1986) recognized that leaves of secondary species in early successional phases display different characteristics in comparison with those thriving in undisturbed mature forests. However, which leaf anatomical aspects are characteristic for montane secondary forests and how foliar parameters differ in comparison to montane primary forests remains unknown. Therefore the present study was initiated as an attempt to contribute in our under-

standing of the foliar anatomy of tree species composing secondary forests in the montane Tropics.

MATERIAL AND METHODS

This study was carried out near Jaboncillo de Dota (2975 m asl) in the 62 000 ha Los Santos Forest Reserve, which is located on the Pacific slope of the Costa Rican Cordillera de Talamanca (9°35'40" N, 83°44'30" W). This mountain range is formed of intrusive and Tertiary volcanic rocks, alternated with marine sediments (Weyl 1980). The study area has a Cf climate according to the Köppen Climate System with a dry season lasting from December to April (Kappelle 1993). At the nearby town of Villa Mills (3000 m asl) the average annual temperature is 10.9 °C and the average rainfall 2812 mm (Anónimo 1988, Kappelle 1993). The topography of the area is

abrupt (slope angles: 20 to 35°). Soils (Andosols; [Histic] Hapludand) are of volcanic origin, rich in organic matter, well-drained and rather acid with a pH ranging from 3.7 to 5.0 (Kappelle *et al.* 1989; J.G. van Uffelen pers. com.). The area has been deforested continuously since the early 1950s (Kappelle and Juárez in prep.).

Before abandonment and forest recovery started, the site of the secondary forest studied had been used for dairy cattle-grazing for a period of about five years, following clearing and burning of mature upper montane *Chusquea* - *Quercus* forest of tall stature. A description of its structure, floristic composition and diversity is given elsewhere (*e.g.* Kappelle 1993).

Mature sun leaves of 31 tree species were sampled in a 0.1 ha plot, which was established in a 30 yr old secondary montane *Quercus* forest. In tall trees sampling was done using alpine rope climbing techniques (Ter Steege and Cornelissen 1988). On basis of an earlier study (Kappelle 1993), species were divided in primary and secondary species, the latter group including pioneers as well as early and late secondary species. *Viburnum costaricanum* and *Miconia schnellii*, formerly placed among secondary species (Kappelle 1993), were included in the set of primary species for they were particularly abundant in nearby undisturbed mature oakforests.

Following Bongers and Popma (1990) one leaf or leaflet of each species was collected and conserved in pure ethanol. In the Hugo de Vries laboratory transversal slices were made manually from the conserved leaves in order to measure lamina (or blade) thickness and thickness of palisade and spongy parenchyma (or mesophyll) tissues using a light microscope. For each species the non-palisade parenchyma / palisade parenchyma ratio was determined and the presence of a hypodermis was recorded. In order to find significant correlations, associations between pairs of leaf anatomical characteristics (interdependence) were established using Pearson product moment correlation coefficients (Sokal and Rohlf 1981, Tanner and Kapos 1982, Bongers and Popma 1990). Measurements of these leaf anatomical characteristics were compared with results from primary forests in the montane Tropics.

RESULTS AND DISCUSSION

Leaves of a total of 14 secondary and 17 primary tree species were sampled and anatomically analyzed (Table 1). Mean lamina thickness was 249.5 μm for secondary species and 220.7 μm for primary species. The average thicknesses of palisade and spongy parenchyma were 85.8 μm and 133.9 μm for secondary species and 75.7 μm and 107.9 μm for primary species, respectively. Thus, the average leaf of the secondary species is relatively thicker than the leaf of the primary species. The non-palisade parenchyma / palisade parenchyma ratio is slightly higher in secondary species than in primary species. This is mainly caused by the high proportion of spongy parenchyma for water storage in leaves of secondary species. Furthermore, seven out of 31 species showed the presence of a hypodermis, five of which were catalogued as primary species (Table 1).

The thickness of palisade parenchyma has a relative large coefficient of variation among both secondary (58.3 %) and primary species (57.2%) due to high values for *Comarostaphylis arbutoides* (190 mm) and *Persea vesticula* (180 mm) as well as the absence of palisade parenchyma in *Ilex pallida*, *Styrax argenteus* and *Verbesina oestadiana*. The relative large coefficient of variation for the non-palisade parenchyma / palisade parenchyma ratio is partly caused by the same species, *Myrcianthes fragrans* (ratio 4.0) and *Zanthoxylum scheryi* (ratio 3.8). Significant coefficients ($p < 0.05$) were found between the thicknesses of palisade and spongy parenchyma for both primary and secondary species and among lamina thickness and the thicknesses of palisade and spongy parenchyma for primary species only (Table 2).

