Major Constituents of the Foliar Epicuticular Waxes of Species from the Caatinga and Cerrado

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The epicuticular waxes of leaves of four species (*Aspidosperma pyrifolium*, *Capparis yco*, *Maytenus rigida* and *Ziziphus joazeiro*) from the Caatinga, (a semi-arid ecosystem of Northeast Brazil) and four species (*Aristolochia esperanzae*, *Didymopanax vinosum*, *Strychnos pseudoquina* and *Tocoyena formosa*) from the Cerrado, (a savanna ecosystem covering one third of the Brazilian territory), were analyzed. Six species contained a high content (above 60 $\mu g.cm^{-2}$) of wax, four of them from the Caatinga. Triterpenoids and *n*-alkanes were the most frequent and abundant constituents found in the species from both habitats. The distribution of *n*-alkanes predominated by homologues with 27, 29, 31 and 33 carbon atoms, displayed no consistent differences between species from the two habitats. Lupeol, β-amyrin, epifriedelinol and ursolic acid were the triterpenoids found. Triterpenoids clearly predominate over alkanes in the waxes from the Cerrado species. The waxes of two evergreen species from the Caatinga yielded *n*-alkanes as predominant constituents. A comparison of foliar epicuticular waxes of native plants from ecosystems with different hydric constraints is discussed.

Introduction

Epicuticular waxes are often complex mixtures of aliphatic long chain and triterpenoid cyclic compounds which line and protect non suberized aerial plant parts. The quantity and composition of the epicuticular waxes are highly variable and dependent on the taxa, organ, phenology, environmental conditions and other internal and external factors inherent to the plant (Baker, 1982; Holloway, 1984; Walton, 1990). Reduction in the amount of water lost by cuticular transpiration is the main physiological role assigned to the epicuticular waxes (Schönherr, 1982).

Several investigations about epicuticular waxes of plants from Brazilian ecosystems have been carried out. For example, Machado and Barros (1996) have described the ultrastructural characteristics of the waxy covering of palms. Amaral *et al.* (1985) and Salatino *et al.* (1985) made observations about the content and composition of leaf epicuticular waxes of plants from the Cerrado, while Salatino *et al.* (1986) studied the morphology of the waxy deposits on leaf surfaces of these plants. Amaral *et al.* (1990) studied the *n*-alkane patterns of epicuticular waxes of aquatic plants. The taxonomic significance of alkane patterns of Velloziaceae, a typical group from the Campos Rupestres, was evaluated by Salatino *et al.* (1989; 1991), while Mimura *et al.* (1998) did the same in connection with plants of the genus *Huberia* (Melastomataceae) from the Campos Rupestres. Varanda and Santos (1996) have compared the alkane patterns of plants from the Cerrado and mesophytic forests. Among these studies, only that of Salatino *et al.* (1985) provided a comprehensive analysis of the foliar epicuticular waxes; the other studies focused only on the composition of the alkane fraction.

The Cerrados are savanna ecosystems of the Brazilian Highland below 1000 m of altitude, characterized by acidic and oligotrophic soils, rich in trivalent (iron and aluminum) cations. The Cerrados cover about one third of the Brazilian territory, mainly in the Central and Southeastern regions. The Caatingas are semi-arid ecosystems from Northeast Brazil, a region with serious social-economic problems stemming chiefly from the lack of enough water, sometimes over long

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periods, for growing crops and raising cattle. So far, no studies have been carried out about epicuticular waxes of plants from the Caatinga.

The present work presents data about the contents and composition of foliar epicuticular waxes of plants from the Cerrado and Caatinga and represents the first report about foliar waxes of Caatinga species and also of Celastraceae and Loganiaceae plants. It may also be regarded as an attempt toward a comparison of foliar epicuticular waxes of native plants from ecosystems inhabited by plants with different hydric relationships.

Material and Methods

Fully expanded, intact leaves of *Aspidosperma pyrifolium* Mart. (Apocynaceae), *Capparis yco* Mart. (Capparaceae), *Maytenus rigida* Mart. (Celastraceae) and *Ziziphus joazeiro* Mart. (Rhamnaceae) were collected in an area of Caatinga in Alagoinha (state of Pernambuco, Northeast Brazil). Leaves of *Aristolochia esperanzae* O. Ktze. (Aristolochiaceae), *Didymopanax vinosum* (Cham. & Schltd.) Marchal (Araliaceae), *Strychnos pseudoquina* A. St.-Hil. (Loganiaceae) and *Tocoyena formosa* (Cham. & Schltd.) K. Schum. (Rubiaceae) were collected in an area of Cerrado in Pirassununga (state of São Paulo, Southeast Brazil). Voucher specimens of the Caatinga and Cerrado species were deposited in the Herbaria of the Federal University of Pernambuco (UFP) and University of São Paulo (SPF), respectively.

