Seasonal and Intraspecific Variation of Flavonoids and Proanthocyanidins in *Cecropia glaziovi* Sneth. Leaves from Native and Cultivated Specimens[§]

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Cecropia glaziovi Sneth. (syn. *C. glaziovii*, *C. glazioui*) (Cecropiaceae) is a South American medicinal plant whose antihypertensive activity is attributed to its flavonoid and proanthocyanidin contents. The seasonal and intraspecific variations of these two classes of compounds in *C. glaziovi* leaves were assayed by spectrophotometry in samples of young and mature leaves collected from native, cultivated and micropropagated trees in the dry and rainy periods of the year. The total flavonoid and proanthocyanidin contents ranged from (0.64 ± 0.21)% to (3.44 ± 0.45)% and (2.23 ± 0.92)% to (5.36 ± 0.95)%, respectively, among the assayed populations. The flavonoid contents in native plants did not differ statistically between young and mature leaves within the same season, whereas it was higher in both young and mature leaves collected in the dry compared to those collected in the rainy period. For cultivated specimens, the results pointed to higher contents in the dry season, whereas no significant difference was observed for leaves of micropropagated (clone) plants collected in both periods. For the assayed populations, higher proanthocyanidin contents were found in the dry season, excepting the micropropagated (clone) plants, whose contents did not differ significantly between the dry and the rainy periods. Leaves of micropropagated (clone) and cultivated specimens showed less intraspecific variation in the flavonoid and proanthocyanidin contents than those from native trees. These features suggest that, as expected, cultivation of *C. glaziovi* is of great interest providing raw herbal material of better uniform quality.

Key words: Cecropia glaziovi Sneth., Flavonoids, Proanthocyanidins, Seasonal and Intraspecific Variation

Introduction

The genus *Cecropia* comprises about 100 species occurring in tropical America, from Mexico to Southern Brazil (Berg, 1996). This genus has been placed in the order Urticales, family Cecropiaceae, which was morphologically considered as intermediate between the Moraceae and Urticaceae (Berg, 1978). Morphological comparisons and recent plastid DNA studies indicate that the Cecropiaceae in the broad sense are more closely related to the Urticaceae than to the Moraceae and are derived from the Urticaceae. This family is constituted by six genera, *Cecropia, Coussapoa, Musanga, Myrianthus, Pourouma* and *Poikilospermum.* Except for this last one, the five genera are morphologically distinct from both Moraceae and Urticaceae (Sytsma *et al*., 2002).*Cecropia glaziovi* Sneth. (syn. *C. glazioui, C. glaziovii*) is popularly named "embaúba" and "torém" in Brazil; "guarumo" and "yarumo" in Colombia and Equator; "yongol" in Peru; and "yagrumo" in Venezuela (Pio Corrêa and Penna, 1969; Pérez-Arbeláez, 1996). This species and other *Cecropia* spp. (*C. adenopus*, *C. pachystachya*, *C. peltata* and *C. hololeuca*) are traditionally used in several Latin American countries as antihypertensive, cardiotonic, diuretic and anti-asthmatic. It is a tree of 8–16 m height, with a slender, hollow, whitish trunk and reddish buds. In Brazil, the species is widely found in the central and southern regions (Pérez-Arbeláez, 1996; Lorenzi and Matos, 2002).

The antihypertensive activity of an aqueous extract and butanolic fraction from *C. glaziovi* leaves

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has been pharmacologically demonstrated in isolated preparations, animal models and humans (Franck, 1998; Franck *et al*., 1996; Lapa *et al*., 1999; Rocha *et al*., 2002). This effect is considered to be related to a blockade of the voltage-gated calcium channels in vascular smooth muscles (Lapa *et al*., 1999; Rocha *et al*., 2002). It is suggested that flavonoids and proanthocyanidins are involved in the antihypertensive acitivity of *C. glaziovi* leaves by acting as inhibitors of angiotensin-converting enzymes (ACEs) (Lacaille-Dubois *et al*., 2001). Anxiolytic-like effects were also reported for extracts of this species (Rocha *et al.*, 2002).