Leaf anatomical data for this Costa Rican montane secondary forest do not differ much from those reported from primary forests in the upland Tropics (Table 3). Mean thickness of lamina and spongy mesophyll for the secondary species *Myrsine coriacea* and the primary species *Cleyera theaeoides* in this study were only slightly greater than values found for these species in a primary montane forest in Jamaica (Tanner and Kapos 1982). The lamina thickness for 31 Costa Rican species is on average similar to the values recorded for different Jamaican montane forests (Tanner and Kapos

TABLE 1

Means of leaf anatomical characteristics of secondary and primary tree species
in a montane secondary *Quercus* forest in Costa Rica

	Family	NPPR	LT (μm)	TPP (μm)	TSP (μm)	PHD
Secondary tree species (14)						
<i>Abatia parviflora</i> Ruiz López & Pavon	Flacourtiaceae	1.2	230	90	110	-
<i>Buddleja nitida</i> Benth.	Loganiaceae	2.4	170	40	95	-
<i>Comarostaphylis arbutoides</i> Lindley	Ericaceae	1.6	385	190	140	-
<i>Cornus disciflora</i> Mociño & Sessé	Cornaceae	1.4	140	50	70	-
<i>Escallonia myrtilloides</i> L.f.	Escalloniaceae	1.4	305	110	150	+
<i>Fuchsia arborescens</i> Sims.	Onagraceae	3.3	195	40	130	-
<i>Hedyosmum mexicanum</i> Cordemoy	Chloranthaceae	0.7	230	110	90	-
<i>Ilex discolor</i> Standley	Aquifoliaceae	0.0	295	80	170	-
<i>Monnina crepinii</i> Chodat.	Polygalaceae	1.0	330	150	150	-
<i>Myrcianthes fragrans</i> McVaugh	Myrtaceae	4.0	345	60	240	+
<i>Myrsine coriacea</i> (Sw.) R. Br. ex R. & Sch.	Myrsinaceae	3.4	260	50	170	-
<i>Oreopanax xalapense</i> (Kunth) Decne. & Pl.	Araliaceae	0.8	125	60	40	-
<i>Symplocos serrulata</i> Kunth	Symplocaceae	2.4	300	80	190	-
<i>Verbesina oerstediana</i> Benth.	Asteraceae	-	185	0	130	-
Values for the total set of 14 secondary species						
Mean		2.0	249.5	85.8	133.9	
± 1 Standard Error		1.1	80.1	50.0	51.5	
Coefficient of Variation		55.0	32.1	58.3	38.5	
Primary tree species (17)						
<i>Cleyera theaeoides</i> (Sw.) Choisy	Theaceae	1.5	220	70	105	-
<i>Drimys granadensis</i> L.f.	Winteraceae	2.7	230	50	135	-
<i>Ilex pallida</i> Standley	Aquifoliaceae	-	235	0	160	+
<i>Miconia schnellii</i> Wurd.	Melastomataceae	2.3	140	30	70	+
<i>Nectandra cufodontisii</i> (Schmidt) Allen	Lauraceae	1.0	260	110	110	-
<i>Ocotea pittieri</i> (Mez) Van der Werff	Lauraceae	1.5	170	60	90	-
<i>Oreopanax capitatus</i> (Jacq.) Decne. & Pl.	Araliaceae	2.3	325	70	160	+
<i>Persea vesticula</i> Standley & Steyerf.	Lauraceae	0.6	335	180	100	-
<i>Quercus copeyensis</i> Corn. Mueller	Fagaceae	0.6	145	80	45	-
<i>Quercus costaricensis</i> Liebm.	Fagaceae	0.5	265	160	80	-
<i>Rhamnus oreodendron</i> L.O. Williams	Rhamnaceae	2.0	140	30	60	-
<i>Schefflera rodriguesiana</i> Frodin	Araliaceae	1.7	232	70	120	+
<i>Styrax argenteus</i> Presl.	Styraceae	-	100	0	80	-
<i>Vaccinium consanguineum</i> Klotzsch	Ericaceae	3.3	270	45	150	+
<i>Viburnum costaricanum</i> (Oersted) Hemsley	Caprifoliaceae	1.5	180	60	90	-
<i>Weinmannia pinnata</i> L.	Cunoniaceae	1.3	200	70	90	-
<i>Zanthoxylum scheryi</i> Lundell	Rutaceae	3.8	305	50	190	-
Values for the total set of 17 primary species						
Mean		1.8	220.7	75.7	107.9	
± 1 Standard Error		0.9	68.6	43.3	39.6	
Coefficient of Variation		50.0	31.1	57.2	36.7	

