Acta bot. bras. 19(4): 727-732. 2005

n-alkanes from *Paepalanthus* Mart. species (Eriocaulaceae)

Lourdes Campaner dos Santos[1,3], Anne L. Dokkedal[2], Miriam Sannomiya[1], Maria Carla Piza Soares[1] and
Wagner Vilegas[1]

Received: February 09, 2004. Accepted: March 04, 2005

RESUMO – (*n*-alcanos de espécies de *Paepalanthus* Mart. (Eriocaulaceae)). Este trabalho apresenta o estudo de substâncias apolares obtidas a partir de plantas pertencentes ao gênero *Paepalanthus* Mart. (Eriocaulaceae). Hidrocarbonetos alifáticos de cadeias longas lineares foram identificados por CG-DIC e CG-EM. Os resultados indicam que as espécies de *Paepalanthus* subg. *Platycaulon* apresentam perfil homogêneo, com cadeias carbônicas de *n*-alcanos variando de C_{25} a $C_{31,}$ com a maioria das amostras apresentando freqüências maiores dos homólogos C_{27} e $C_{29.}$ As espécies do subgênero *Paepalocephalus* podem ser diferenciadas pela distribuição dos *n*-alcanos principais. *P. macrocephalus*, uma espécie da subseção *Aphorocaulon*, apresenta perfil com alcanos de cadeia ímpar, enquanto *P. denudatus* e *P. polyanthus*, espécies da seção *Actinocephalus*, apresentam perfil bem distinto, com grande número de cadeias mais curtas e alta freqüência de cadeias com número par de carbonos, especialmente *P. polyanthus*. Os resultados obtidos indicam que a distribuição de *n*-alcanos pode ser útil como caráter taxonômico, assim como as substâncias mais polares, como os flavonóides glicosilados.

Palavras-chave: *Paepalanthus*, *n*-alcanos, GC-FID, GC-MS, quimiotaxonomia

ABSTRACT – (*n*-alkanes from *Paepalanthus* Mart. species (Eriocaulaceae)). This work presents the study of nonpolar compounds from plants belonging to the genus *Paepalanthus* Mart. (Eriocaulaceae). Long-chain linear aliphatic hydrocarbons were identified by GC-FID and GC-MS. The results indicate that *Paepalanthus* subg. *Platycaulon* species present a very homogenous profile, with carbon chains of *n*-alkanes ranging from C_{25} to C_{31}, most samples presenting higher frequencies of C_{27} and C_{29} homologues. *Paepalanthus* subg. *Paepalocephalus* species may be distinguished from one another by the distribution of main *n*-alkanes. *P. macrocephalus*, subsect. *Aphorocaulon* species, presents alkanes with odd-carbon numbers and *P. denudatus* and *P. polyanthus*, *Actinocephalus* species, present alkanes with quite distinctive profiles, with many shorter chains and a high frequency of even-carbon number, especially *P. polyanthus*. The results obtained indicate that the distribution of alkanes can be a useful taxonomic character, as do polar compounds like flavonoid glycosides.

Key words: *Paepalanthus*, *n*-alkanes, GC-FID, GC-MS, chemotaxonomy

Introduction

Eriocaulaceae comprises around 1,200 species in 10 genera (Giulietti *et al.* 2000). It is a natural group of herbaceous monocotyledons, characterized by small flowers densely arranged in capitula. Despite the low number of genera when compared to other plant families, the Eriocaulaceae encompasses many infrageneric levels. *Paepalanthus* is the largest genus of this family, comprising about 500 species (Giulietti & Hensold 1990) and it is subdivided in many subgenera, sections and subsections (Sano 2004). Although the huge amount of botanical work on this group, it is still difficult to clearly define these levels (Giulietti *et al.* 1995; Sano 2004). On the other hand, little is known about the chemical composition of the plants from this family. Previous reports (Andrade *et al.* 1999; Vilegas *et al.* 1999a; Vilegas *et al.* 1999b; Dokkedal *et al.* 2004) have shown that polar compounds like flavonoids glycosides can be usefull as a taxonomic character.