It is well known that the qualitative and quantitative contents of secondary metabolites in a plant show marked variation, that is regulated by intrinsic factors (ontogeny and phenology) and also by abiotic (*e.g.* light, moisture, nutrient availability) and biotic factors such as different physiological and growth stages (Harborne, 1993; Brooks and Feeney, 2000; Sosa *et al*., 2005; Calixto, 2000). In the case of plants used for medicinal purposes, all these factors must be considered, besides the post-harvesting managements (Sharapin, 2000).

Flavonoids are ubiquitous secondary products of plants and about 4,000 representatives are known (Harborne, 1977; Witzell *et al*., 2003). The production of flavonoids shows a wide qualitative and quantitative variation between plant organs and is dependent on plant growth, environmental factors and stress stimuli such as UV irradiation, drought and high temperatures (Sosa *et al*., 2005; Witzell *et al*., 2003). Besides the physiological role of plant flavonoids, they have been recognized as part of the defense strategies being responsible for important ecological functions such as antimicrobial, antifungal, antioxidant, allelopatic and detoxificant of heavy metals. Consequently, they are involved in plant-animal and plant-plant biochemical relationships, as well as in nutrient cycles (Sosa *et al*., 2005; Simmonds, 2003). Flavonoids are frequently found in fruits and vegetables and therefore are part of the human diet. They are also responsible for the pharmacological effects of several medicinal plants. As a consequence of their chemical diversity and biological functions, there is an increasing interest in this group of phytochemicals as chemotaxonomic markers, as well as in their ecological role and beneficial health effects in chronic and degenerative diseases. They disclose a wide pharmacological profile including antioxidant, free radical scavenger, lipid peroxidation inhibition, anti-inflammatory, anti-allergic, anticarcinogenic, anti-arthrithic, anxiolytic and antihypertensive activities (Di Carlo *et al*., 1997; Robards and Antolovich, 1997). Recent advances in the knowledge on the neuropharmacological and cardiac effects of flavonoids point out to their potential for the management of various psychiatric conditions and cardiac insufficiencies including the treatment of hypertension, arrhythmia and tachycardia (Johnson and Beart, 2004; WHO, 2002).

Considering the importance of *C. glaziovi* as a Brazilian medicinal plant and the previous pharmacological investigations that have confirmed its antihypertensive and anxiolytic effects, possibly related to flavonoids and proanthocyanidin, along with the lack of information on chemical parameters for drug quality control, the main goal of the present study was to evaluate the seasonal and intraspecific variation of the total flavonoid and proanthocyanidin contents in the leaves of this species.

Materials and Methods

Plant material

Five different populations of *C. glaziovi* were used in the quantitative assays. Three populations of adult native specimens were harvested in the state of Minas Gerais, Brazil: 1) Parque Estadual do Rio Doce (PERD); 2) campus Pampulha, Universidade Federal de Minas Gerais (UMFG), Belo Horizonte; and 3) Serra da Piedade, Caeté, in two periods within a year, at the end of the rainy period (RP; March–April) and of the dry period (DP; September–October). Two other groups of trees of similar ages were selected from populations of cultivated *C. glaziovi*. The first one was harvested in the vicinity of Ubatuba, state of São Paulo, Brazil, and the second one was originated from micropropagation of a plant obtained by tissue culture methods. Initial plant material for *in vitro* assays was obtained from one plantlet derived from seed germination as previously described (Alves, 1993). All plants derived from this process resulted in a clone. These cultivated plants were established at the experimental field of Centro de Pesquisas Químicas, Biológicas e Agronômicas (CPQBA), Campinas, state of São Paulo, Brazil. Young (from buds; YL) and mature leaves (from lower half part of the trees; ML) were collected from native, cultivated and micropropagated trees. Sampling was of 1 to 10 individuals

Table I. Collection data for *Cecropia glaziovi* samples.