The epicuticular waxes were extracted according to Silva Fernandes *et al.* (1964). In short, after drying in the shade, 500 g of leaves were immersed three consecutive times in redistilled $CHCl_3$ for 30 sec with constant stirring. The pooled extracts were concentrated to dryness under reduced pressure. The total amount of epicuticular wax was determined by the ratio between the amount of wax (μg) and the foliar surface area (cm^2). The latter was measured by digitalization of 30 dried leaves, using a tabletop scanner (HP Deskscan II) and the software Siarcs v. 3.0 (EMBRAPA, CNPDIA, 1997).

The classes of constituents of the waxes were separated over CC using silicagel (Merck). The alcoholic triterpenoids and the classes of aliphatic compounds, except free carboxylic acids, were purified over TLC using silicagel G Typ 60 (Merck) impregnated with 0.02% sodium fluoresceine using cyclohexane:$CHCl_3$ (73:27 v/v) or $CHCl_3$ as solvent, followed by visualization under long wave UV. Triterpenic acids and free aliphatic carboxylic acids were purified over TLC using silicagel and $CHCl_3$:AcOEt:Et_2O (3:4:4 v/v) as solvent. Visualization of the triterpenic acids was achieved by spraying a solution of sulfuric vanillin 5% and heating at 110 °C for 10 min. The aliphatic carboxylic acids were visualized by spraying with a solution of 0.2% ethanolic sodium fluoresceine followed by observation under long wave UV. All fractions of the separated and purified constituent classes by TLC were weighed with the purpose of establishing estimates of their concentration in the waxes.

For identification, triterpenic acids were derivatized with diazomethane. The methyl esters of triterpenic acids, alcoholic triterpenoids, aliphatic ketones and *n*-alkanes were analyzed by GC/EIMS at 70 eV using an HP 5890 ser. II Plus chromatograph and an HP 5989B ChemStation mass spectrometer. GC involved the use of an HP-5MS capillary column (30 m x 0.30 mm id). The initial temperature of the column was 150 °C (2 min), followed by a gradient to 300 °C at 10 $°C.min^{-1}$. The temperature of the injector and detector were 300 °C. Helium was used as carrier gas at a flux of 1 $cm^3.min^{-1}$. Identification of the compounds was based on direct comparisons with authentic samples, analyses of the mass spectra and comparison of the latter with data from the Wiley275-pc library.

Results and Discussion

The content of epicuticular waxes of the eight species studied ranged from 40.4 $\mu g.cm^{-2}$ to 82.3 $\mu g.cm^{-2}$ (Table I). The data do not suggest consistent differences in wax contents between plants from the two habitats. Contents above 60 $\mu g.cm^{-2}$ have been considered exceptionally high (Baker, 1982). Thus all species from the Caatinga analyzed, besides *Didymopanax vinosum* and *Tocoyena formosa* from the Cerrado, may be regarded as bearing exceptionally thick foliar waxy deposits. Environmental conditions such as high light intensities, low air humidity and high temperatures have been pointed out as factors favoring high production of epicuticular waxes

Table I. Total content ($\mu g.cm^{-2}$) and percent composition (w/w) of foliar epicuticular waxes of species from the Caatinga and Cerrado. Dashes correspond to undetected constituents.

		Aliphatic constituents					Triterpenoids			
Species	Total Wax Content*	Fatty acids	Primary alcohols	Secondary alcohols	Ketones	*n*-Alkanes	Lupeol	β-Amyrin	Epifriede-linol	Ursolic acid
Caatinga										
Aspidosperma tomentosum	70.1 ± 2.4	4.6	3.1	–	–	21.3	23.0	–	–	46.4
Capparis yco	60.3 ± 3.4	6.8	2.4	2.8	–	76.5	–	–	–	–
Maytenus rigida	75.2 ± 2.5	2.2	–	–	–	11.5	–	–	72.2	–
Ziziphus joazeiro	72.2 ± 2.8	2.8	2.7	2.5	2.1	78.6	–	–	–	–
Cerrado										
Aristolochia esperanzae	40.4 ± 3.5	7.3	8.3	1.9	62.6	16.7	–	–	–	–
Didymopanax vinosum	75.3 ± 2.4	6.2	3.3	–	–	27.8	53.7	–	–	–
Strychnos pseudoquina	51.6 ± 1.9	6.6	2.6	–	–	40.2	26.5	21.7	–	–
Tocoyena formosa	82.3 ± 1.8	5.6	–	2.3	2.0	25.3	–			54.6

* Values represent the means of thirty adult leaves for each specie ± standard derivation.

(Baker, 1974). They prevail in the Cerrado and Caatinga, being yet more pronounced and longer lasting in the latter habitat. The wax contents of the Cerrado plants on Table I are similar to values reported by Amaral *et al.* (1985) and Salatino *et al.* (1985).

