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## LEAF LIFE SPAN AND NITROGEN CONTENT IN SEMIDECIDUOUS FOREST TREE SPECIES (*Croton priscus* AND *Hymenaea courbaril*)

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**ABSTRACT:** In comparison to deciduous species, evergreen plants have lower leaf nutrient contents and higher leaf life span, important mechanisms for nutrient economy, allowing the colonization of low fertility soils. Strategies to conserve nitrogen in two semideciduous tropical forest tree species, with different leaf life spans were analyzed. The hypothesis was the fact that the two species would present different nitrogen conservation mechanisms in relation to chemical (total nitrogen, protein, chlorophyll, and proteolytic activity), functional (leaf life span, N-use efficiency, and N-resorption efficiency), morphological (specific leaf mass) leaf characteristics, and total nitrogen in the soil. *Hymenaea courbaril* L. presented lower nitrogen compounds in leaves, longer leaf life span, higher N-use efficiency, and higher specific leaf mass, while absorbing proportionally less nitrogen from the soil than *Croton priscus* Croizat. These characteristics can contribute for a better nitrogen economy strategy of *H. courbaril*. No relationship was found between leaf life span and N resorption efficiency, nor between leaf life span, protease activity and nitrogen mobilization. The electrophoretic profiles of proteolytic enzymes in young leaves of the two species presented more bands with enzymatic activity than other kinds of leaves.

Key words: leaf life span, specific leaf mass, N-resorption efficiency, N-use efficiency, Atlantic forest

## DURAÇÃO DA VIDA DA FOLHA E CONTEÚDO DE NITROGÊNIO EM ESPÉCIES ARBÓREAS (*Croton priscus* E *Hymenaea courbaril*) DE FLORESTA SEMIDECÍDUA

**RESUMO:** Comparadas a espécies decíduas, as sempre-verdes têm menos nutrientes nas folhas, que também são mais longevas. Estes mecanismos são importantes para economia de nutrientes, e permitem a colonização de solos com baixa fertilidade. Foram analisadas estratégias de conservação de nitrogênio em duas espécies de floresta semidecídua, que aparentemente apresentavam longevidades foliares diferentes. Para isto foram comparados mecanismos químicos (concentrações de nitrogênio total, de proteína e clorofila e atividade proteolítica), funcionais (duração de vida das folhas, eficiência do uso de nitrogênio e eficiência de redistribuição de nitrogênio) e morfológico (massa foliar específica) de conservação de nitrogênio nas folhas e a concentração de nitrogênio no solo. *Hymenaea courbaril* L. apresentou menor concentração de compostos nitrogenados nas folhas, maior longevidade foliar, maior eficiência do uso do nitrogênio e maior massa foliar específica, além de ter retirado proporcionalmente menos nitrogênio do solo do que *Croton priscus* Croizat. Estas características podem contribuir com uma melhor estratégia de conservação de nitrogênio em *H. courbaril*. Não foi encontrada relação entre a duração de vida da folha e a eficiência de redistribuição de nitrogênio, ou entre a duração de vida da folha, atividade de proteases e mobilização de nitrogênio. As folhas jovens das duas espécies apresentaram mais bandas com atividades enzimáticas nos perfis eletroforéticos de enzimas proteolíticas.

Palavras-chave: longevidade foliar, massa foliar específica, eficiência de redistribuição de nitrogênio, eficiência de utilização do nitrogênio, floresta Atlântica

### INTRODUCTION

For low-nutrient soils the dominant species tend to be the evergreen (Chabot & Hicks, 1982; Aerts, 1996). In comparison to deciduous species, evergreen plants have lower leaf nutrient contents and higher leaf

life span, important mechanisms for nutrient economy, allowing the colonization of low fertility soils (Reich et al., 1995; Aerts, 1996; Eamus & Prichard, 1998). The leaf life span may range from a few weeks to several years, according to the plant species (Reich et al., 1991).

During leaf senescence, cell constituents such as proteins and chlorophyll are degraded and transported to younger leaves for building up of new structures (Himelblau & Amasino, 2001; Reyes-Arribas et al., 2001). This process is called nutrient resorption, and is a form of adaptation in low nutrient environments, since it reduces nutrient loss (Pugnaire & Chapin, 1993). Nevertheless, comparison of resorption efficiency between deciduous and evergreen species is still conflicting (Aerts, 1996; Lal et al., 2001).