Among plant secondary metabolites, alkanes from epicuticular waxes have acquired a very consolidated condition as indicators of taxonomic relations between plant groups. Considerable interest has been shown in the systematic distribution of such compounds throughout the plant kingdom. Attention has been directed towards the possibility of using their distribution as a means of establishing a taxonomic system based on chemical characteristics (Eglinton *et al.* 1962a;

---

[1] Universidade Estadual Paulista, Departamento de Química Orgânica, Instituto de Química, C. Postal 355, CEP 14800-900, Araraquara, SP, Brasil (FAPESP, CNPq)

[2] Universidade Estadual Paulista, Faculdade de Ciências, Departamento de Ciências Biológicas, C. Postal 473, CEP 17033-360, Bauru, SP, Brasil (FUNDUNESP)

[3] Corresponding Author: loursant@iq.unesp.br

Eglinton *et al.* 1962b; Eglinton & Hamilton 1963; Herbin & Robins 1968; Manners & Davis 1984; Gneco *et al*. 1988; Salatino *et al*. 1988; Salatino *et al*. 1989). However, inconsistencies of the alkanes of plant waxes as taxonomic markers have been pointed out; some authors have observed that the alkane distribution could be strongly affected by several factors, among them the age of the plant organ (Wilkinson & Kasperbauer 1972; Stocker & Wanner 1975; Nordby & Nagy 1977). Others (e.g. Smith & Martin-Smith 1978) concluded that no chemotaxonomic relationship could be derived from the composition of *n*-alkanes as the intraspecific variation was greater than the interspecific variations. Gradually, it seems that alkanes have regained the chemotaxonomists confidence (Sorensen *et al*. 1978; Faboya *et al*. 1980; Cowlishaw *et al*. 1983; Broschat & Bogan 1986). Salatino *et al*. (1991) have analysed a variable number of individuals of 12 species of Velloziaceae. Three degrees of plasticity of alkane profiles were recognized, depending on the species considered. They found that for most taxa, alkanes may be taxonomically useful at the species level if some precautions are taken. Skorupa *et al*. (1998) found that foliar epicuticular hydrocarbon patterns of 11 species of *Pilocarpus* represent useful evidences for its taxonomy at the interspecific, specific e infraspecific hierarchic levels. Merino *et al*. (1999) observed that alkane patterns are taxonomically valuable to explain *Lupinus* species relationships.

In the present study we have undertaken the investigation of nonpolar extracts by GC-FID and GC-MS from capitula of 11 additional species of *Paepalanthus*, distributed in two subgenera as follows: *P*. subg *Platycaulon* (*P. bromelioides*, *P. latipes*, *P. planifolius*, *P. speciosus*, *P. vellozioides*) and *P*. subg. *Paepalocephalus* (*P. macrocephalus*, *P. denudatus*, *P. hilairei*, *P. polyanthus*, *P. ramosus* and *P. robustus*).

Material and methods

The plants were collected in February 1995 in Serra do Cipó, Minas Gerais state, Brazil and were identified by Prof. Dr. Paulo Takeo Sano and by Prof. Dr. Ana Maria Giulietti, of the Instituto de Biociências - USP, where the exsicatas were kept: *P. denudatus* Koern. (CFSC 13853); *P. hilairei* Koern. (CFSC 13843); *P. polyanthus* (Bong.) Kunth (CFSC 13849); *P. ramosus* (Wilkstr.) Kunth (3210 HUEFS, SPF); *P. robustus* Silv. (CFSC 13840) belonging to *P*. sect. *Actinocephalus* and *P. macrocephalus* Ruhl. (CFSC 13847) of *P*. subsect. *Aphorocaulon* (all species of *P*. subg. *Paepalocephalus*); *P. bromelioides* Silv (CFSC 13856); *P. latipes* Silv. (CFSC 13846); *P. planifolius* (Bong.) Koern. (CFSC 13848); *P. speciosus* Gardner (CFSC 13851); *P. vellozioides* Ruhl. (CFSC 13842), of *P*. subg. *Platycaulon*.