	Collection site[a]	Collection period[b]	Development stage of leaves	Sample code[c]	Herbarium voucher number
Native trees	PERD/Caratinga (MG)	RP	Young Mature	RDYL RDML	BHCB50781
	UFMG/Belo Horizonte (MG)	RP	Young Mature	UFMGYL UFMGML	BHCB53578
		DP	Young Mature	UFMGYL UFMGML	
	Serra da Piedade/Caeté (MG)	RP	Young Mature	SPYL SPML	BHCB53726
		DP	Young Mature	SPYL SPML	
Cultivated trees	CPQBA/Campinas (SP)	RP	Young Mature	CTYL CTML	CPQBA78
		DP	Young Mature	CTYL CTML	
Micro-propagated trees (clone)	CPQBA/Campinas (SP)	RP	Young Mature	CLYL CLML	CPQBA78
		DP	Young Mature	CLYL CLML	

[a] PERD, Parque Estadual do Rio Doce; MG, state of Minas Gerais; UFMG, campus Pampulha of the Universidade Federal de Minas Gerais; CPQBA, Centro de Pesquisas Químicas, Biológicas e Agronômicas; Campinas, state of São Paulo.
[b] RP, rainy period (March–April); DP, dry period (September–October).
[c] CT, cultivated trees; CL, micropropagated (clone) trees; YL, young leaves; ML, mature leaves.

randomly collected from different populations, each specimem being labeled for data comparison purposes. Collection and identification of leaves from native plants were carried out by Dr. Júlio Antônio Lombardi, Departamento de Botânica, UFMG, Belo Horizonte, state of Minas Gerais, Brazil (present address: Departamento de Botânica, Instituto de Biociências de Rio Claro, UNESP, campus de Rio Claro, Av. 24-A 1515, Bela Vista, Caixa Postal 199, 13506-900, Rio Claro, SP, Brazil). Leaves from cultivated plants were kindly supplied by Dr. Pedro Melillo de Magalhães, CPQBA – IB, UNICAMP, Campinas, state of São Paulo, Brazil. Collection site and period, kind of leaves, sample code and herbarium voucher numbers are shown in Table I.

Stabilization and preparation of leaves powder

Young and mature leaves were separated and the leaf-blades were detached from the petioles. Only the leaf-blades were analyzed and the stipules were removed from the young leaves. The botanical material was dried in an oven with circulating air at 40 °C for 72 h. The dried material was powdered in a cutting mill.

HPLC analysis of the extracts

Powder of dried leaves was weighed (approx. 500 mg) and extracted, in an ultrasonic bath at room temperature, with etanol/water (7:3) (3 × 25 ml). After filtration, the extract was concentrated in a rotary evaporator (60 °C), the residue was dissolved in methanol/water (1:1) (5 ml) and centrifuged at 10,000 rpm for 10 min. The solution was filtered on Adsorbex RP-18 cartridges (Merck, Darmstadt, Germany), previously conditioned with methanol (2 ml), prior to injection into the HPLC apparatus. HPLC fingerprints were obtained on a Waters 2695 apparatus with a UV-DAD detector (Waters 2996). A Lichrospher 100 RP-18 column (125 × 4.0 mm i.d., 5 μm) (Merck) was employed at a temperature of 40 °C, flow rate of 1.0 ml/min and wavelengths of 280 and 350 nm. A linear gradient elution of water/acetic acid 2% (A) and methanol/acetic acid 2% (B) was employed (from 17% to 90% B in 20 min). Analyses

were performed in triplicate and each sample (10 μl) was injected twice into the HPLC apparatus. Solvents used were of HPLC grade (Merck) and were degassed by sonication before use. The identification of peaks was accomplished by comparison with the retention time (RT) of reference compounds injected under the same conditions, as well as by analysis of UV spectra recorded online. The reference compounds orientin, isoorientin, vitexin, isovitexin and isoquercitrin were purchased from Extrasyntése (Genay, France) and chlorogenic acid from Sigma (St. Louis, USA).

Spectrophotometric quantification of total flavonoids

Total flavonoid contents in the dry leaves of *C. glaziovi* were assayed by measuring, at 420 nm, the absorbance of the complex formed with aluminium chloride in acidic medium, according to Rusak *et al.* (1993). A four-point calibration curve was constructed by plotting the absorbance data and concentrations of standard rutin solutions (0.005 to 0.030 mg/ml). Linear regression analysis showed a satisfactory linearity within the analyzed range ($r^2 = 0.99664$) (Sigma Plot 2.7, Jandel Co., USA). Analyses of individual samples were performed in triplicate and the results are presented as mean ± standard deviation (s.d.) for each population.