Triterpenoids and *n*-alkanes often appear as important constituents of the studied waxes (Table I). Only leaves of *A. esperanzae* yielded a ketone (hentriacontane-16-one) as main wax constituent. In the waxes of the other species from the Cerrado triterpenoids predominate over *n*-alkanes. Triterpenoids also predominated in the waxes of the leaves of two species from the Caatinga (*Aspidosperma pyrifolium* and *Maytenus rigida*). The leaf waxes of *Capparis yco* and *Ziziphus joazeiro* contained *n*-alkanes as main constituents (Table I). Triterpenoids have generally been regarded as low efficient anti-transpiring barriers (Grncarevic and Radler, 1967; Holloway, 1969). On the other hand, they may exhibit pronounced anti-herbivore activity (Varanda *et al.*, 1992; Kombargi *et al.*, 1998; Salatino *et al.*, 1998). Xerophytes from other ecosystems are also known to possess pentacyclic triterpenoids with anti-herbivore activity (Heinzen and Moyna, 1993). The high herbivory pressure on plants from the Cerrado (Fowler and Duarte, 1991) and the common and abundant occurrence of triterpenoids in their leaf waxes is an intriguing connection that deserves further consideration.

Alcoholic triterpenoids (lupeol, epifriedelinol and β-amyrin) appeared in waxes of four species (*Aspidosperma pyrifolium*, *Didymopanax vinosum*, *Maytenus rigida* and *Strychnos pseudoquina*), while ursolic acid was found in only two species (*A. tomentosum* and *Tocoyena formosa*), always as the main constituent (Table I). In *D. vinosum* lupeol has been related to a deterrence against leaf-cutter ants (Salatino *et al.*, 1998). This compound has often been found as a wax constituent of many species, commonly in association with α- and β-amyrin (Wollenweber and Dörr, 1995). Epifriedelinol appears as the main constituent of the foliar wax of *M. rigida* (Table I). This species has been regarded as highly resistant to drought, although its adaptative mechanisms are still unknown (Sampaio, 1995). Friedelanes have been found in foliar waxes of other taxa (Gülz *et al.*, 1992).

Hentriacontan-16-one is sometimes found in waxes possessing hentriacontane as dominant hydrocarbon (Kollatukudy, 1970). Such is the case of *A. esperanzae* (Table II), a fact that strengthens

Table II. Percent distribution of *n*-alkanes from foliar epicuticular waxes of species from the Caatinga and Cerrado. Dashes correspond to undetected homologues.

Species	Number of carbon atoms									
	25	26	27	28	29	30	31	32	33	Others
Caatinga										
Aspidosperma pyrifolium	3	-	22	2	32	2	5	2	29	3
Capparis yco	2	-	5	-	43	2	19	-	27	2
Maytenus rigida	2	2	13	-	51	3	16	3	4	6
Ziziphus joazeiro	2	-	9	2	11	-	42	-	32	2
Cerrado										
Aristolochia esperanzae	2	2	11	-	27	-	43	-	7	8
Didymopanax vinosum	2	-	25	-	32	-	22	5	10	4
Strychnos pseudoquina	-	3	32	-	44	-	12	-	8	2
Tocoyena formosa	3	-	40	4	32	-	7	-	9	5

proposed biogenetic relationships between wax alkanes and ketones. Hydrocarbons were obtained from the foliar waxes of all species studied (Table I). The distribution of the *n*-alkanes follow the general rule for most angiosperms, with predominance of the odd numbered carbon chains C_{27}, C_{29}, C_{31} and C_{33} (Table II). As is also very common in angiosperms, nonacosane was the most frequent main homologue. Quite different *n*-alkane patterns characterize *Aspidosperma tomentosum* (Cerrado) and *A. pyrifolium* (Caatinga), since the former contains heptatriacontane as the main homologue (Salatino *et al.*, 1985) and the latter nonacosane and hentriacontane (Table II). Data of Table II suggest that probably no differences between alkane patterns distinguish foliar waxes of plants from the Cerrado and Caatinga. Two species from the latter, *Capparis yco* and *Ziziphus joazeiro*, stand out for their high yields of alkanes (Table I). The latter species is evergreen, rarely shedding leaves, probably because of a suite of factors (Ferri, 1955; Sampaio, 1995; Machado *et al.*, 1997). *C. yco* is not known to possess similar mechanisms for drought resistance; nonetheless, it is also evergreen, retaining leaves even along the harshest drought periods (Ferri, 1955). Alkanes have been shown to be highly efficient anti-transpiring barriers (Grncarevic and Radler, 1967; Holloway, 1969). Could it be that for those xerophytes from the Caatinga *n*-alkanes bear a higher survival value than for most species from the Cerrado?

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