Characteristics given for N = 1 leaf or leaflet are: Non-Palisade Parenchyma / Palisade Parenchyma Ratio (NPPR), Lamina (Blade) Thickness (LT), Thickness of Palisade Parenchyma (TPP), Thickness of Spongy Parenchyma (TSP) and the Presence of a Hypodermis (PHD with '+' is present and '-' is absent).

TABLE 2

Correlations between leaf anatomical characteristics for secondary and primary tree species in a secondary montane *Quercus* forest in Costa Rica

	NPPR	LT	TPP	TSP
Secondary species (14)				
NPPR	\	-	-	-
LT		\	-	-
TPP			\	.69
TSP				\
Primary species (17)				
NPPR	\	-	-	-
LT		\	.56	.70
TPP			\	.85
TSP				\

Characteristics given for N= 1 leaf or leaflet are: Non-Palisade Parenchyma / Palisade Parenchyma Ratio (NPPR), Lamina (Blade) Thickness (LT), Thickness of Palisade Parenchyma (TPP) and Thickness of Spongy Parenchyma (TSP). Pearson product moment correlation coefficients with '-' not significant and 'values' significant at $p < 0.05$.

1982) and close to values found in African montane forests (Malaisse and Colonval-Elenkov 1981, 1982), but considerably lower than in several other, probably more wind-exposed high-elevation forests (*e.g.* Grubb 1974). Cavelier and Goldstein (1989) reported extraordinarily high values for the lamina thickness for ten species in a Panamanian elfin cloud forest (595 m) and concluded that thick leaves (high lamina thickness) seem to have more physiological characteristics of drought tolerance than thin leaves (low lamina thickness). Buckley *et al.* (1980) pointed out how the overall lamina thickness increases with altitude. Earlier, Grubb (1974, 1977) introduced the term 'pachyphyll' for high-altitude species with (i) extremely thick leaves (lamina > 300 μ m), (ii) a well developed palisade (thickness of palisade parenchyma > 0.5 but < 0.75 of thickness of spongy parenchyma), (iii) outer walls of the epidermides markedly thickened, and (iv) a hypodermis frequently present. In the case of the Costa Rican montane secondary for-

TABLE 3

Leaf anatomical characteristics of some tropical montane forests

Country/Source	Altitude (m alt.)	Spp. Nr.	LT (μ m)	NPPR	>2LPP	HD
<i>Costa Rica</i>						
This study (Secondary)	2975	31	235	1.9	10.0	22.6
<i>Panama</i>						
Cavelier & Goldstein 1989	980	10	595	2.1	10.0	50.0
<i>Venezuela</i>						
Sugden 1985 (Wet Thicket)	900	23	397	1.4	34.8	43.5
Sugden 1985 (Cloud)	700	22	300	1.3	36.4	22.7
Sugden 1985 (Transition)	500	23	284	1.5	8.7	30.4
<i>Jamaica</i>						
Tanner & Kapos 1982 (Gap Gully)	1550	20	247	2.2	10.0	30.0
Tanner & Kapos 1982 (Mature, Mull Ridge)	1550	24	247	2.1	4.2	37.5
Tanner & Kapos 1982 (Mature, Mor Ridge)	1550	11	295	2.1	0.0	36.4
Tanner & Kapos 1982 (Mature, Wet Slope)	1550	23	229	2.1	8.7	26.1
<i>Puerto Rico</i>						
Howard 1969	1050	24	377	2.0	-	46.0
<i>Malawi-Zimbabwe</i>						
Malaisse & Colonval-Elenkov 1982	1585	19	250	2.7	5.0	42.0
<i>Zaire</i>						
Malaisse & Colonval-Elenkov 1981 (Dry)	1210	23	260	-	-	22.0
<i>New Guinea</i>						
Grubb 1974 and 1977	3300	61	376	1.9	-	56.0
Grubb 1974 and 1977	2500	55	200	-	-	46.0

Characteristics given for N = 1 leaf or leaflet are: Lamina (Blade) Thickness (LT), Non-Palisade Parenchyma / Palisade Parenchyma Ratio (NPPR), Percentage of species with more than two layers of palisade parenchyma (>2LPP) and Percentage of species with a hypodermis present (HD).