The total flavonoid content, expressed as rutin, was calculated using the expression

$$\% = \frac{A_{\text{sample}} - A_{\text{blank}} \times 3.1056}{m_{\text{sample}}},$$

where A is the measured absorbance at 420 nm and m_{sample} the sample mass in g.

Spectrophotometric quantification of total proanthocyanidins

Total proanthocyanidin contents in the dry leaves were determined by measuring, at 540 nm, the absorbance of the resulting cyanidin chloride, after acid-catalyzed solvolysis with *n*-BuOH/HCl 37% (95:5), according to the method described by Hiermann *et al.* (1986). Each sample was analyzed in triplicate and the results are expressed as mean ± s.d., for each population. The total proanthocyanidin content, expressed as cyanidin chloride, was calculated using the expression

$$\% = \frac{A_{\text{sample}} - A_{\text{blank}} \times 4.115}{m_{\text{sample}}},$$

where A is the measured absorbance at 540 nm and m_{sample} the sample mass in g.

Statistical analysis

The statistical analyses were performed by ANOVA and considered significantly different at the level of $p < 0.05$.

Results and Discussion

Several analytical methods are available for the quantitative determination of secondary metabolites in plants and plant drugs, the pharmacopoeial assays being usually based on spectrophotometric methods (UV-VIS). HPLC techniques are becoming more and more used for the fingerprint characterization of extracts, as well as for the quantification of components, once an adequate resolution is achieved (Heigl and Franz, 2003; Rehwald *et al.*, 1994; Blázovics *et al.*, 2003). Furthermore, capillary electrophoresis has been applied to the separation and quantification of flavonoid glycosides in commercial samples of *Passiflorae herba*. The total flavonoid contents determined by this method correlated satisfactorily with those achieved by the spectrophotometric assays carried out according to the European Pharmacopoeia (Marchart *et al.*, 2003).

Crude hydroethanol extracts of *C. glaziovi* leaves were initially characterized by their RP-HPLC profiles at 280 and 350 nm (Fig. 1). Both chromatograms show a major peak corresponding to isoorientin. The main difference between these chromatograms is the presence of an intense peak of a polar constituent (RT 1.22 min) at 280 nm, which is substantially reduced in the chromatogram recorded at 350 nm. Based on these findings, it might correspond to a proanthocyanidin (Franck, 1998). The HPLC fingerprints registered at 280 and 350 nm and co-injection of reference compounds allowed identifying peaks corresponding to chlorogenic acid (RT 3.68 min), orientin (RT 9.42 min), isoorientin (RT 10.44 min), vitexin (RT 12.58 min), isovitexin (RT 13.91 min) and isoquercitrin (RT 14.52 min). Compounds previously isolated from *C. galziovi* leaves comprise chlorogenic acid, (+)-catechin, procyanidins B_2 and C_1, (−)-epicatechin, orientin, isoorientin and isoquercitrin (Franck, 1998; Lacaille-Dubois *et al.*, 2001). The obtained HPLC profile at 280 nm does not show peaks corresponding to (+)-catechin and (−)-