Proteolyses plays an important role in nitrogen mobilization from senescent leaves to growing tissues and, consequently, in nutrient conservation within the plant (Callis, 1995). Therefore, it can be expected that individuals with higher protease activity, mobilize more nitrogen, as it has been demonstrated for different *Molinea caerulea* clones (Thornton & Bausenwein, 2000).

There are indications that plants grown in low fertility environments produce more organic matter per unit of mineral nutrient than plants grown on eutrophic soils. This ratio is defined as nutrient use efficiency (Vitousek, 1982); Chabot & Hicks (1982) explained that evergreen species dominate low fertility habitats because of their efficiency in nutrient use and owing to their long leaf life span.

The leaf life span is related to morphological characteristics, such as specific leaf mass. Species with higher leaf life span tend to have thicker leaves with higher specific leaf mass (Chabot & Hicks, 1982; Cornelissen et al., 1999). Gates (in Chabot & Hicks, 1982) suggested that the hardy construction of evergreen leaves might be a necessity, because they have to go through several years of environmental stress.

The aim of this study was to ascertain, through comparison, whether two semideciduous forest tree species with different leaf life spans, fit the model proposed for leaf life spans and nitrogen conservation in evergreen and deciduous species.

## MATERIAL AND METHODS

*Croton priscus* Croizat and *Hymenaea courbaril* L. var. *stilbocarpa* (Hayne) Lee et Lang are tree species from a semideciduous forest, a 251,000 m<sup>2</sup> remnant of the Atlantic Forest, Santa Genebra Reserve (SGR), Campinas, São Paulo State, Brazil (22°49'45"S; 47°06'33"W). *Croton priscus* is a species of *Euphorbiaceae* and *H. courbaril* is a leguminous tree that has no association with N<sub>2</sub>-fixing symbiotic bacteria (Moreira et al., 1992).

The experiment was carried out within a greenhouse, at Campinas, SP, Brazil, during 2000-2002. Plants were exposed to natural temperature and sunlight. Seeds from both species were sown in 4.5-L plastic pots filled

with soil derived from their growing sites in the SGR forest. The plastic pots were maintained inside plastic dishes. Irrigation was performed daily through sprinkling. The number and length of sprinklings were graded according to necessities during the different seasons, to maintain the soil moisture while preventing pot overflow. Plants were cultivated for two years without fertilization prior to biochemical analyses. During this period, leaf life span was monitored using dated, 2 cm-long plastic tape placed on petioles of very young leaves of ten plants of each species.

Leaves at three shoot positions (apical / young, median / mature green, and basal / senescent) were used in all chemical analyses. The mean values of ten replications per leaf area of leaves used in chemical analysis was 33.4 (±8.8), 51.0 (±16.8), and 47.9 (±19) cm<sup>2</sup> for young, mature green and senescent leaves of *H. courbaril*, respectively, and 59.0 (±15), 88.4 (±22), and 73.3 (±23) cm<sup>2</sup> for young, mature green and senescent leaves of *C. priscus*, respectively. Mature green leaves had reached the maximum leaf area, whereas senescent leaves were all totally yellow and fell when touched. Specific leaf mass (dry mass/leaf area) was calculated for mature green leaves, from ten plants of each species, whereas leaf area was estimated with a leaf area meter (Model Li 3100, Li-Cor), and the dry mass obtained at 60°C.

Total soil nitrogen was determined (Bremner, 1996) using 0.200-kg soil samples collected from both, the soil used to fill the pots, and that after two years of plant cultivation. The ratio between soil nitrogen at the outset of the experiment, and after two years of cultivation, was calculated in order to know by what factor nitrogen had decreased over this period.

For chlorophyll extraction, leaf pieces of 40 mg were extracted with 95 % ethanol at 60 °C. Leaf pieces with a similar fresh weight were taken from the same leaves to obtain dry weight by placing in an oven at 80°C for 48 h. The chlorophyll concentrations were calculated according to Lichtenthaler & Wellburn (1983).