Capitula of each plant (1g) were extracted with 10 mL of hexane by maceration for one week. The extracts were concentrated at 40 °C in a rotary evaporator and the final solutions were evaporated under a gentle nitrogen flow until almost dry, and diluted to 200 µL with hexane. The extracts were transferred onto a silica gel Sep-Pak cartridge (690 mg 8 µm), which was previously conditioned with 5 mL of hexane and sequentially eluted with 1.5 mL of hexane. Fractions from each plant were evaporated under a stream of nitrogen until almost dry. The residues were dissolved in 100 µL of hexane and then analyzed by GC-FID with standards of *n*-alkanes (C_{20}, C_{26} and C_{32} - Aldrich Chemie) and GC-MS. Gas chromatography (GC) analyses were performed using a Varian 3380 gas chromatograph equipped with a fused silica CBP-5 capillary column (25m×0.33 mm i.d.; film thickness 0.5 m) and a flame ionization detector (FID). Hydrogen was used as the carrier gas (60 Kpa), and the injection split ratio was 1:30. The injection temperature was 250 °C; the column temperature was held at 100 °C for 2 min, then increased to 280 °C at 10 °C/min, and this temperature was held for 15 min; the detector temperature was 280 °C. Samples of 1 µL were injected using a 10 µL Hamilton syringe. High resolution (HR) chromatography-mass spectrometry (GC-MS) analyses were performed using a Hewlett Packard (HP) 5970 MSD, with electron impact ionization (70eV), coupled to an HP 5890 GC. The column was a 25 m× 0.25 mm i.d. HP-1 (cross-linked methyl silicon; 0.3 µm film thickness). Samples of 1 µL were injected using the split mode (split ratio 1:30), with the injector and the interface both maintained at 280°C. The temperature program used was the same as described above. Hydrogen was used as carrier gas (100 Kpa). The MS scan range was 50-500 a.m.u. Data were processed on an HP 7946/HP 9000-300 CPU.

The calibration curve was constructed injecting standard hydrocarbons C_{20}, C_{26}, C_{32}. The calibration curve graph was constructed using log Retention Time *vs* Carbon atom number. The first fractions of each hexane extract from each *Paepalanthus* species were then analyzed by HRGC-FID under the same condition as that of the hydrocarbons standard aforementioned to obtain the chromatograms.

The first fractions of each hydrocarbon standard were injected into the GC-MS equipment using the same conditions as described above. The identification of the compounds was based on interpretation of the fragmentograms and retention index calculations.

Results and discussion

The chromatograms of the fractions analyzed by GC-FID and GC-MS show a typical profile of well-resolved peaks separated by 14 a.m.u (CH_2) corresponding to long-chain aliphatic hydrocarbons. The molecular ion is weak in every case. Major peaks were identified as being $C_{25}H_{52}$, $C_{27}H_{56}$, $C_{29}H_{60}$ and $C_{31}H_{64}$, respectively. Figures 1 and 2 show the *n*-alkanes profile of each plant based on the intensity of the peaks obtained from GC-FID analyses.

The results indicate that *Paepalanthus* subg. *Platycaulon* (Fig. 1) species present a very homogenous profile, with carbon chains of the *n*-alkanes ranging from C_{25} to C_{31}, most samples presenting higher frequencies of C_{27} and C_{29} homologues. In all species C_{27} is the main alkane, excluding *P. latipes*, where C_{29}