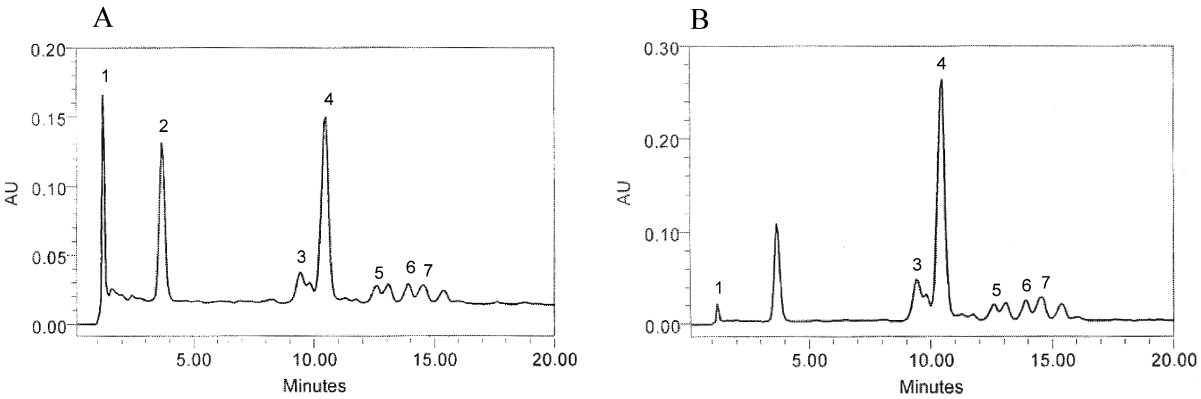

Fig. 1. RP-HPLC fingerprints for crude ethanol/water (7:3) extracts of *Cecropia glaziovi* leaves, registered at (A) 280 nm and (B) 350 nm. Chromatographic conditions: see Materials and Methods. Identified peaks: 1, proanthocyanidin; 2, chlorogenic acid; 3, orientin; 4, isoorientin; 5, vitexin; 6, isovitexin; 7, isoquercitrin.

Table II. Total contents of proanthocyanidins and flavonoids in dry leaves of *Cecropia glaziovi* collected from native, cultivated and micropropagated (clone) plants, in the dry and rainy periods of a year.

Population[a]	Total proanthocyanidins (% ± s.d.)		Total flavonoids (% ± s.d.)	
	Dry period	Rainy period	Dry period	Rainy period
NYL	4.42 ± 1.28	2.23 ± 0.92	1.50 ± 0.44	0.77 ± 0.40
NML	4.92 ± 1.45	2.54 ± 0.66	1.92 ± 0.95	0.64 ± 0.21
CTYL	5.36 ± 0.95	ND[b]	2.48 ± 0.48	ND
CTML	3.96 ± 0.64	3.28 ± 0.61	1.80 ± 0.34	1.40 ± 0.22
CLYL	4.93 ± 0.59	ND	3.44 ± 0.45	ND
CLML	4.07 ± 0.38	3.58 ± 0.43	2.07 ± 0.32	1.76 ± 0.37

[a] NYL, native young leaves; NML, native mature leaves; CTYL, cultivated young leaves; CTML, cultivated mature leaves; CLYL, micropropagated (clone) young leaves; CLML, micropropagated (clone) mature leaves.
[b] ND, not determined.

epicatechin, thus indicating their low relative contents.

Considering the complex HPLC profiles of flavonoids in the crude extract of *C. glaziovi* leaves, the spectrophotometric quantification would be undoubtedly a more convenient method for the quality control of the plant drug. Besides, it has been demonstrated that several compounds belonging to this class show angiotensin-converting enzyme (ACE) inhibitory activity and vasorelaxant effect (Franck, 1998; Franck *et al.*, 1996; Lacaille-Dubois *et al.*, 2001), what would explain the observed antihypertensive activity of *C. glaziovi* leave extracts (Lapa *et al.*, 1999). Hence, the quantification of the total contents of these metabolites was carried out according to previous described spectrophotometric methodologies (Rusak *et al.*, 1993; Hiermann *et al.*, 1986). The contents of total flavonoids and proanthocyanidins in *C. glaziovi* leaves were assayed for native, cultivated and micropropagated (clone) specimens. The amounts of these metabolite classes, determined in young and mature leaves, harvested during the dry and rainy seasons, are shown in Table II and Figs. 2–5. For the analysis and discussion of the results, the sample codes adopted in Table I were simplified and the specimens were grouped as native, cultivated and micropropagated (clone) trees.

Total flavonoids

The analyses were initially focused on the results obtained for plants collected within the same period. In the dry season, no significant difference was found between the total flavonoid content of young [(1.50 ± 0.44)%] and mature [(1.92 ±