Total leaf nitrogen was determined in 100 mg leaf samples, dried at 60°C for seven days. The nitrogen concentration was obtained according to Nelson & Sommers (1973). Nitrogen resorption efficiency (NRE) was calculated as  $NRE = (NG - NS) / NG \times 100$  (NG = nitrogen of mature green leaves, NS = total nitrogen of senescent leaves) (Pugnaire & Chapin, 1993). Nutrient use efficiency (NUE) for nitrogen was calculated as DM / N (DM = dry mass of mature green leaves, N = total nitrogen of mature green leaves) (Chabot & Hicks, 1982).

Protease activities were determined by partially denaturing SDS-PAGE. Proteins were extracted with (1g per 10 mL) 100 mmol L<sup>-1</sup> phosphate buffer (pH 7), 0.25% ascorbic acid, 5% glycerol, 5 mmol L<sup>-1</sup> 2-

mercaptoetanol, and 100 mg polivinylpolypyrrolidone. From the supernatants obtained through centrifugation (15,000 rpm, 15 min, 4 °C), equal amounts (Bradford, 1976) of protein were loaded in denaturing, 10 % polyacrylamide gel electrophoresis containing 0.15 % bovine serum albumin (Jiang et al., 1999). After the development of the electrophoresis and incubation in the activity buffer, the gel was stained with Comassie Blue. Following destaining, protease activities in the gel were observed as white bands. The negative image was recorded in photography.

Statistical analyses were performed with the SAS/STAT software (version 8). Normality of data distribution was determined with the Kolmogorov-Smirnov test. Means were compared by ANOVA when the assumption of normality was met. Differences among means were identified by the Tukey test. Nonparametric tests (Mann-Whitney for soil nitrogen analysis, and Wilcoxon Signed-Rank Test for soil nitrogen ratio N1/N2) were used when data were not normally distributed (Woolson, 1987; Montgomery, 1993). The number of replications for chlorophyll analysis, nitrogen resorption efficiency, nutrient use efficiency, specific leaf mass, and leaf life span was ten; for total nitrogen in leaves, three; for both protein analysis and protease activities, six; and for total nitrogen in soil samples, four replications.

## RESULTS AND DISCUSSION

*H. courbaril* presented a leaf life span ( $430 \pm 24.7$  days) approximately 3.5 times longer than *C. priscus* ( $125 \pm 62.5$  days), and specific leaf mass ( $10.54 \pm 1.3$  g cm<sup>-2</sup>) approximately twice that of *C. priscus* ( $4.96 \pm 1.8$  g cm<sup>-2</sup>). Data are consistent with previous observations, since species of longer leaf life span present higher specific leaf mass (Chabot & Hicks, 1982).

*H. courbaril* had lower content of chlorophyll, total nitrogen and protein than *C. priscus* (Table 1). Therefore, based on these parameters, these two semideciduous forest tree species, with different leaf life spans, follow the model established for evergreen and deciduous species, in which species with higher leaf life span present lower leaf nutrient concentrations (Reich et al., 1995; Eamus & Prichard, 1998).

There was no underlying relationship between leaf life span and soil nitrogen availability, since there was no difference in the nitrogen content of the soil of both sites in the SGR forest (Table 2). Different results were obtained from evergreen and deciduous species, evergreens being more abundant in low fertility soil (Aerts, 1996; Eckstein et al., 1999). After two years of cultivation in plastic pots, the soils were analyzed again for total nitrogen (Table 2). The soil from pots planted with *H. courbaril* had more nitrogen than the soil from pots planted with *C. priscus*. The ratio between soil nitrogen at the outset of the experiment, and after two years of cultivation reveals that *H. courbaril* took up less nitrogen from the soil than *C. priscus*. This feature associated to the lower leaf nitrogen concentration and higher leaf life span of *H. courbaril* can contribute for a better nitrogen economy strategy.

No relationship was found between leaf life span and N resorption efficiency, since *H. courbaril* did not present higher N resorption efficiency (49.31%) than *C. priscus* (51.52%). In fact, several authors agree that low nutrient concentration in mature leaves along with great leaf longevity, are more important factors in plant nutrient conservation than the recycling of senescent leaf nutrients (Escudero et al., 1992; Reich et al., 1995; Aerts, 1996).

There is a relationship between leaf life span and nutrient use efficiency, since the value for *H. courbaril* ( $80.3 \pm 10.5$  g g<sup>-1</sup>) was twice that of *C. priscus* ( $40.6 \pm 5.7$  g g<sup>-1</sup>). This result is consistent with previous observations, whereby species with longer leaf life spans have higher nutrient use efficiency (Aerts et al., 1999).