est, such pachyphyllous, thick-leaved species with an assumed good drought tolerance are, for instance, *Myrcianthes fragrans* and *Oreopanax capitatus* and to a lesser extent species such as *Comarostaphylis arbutoides* and *Persea vesticula*. Especially those species with a hypodermis (see Table 1) are better adapted to resist severe drought and transpire at maximum rates for longer periods (Cavelier and Goldstein 1989), because the hypodermis serves as a water reservoir, protecting the underlying palisade parenchyma against desiccation and excessive insolation (Roth 1984, Cavelier and Goldstein 1989). Moreover, the very thick outer walls of pachyphylls may well be a major adaptation to minimize invasion of the leaf by epiphyllous fungi, which are prevented from destroying the plant's protection against water loss under drying conditions (Grubb 1977). In the same way, epiphyllous hepatics are stopped from shading the leaves.

The mean ratio of non-palisade parenchyma / palisade parenchyma tissue in this study is equal to that ratio in a New Guinean montane primary forest at 3300 m asl (Grubb 1974, 1977) and also very close to ratios for Puerto Rican, Jamaican and Panamanian, primary forests (Howard 1969, Tanner and Kapos 1982, Cavelier and Goldstein 1989, respectively). The relatively high non-palisade parenchyma / palisade parenchyma ratio found in a Puerto Rican elfin forest made Howard (1969) suggest that plant species surviving there have adjusted to low light values through a reduction in the palisade parenchyma and an increase in the amount of spongy parenchyma. This may also be the case in Costa Rican montane forests. The percentage of species with more than two layers of palisade parenchyma found in this study is the same as in a Jamaican gap forest at 1550 m asl (Tanner and Kapos 1982) and a Panamanian elfin cloud forest at 980 m asl (Cavelier and Goldstein 1989).

In comparison to tropical montane primary forests, the percentage of species with a hypodermis present seems relatively low in the Costa Rican secondary forest studied. However, it is certainly very similar to the proportion recorded for a Venezuelan cloud forest at ca. 700 m asl (Sugden 1985), a mature wet slope forest at 1550 m asl in Jamaica (Tanner and Kapos 1982) or a Zambesian dry evergreen forest at 1210 m asl (Malaisse and Colonval-

Elenkov, 1981). Moreover, if only the primary species with a hypodermis present are considered, the percentage calculated (29.4 %) comes much closer to the values for montane primary forests throughout the Neotropics.

In conclusion, it was found that the mean anatomical leaf structure in a tropical montane secondary forest does not necessarily differ much from that in tropical montane primary forests. However, the presence of a large amount of secondary species may contribute to relatively higher means for lamina and mesophyll thickness. Like in primary tropical montane forests (Tanner and Kapos 1982, Sugden 1985), in this Costa Rican montane secondary forest there is considerable variation in leaf anatomical characters among species. Further studies are needed to find out how leaf anatomy changes along a complete successional gradient in tropical montane forests, from the early stages of recovery towards the mature forest phase.

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RESUMEN

Se estudió la anatomía foliar de varias especies de (14) árboles secundarios y (17) primarios en un roble secundario tardío de 30 años en la Cordillera de Talamanca, Costa Rica. En general, los tejidos foliares son más gruesos en especies secundarias, debido a la alta proporción de parénquima esponjoso para el almacenamiento de agua. En especies secundarias, el grosor promedio de la lamina foliar es de 249.5 μm y el grosor promedio del parénquima en palisada y del parénquima esponjoso es de 85.8 μm y de 133.9 μm , respectivamente. En especies primarias, el grosor promedio de la

lamina foliar es de 220.7 μm y el grosor promedio del parénquima en palisada y del parénquima esponjoso, es de 75.7 μm y de 107.9 μm , respectivamente. La fracción del grosor 'parénquima no en-palisada / parénquima en palisada' fue un poco más alto para especies secundarias. Se encontraron correlaciones significativas entre el grosor promedio del parénquima en palisada y de parénquima esponjoso para especies primarias y secundarias, y también entre el grosor promedio de la lámina foliar y el grosor promedio del parénquima en palisada y parénquima esponjoso para especies primarias solamente. Los valores de las características anatómicas foliares de este bosque secundario montano se ajustan en principio a los conocidos para bosques primarios en otras regiones tropicales de montaña.

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