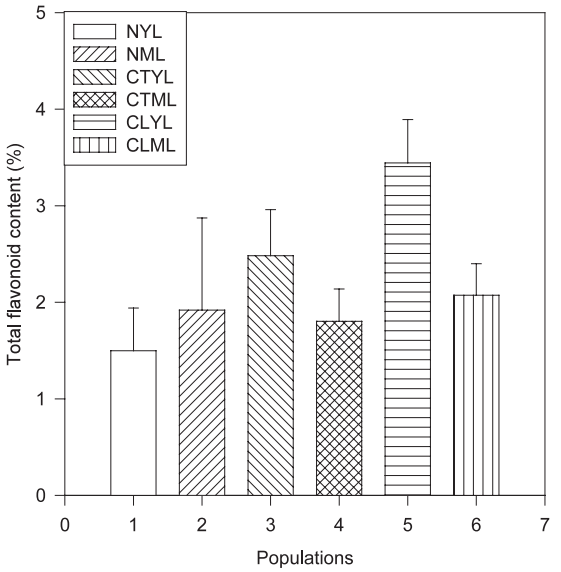

Fig. 2. Total flavonoid content of *Cecropia glaziovi* leaves collected from native, cultivated and micropropagated (clone) plants during the dry season. NYL, native young leaves; NML, native mature leaves; CTYL, cultivated young leaves; CTML, cultivated mature leaves; CLYL, micropropagated (clone) young leaves; CLML, micropropagated (clone) mature leaves.

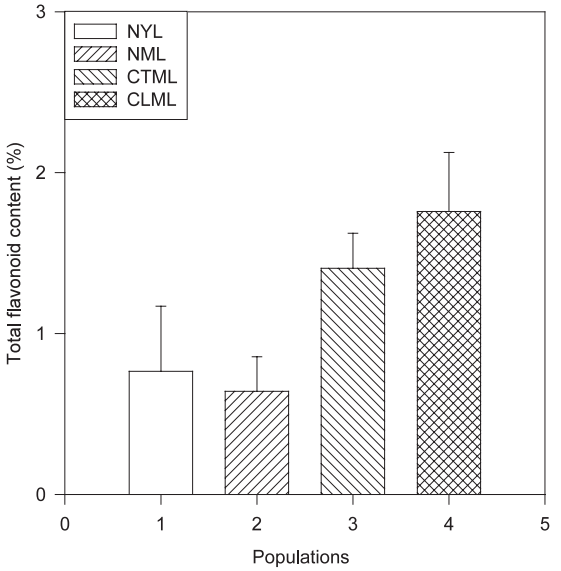

Fig. 3. Total flavonoid content of *Cecropia glaziovi* leaves collected from native, cultivated and micropropagated (clone) plants during the rainy season. NYL, native young leaves; NML, native mature leaves; CTML, cultivated mature leaves; CLML, micropropagated (clone) mature leaves.

0.95)%] leaves from native specimens ($p > 0.05$), whereas those from cultivated and micropropagated plants did show distinct amounts ($p < 0.05$), with higher contents found for the young leaves of both groups, respectively, (2.48 ± 0.48)% and (3.44 ± 0.45)% (Fig. 2). The flavonoid content in the young leaves from native, cultivated and micropropagated plants was significantly distinct, being highest for the specimens from the last group [(3.44 ± 0.45)%] (Table II). In mature leaves, the medium flavonoid content was not distinct for clones [(2.07 ± 0.32)%], cultivated species [(1.80 ± 0.34)%] and native specimens [(1.92 ± 0.95)%] ($p > 0.05$) (Fig. 2). It is worth mentioning that the total flavonoid content in the young leaves of clones was twice higher than that determined for young native plants (Table II).

For native plants collected during the rainy season, the flavonoid content did not differ statistically between young [(0.77 ± 0.40)%] and mature [(0.64 ± 0.21)%] leaves ($p > 0.05$) (Fig. 3). Samples of young leaves from micropropagated plants and cultivated specimens were not available, impairing the comparison with the content determined for the corresponding mature leaves. The total flavonoid amount [(0.64 ± 0.21)%] in mature leaves from native plants was significantly lower than those for leaves of clones [(1.76 ± 0.37)%] and cultivated [(1.40 ± 0.22)%] specimens ($p < 0.05$), collected in the rainy period (Fig. 3).