The protease electrophoresis profile was similar for *H. courbaril* and *C. priscus* (Figure 1). Therefore a higher protease activity in senescent leaves cannot be related to a higher nutrient economy of long life span species. Higher protease activities were observed in extracts from young leaves, which can be explained by the fact that proteases are also present in young tissues. Young tissues have intense metabolic activity, resulting in a continuous synthesis and degradation of proteins (Messdaghi & Dietz, 2000).

Table 1 - Chlorophyll, nitrogen and protein contents in young (Y), mature green (M) and senescent (S) leaves of *H. courbaril* and *C. priscus*. DM = dry mass, FM= fresh mass, and L= leaf type.

L	Chlorophyll		Nitrogen		Protein	
	<i>C. priscus</i>	<i>H. courbaril</i>	<i>C. priscus</i>	<i>H. courbaril</i>	<i>C. priscus</i>	<i>H. courbaril</i>
	----- g kg <sup>-1</sup> DM -----				----- g kg <sup>-1</sup> FM -----	
Y	6.9 ± 0.9 a*	4.3 ± 1.4 b	15.6 ± 1.9 a	8.0 ± 0.4 b	75.3 ± 9 a	48.5 ± 10 b
M	9.9 ± 2.3 a	3.5 ± 2.2 b	13.7 ± 1.5 a	7.7 ± 0.6 b	71.9 ± 7 a	38.6 ± 14 b
S	4.9 ± 2.1 a	2.5 ± 0.3 b	12.5 ± 1.7 a	8.3 ± 1.0 b	59.4 ± 23 a	53.7 ± 11 a

Data represent means ± SD. \*Means followed by different letters in the line are different (Tukey test; P = 0.05).

Table 2 - Total nitrogen in soil samples from SGR forest taken under *H. courbaril* and *C. priscus* trees (N 1). Total nitrogen of the same soils, after the two species were grown in pots over 2 years, without receiving fertilization (N 2). DM = dry mass.

Soil	N 1	N 2	N 1 / N 2
	----- g kg <sup>-1</sup> DM -----		
<i>H. courbaril</i>	2.120 a*	0.0373 a	56.84 b
<i>C. priscus</i>	1.505 a	0.0165 b	91.21 a

\*Means followed by different letters within the column are different. P-value < 0.05 for N 1 and N 2, and = 0.0625 for N 1 / N 2.

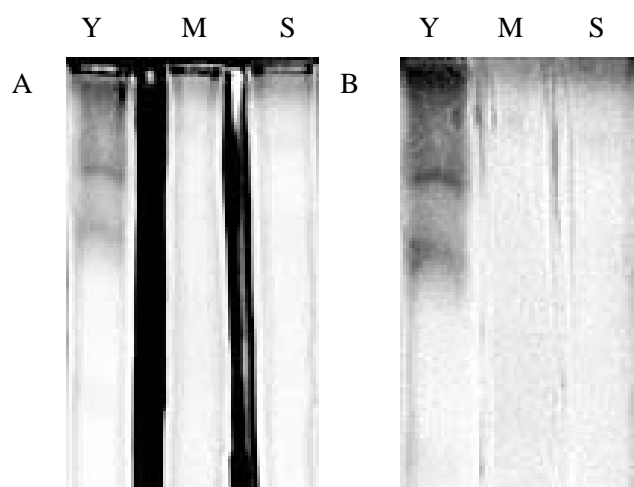


Figure 1 - Protease activities in partially denaturing SDS-PAGE of young (Y), mature green (M) and senescent (S) leaves of *H. courbaril* (A) and *C. priscus* (B).