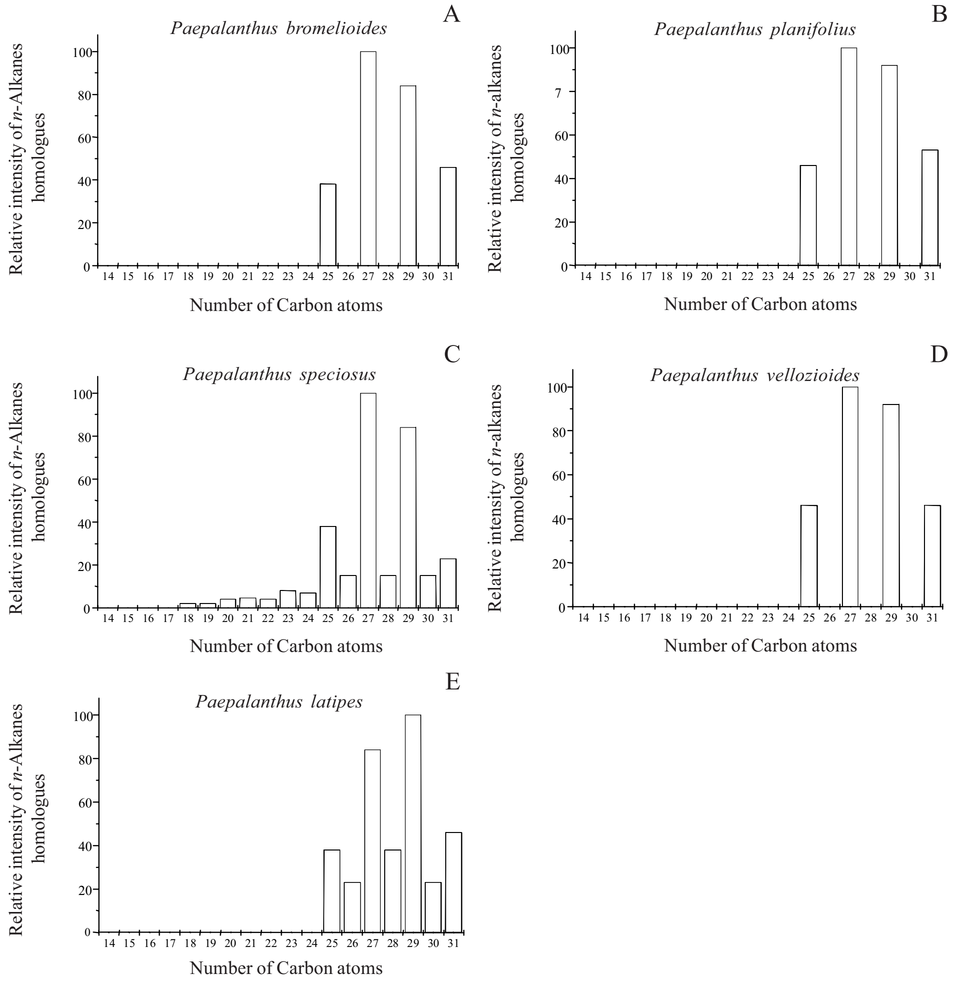

Figure 1. *n*-Alkanes profile of plants of *Paepalanthus* subg. *Platycaulon* based on the intensity of the peaks obtained from GC-FID analyses.

is the main one. These results agree with data of previous reports using polar compounds like flavonoid glycosides (Vilegas *et al*. 1999a; Vilegas *et al*. 1999b) and reinforce the homogeneity of these taxa, since subg. *Platycaulon* is characterized by naphthopyranone derivatives and 7-methoxy flavonol derivatives. On the other hand, in the subg. *Paepalocephalus* species (Fig. 2), although C_{27}-C_{29} homologues present higher frequencies in all samples, they may be distinguished from one another by the distribution of *n*-alkanes, taking into account the main alkanes quoting the main one outside and the second one inside parentheses: *P. macrocephalus* - C_{21}, C_{27} and C_{29} (no real predominance of either alkane); *P. hilairei* - C_{27} (C_{29});