Analysis of variation in the flavonoid contents between the evaluated seasons, for native specimens, indicated higher contents for both young [(1.50 ± 0.44)%] and mature [(1.92 ± 0.95)%] leaves collected in the dry period, in comparison to the rainy season [(0.77 ± 0.40)% and (0.64 ± 0.21)%, respectively] ($p < 0.05$) (Table II). Similarly, for mature leaves from cultivated plants, a higher flavonoid content was found during the dry period [(1.80 ± 0.34)%], than in the rainy season [(1.40 ± 0.22)%] ($p < 0.05$). Otherwise, mature leaves from micropropagated plants did not show significant variation in the flavonoid content during the dry [(2.07 ± 0.32)%] and rainy seasons [(1.76 ± 0.37)%] ($p > 0.05$).

Considering the obtained results, it can be concluded that either young or mature leaves from native plants might be collected in the dry season in the case of a future industrial use, once they do not differ significantly in their flavonoid content. For cultivated specimens, the results also pointed out to higher contents in the dry season, at least

for mature leaves, whereas no significant difference was observed for leaves collected in both periods in micropropagated plants.

Total proanthocyanidins

For plants collected in the dry season (Fig. 4), no significant difference was found between young [(4.42 ± 1.28)%] and mature leaves [(4.92 ± 1.45)%] from native specimens, as well as from micropropagated plants, respectively, (4.93 ± 0.59)% and (4.07 ± 0.38)%; $p > 0.05$. In this period, for cultivated plants, young leaves [(5.36 ± 0.95)%] showed a higher proanthocyanidin content than the mature ones [(3.96 ± 0.64)%] ($p < 0.05$). Comparison of the medium proanthocyanidin contents determined for young leaves of the three populations revealed no significant difference between them, respectively, (4.42 ± 1.28)%, (5.36 ± 0.95)% and (4.93 ± 0.59)% for native, cultivated and micropropagated plants ($p > 0.05$) (Fig. 4). Similarly, the proanthocyanidin contents assayed in mature leaves did not show any significant difference between native [(4.92 ± 1.45)%], cultivated [(3.96 ± 0.64)%] and micropropagated plants [(4.07 ± 0.38)%] ($p > 0.05$).

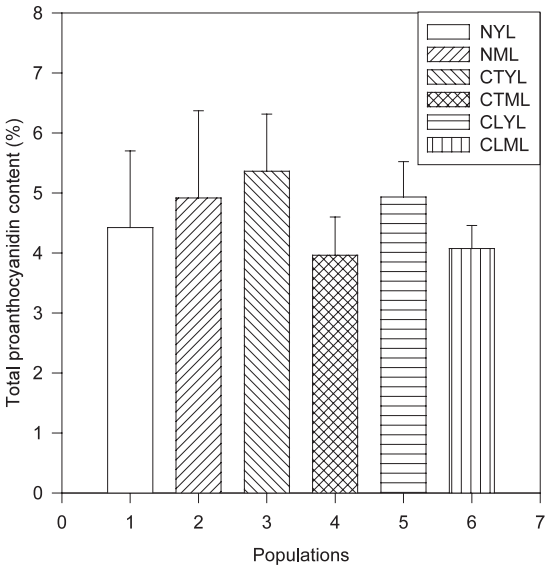

Fig. 4. Total proanthocyanidin content of *Cecropia glaziovi* leaves collected from native, cultivated and micropropagated (clone) plants during the dry season. NYL, native young leaves; NML, native mature leaves; CTYL, cultivated young leaves; CTML, cultivated mature leaves; CLYL, micropropagated (clone) young leaves; CLML, micropropagated (clone) mature leaves.

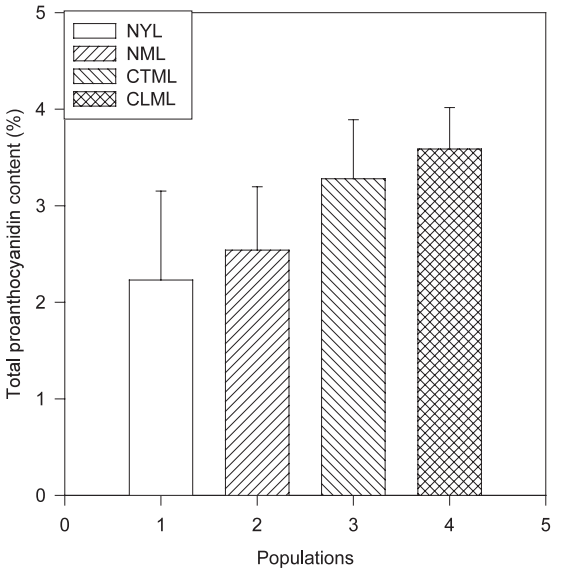

Fig. 5. Total proantocyanidin content of *Cecropia glaziovi* leaves collected from native, cultivated and micropropagated (clone) plants during the rainy season. NYL, native young leaves; NML, native mature leaves; CTML, cultivated mature leaves; CLML, micropropagated (clone) mature leaves.