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## REFERENCES

- AERTS, R. Nutrient resorption from senescing leaves of perennials: are there general patterns? **Journal of Ecology**, v.84, p.597-608, 1996.
- AERTS, R.; VERHOEVEN, J.T.A.; WHIGHAM, D.F. Plant-mediated controls on nutrient cycling in temperate fens and bogs. **Ecology**, v.80, p.2170-2181, 1999.
- BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v.72, p.248-254, 1976.
- BREMNER, J.M. Nitrogen-total. In: KLUTE, A.; WEAVER, R.W.; MICKELSON, S.H.; SPARKS, D.L.; BARTELS, J.M. **Methods of soil analysis**. Madison: SSSA, 1996. Pt. 3, p.1085-1121. (Chemical Methods. Book Series, 5)

- CALLIS, J. Regulation of protein degradation. **Plant Cell**, v.7, p.845-857, 1995.
- CHABOT, B.F.; HICKS, D.J. The ecology of leaf life spans. **Annual Review of Ecology and Systematics**, v.13, p.229-259, 1982.
- CORNELISSEN, J.H.C.; DIEZ, P.C.; HUNT, R. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. **Journal of Ecology**, v.84, p.755-765, 1999.
- EAMUS, D.; PRICHARD, H. A cost-benefit analysis of leaves of four Australian savanna species. **Tree Physiology**, v.18, p.537-545, 1998.
- ECKSTEIN, R.L.; KARLSSON, P.S.; WEIH, M. Leaf life span and nutrient resorption as determinants of plant nutrient conservation in temperate-arctic regions. **New Phytologist**, v.143, p.177-189, 1999.
- ESCUADERO, A.; del ARCO, J.M.; SANZ, I.C.; AYALA, J. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the biomass of different woody species. **Oecologia**, v.90, p.80-87, 1992.
- HIMELBLAU, E.; AMASINO, R.M. Nutrient mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. **Journal of Plant Physiology**, v.158, p.1317-1323, 2001.
- JIANG, W.B.; LERS, A.; LOMANIEC, E.; AHARONI, N. Senescence-related serine protease in parsley. **Phytochemistry**, v.50, p.377-382, 1999.
- LAL, C.B.; ANAPURNA, C.; RAGHUBANSHI, A.S.; SINGH, J.S. Effect of leaf habit and soil type on nutrient resorption and conservation in woody species of a dry tropical environment. **Canadian Journal of Botany**, v.79, p.1066-1075, 2001.
- LICHTENTHALER, B.K.; WELLBURN, A.R. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. **Biochemical Society Transactions**, v.11, p.591-592, 1983.
- MESSDAGHI, D.; DIETZ, K.J. Characterization of an extracellular chymostatin-sensitive serine protease preferentially expressed in young plant tissues. **Biochimica et Biophysica Acta**, v.1480, p.107-116, 2000.
- MONTGOMERY, D.C. **Designs and analysis of experiments**. 3.ed. New York: Wiley & Son, 1993. 649p.
- MOREIRA, F.M.S.; SILVA, M.F.; FARIA, S.M. Occurrence of nodulation in legume species in the Amazon region of Brasil. **New Phytologist**, v.121, p.563-570, 1992.
- NELSON, D.W.; SOMMERS, L.E. Determination of the total nitrogen in plant material. **Agronomy Journal**, v.65, p.109-112, 1973.
- PUGNAIRE, F.I.; CHAPIN, F.S. Controls over nutrient resorption from leaves of evergreen Mediterranean species. **Ecology**, v.74, p.124-129, 1993.
- REICH, P.B.; UHL, C.; WALTERS, M.B.; ELLSWORTH, D.S. Leaf life-span as a determinant of leaf structure and function among 23 Amazonian tree species. **Oecologia**, v.86, p.16-24, 1991.
- REICH, P.B.; ELLSWORTH, D.S.; UHL, C. Leaf carbon and nutrients assimilation and conservation in species of differing successional status in an oligotrophic Amazonian forest. **Functional Ecology**, v.9, p.65-76, 1995.
- REYES-ARRIBAS, T.; BARRET, J.E.; HUBER, D.J.; NELL, T.A.; CLARK, D.G. Leaf senescence in a non-yellowing cultivar of chrysanthemum (*Dendranthema grandiflora*). **Physiologia Plantarum**, v.111, p.540-544, 2001.
- SAS/STAT Users guide. Version 8. Cary: SAS Institute Inc., 1990. Cd-Room.
- THORNTON, B.; BAUSENWEIN, U. Seasonal protease activity in storage tissue of the deciduous grass *Molinia caerulea*. **New Phytologist**, v.146, p.75-81, 2000.
- VITOUSEK, P. Nutrient cycling and nutrient use efficiency. **The American Naturalist**, v.119, p.553-572, 1982.
- WOOLSON, R.F. **Statistical methods for the analysis of biomedical data**. New York: John Wiley & Sons, 1987. 513p.

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