The proanthocyanidin content of young [(2.23 ± 0.92)%] and mature [(2.54 ± 0.66)%] leaves from native populations, collected in the rainy season, did not differ statistically ($p > 0.05$) (Fig. 5). As previously discussed, this analysis was not feasible for micropropagated plants and cultivated specimens, due to the lack of samples of young leaves collected in this period. The proanthocyanidin contents determined for mature leaves from micropropagated [(3.58 ± 0.43)%] and cultivated [(3.28 ± 0.61)%] plants did not differ (Fig. 5), but were considerably higher than the amounts found in mature leaves from native specimens [(2.54 ± 0.66)%] ($p < 0.05$). Besides, for the assayed populations, higher proanthocyanidin contents were found in the dry season ($p < 0.05$) (Table II), except for the clones, whose contents did not differ significantly between the dry [(4.07 ± 0.38)%] and the rainy [(3.58 ± 0.43)%] periods ($p > 0.05$).

These data indicate that leaves from native populations of *C. glaziovi* showed higher proanthocyanidin contents during the dry season, and that no significant variation was observed between the proanthocyanidin content in young and mature leaves of native specimens.

Intraspecific variation

The obtained data also allowed a comparison of the intraspecific variation between the assayed populations. As expected, the native specimens presented a more pronounced intraspecific variation, indicated by the higher values of relative standard deviation (RSD) in the amounts of flavonoids and proanthocyanidins, reaching maxima of 59% and 41%, respectively (Table II). The flavonoid content within this group ranged from (0.27 ± 0.04)% to (3.83 ± 0.35)%, while the proanthocyanidin amount was in the range of (1.22 ± 0.17)% to (7.49 ± 0.87)% (primary data not shown). On the other hand, as expected, the intraspecific variation observed for micropropagated plants was considerably lower, with maximal RSD values of 12% and 21%, respectively, for proanthocyanidin and flavonoids, whereas cultivated specimens presented a maximal RSD of 19% for both classes of the assayed metabolites (Table II). Such differences could be related to the genetic basis of the populations, whereas the higher contents in the dry season, within each population, are probably a consequence of environmental factors, specially climatic conditions, as previous studies have demonstrated: UV light and stress moisture synergistically induce flavonoid synthesis (Sosa *et al*., 2005).

The results here reported indicate that both young and mature leaves from native *C. glaziovi* trees show higher contents of flavonoids and proanthocyanidins in the dry period of the year than those observed for leaves collected in the rainy season, what is a clear evidence for abiotic factors influencing the plant physiology. Compared to native plants, the cultivated and micropropagated (clone) trees showed similar or higher amounts of flavonoids and proanthocyanidins, besides a less intraspecific variation. As expected, these features suggest that cultivation of *C. glaziovi* is of great interest providing raw herbal material of better uniform quality.

It should be emphasized that the phytochemical profile observed for *C. glaziovi* leaves is similar to that of hawthorn [*Crataegus oxyacantha* L., synon. *C. monogina* Jacq. (Lindm.)], a herbal drug used for the treatment of mild to moderately severe heart failure and coronary heart disease. *Crataegus* is one of the few antihypertensive plant drugs whose medicinal use was confirmed by pre-clinical and clinical assays (Vierling *et al*., 2003; WHO, 2002), what discloses the potential of *C. glaziovi* as a candidate for the development of phytomedicines.

As far as we are aware, these are the first data on the contents as well as on the seasonal and intraspecific variation of flavonoids and proanthocyanidins in the leaves of native and cultivated specimens of *C. glaziovi* and these data can be useful for the pharmacopoeial characterization of the plant drug and to guide the standardization of extracts.

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