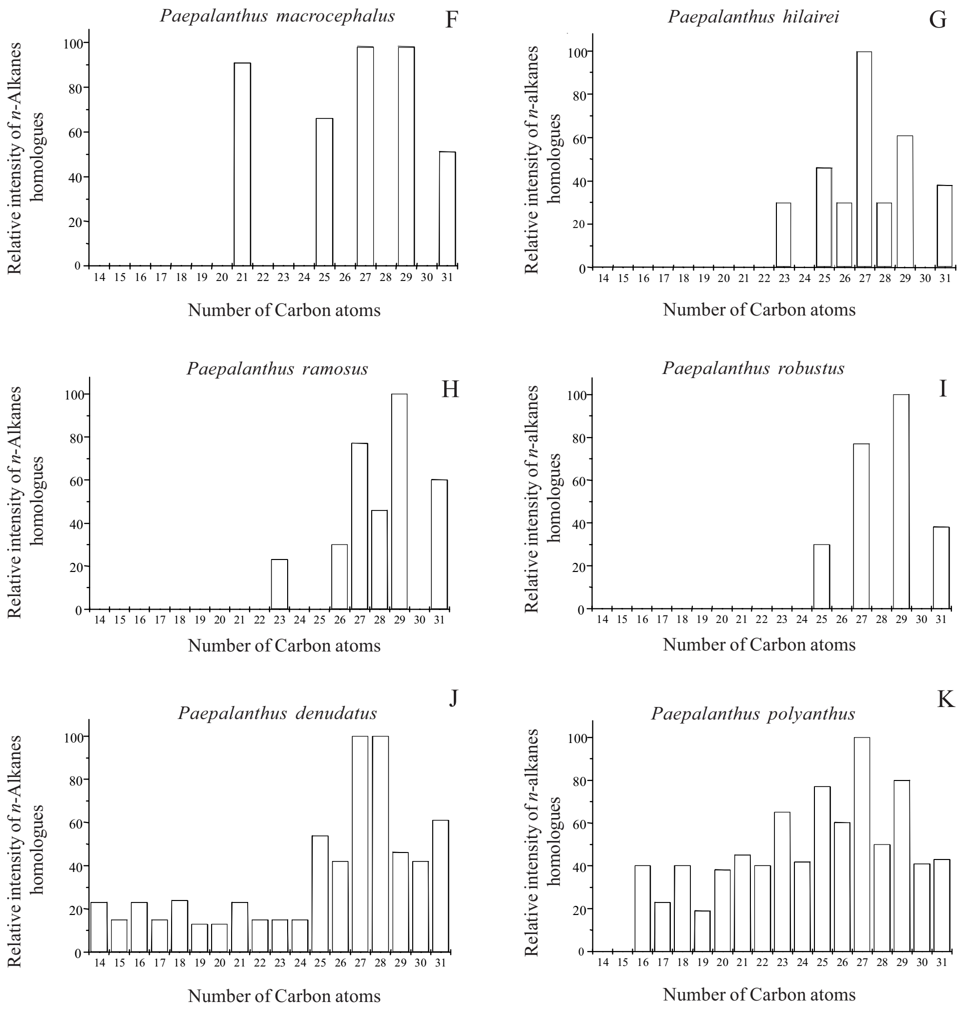

Figure 2. *n*-Alkanes profile of plants of *Paepalanthus* subg. *Paepalocephalus* based on the intensity of the peaks obtained from GC-FID analyses (F-K: F- *P.* subsect. *Aphorocaulon*; G-K, *P.* sect. *Actinocephalus*).

P. ramosus - C_{29} (C_{27}); *P. robustus* - C_{29} (C_{27}); *P. denudatus* - C_{27}, C_{28}; *P. polyanthus* - C_{27} (C_{25}, C_{29}). We also can see that *P. macrocephalus* (subsect. *Aphorocaulon* species) presents just alkanes with odd-carbon numbers and *P. denudatus* and *P. polyanthus* (*Actinocephalus* species) present a quite distinctive profile, with many shorter chains and a high frequency of alkanes with even-carbon numbers, especially *P. polyanthus*. These results are different from those described by Salatino *et al*. (1988), who detected C_{27} as the main homologue in all species of *P*. subg. *Paepalocephalus.* These data are in agreement with the cladistic analysis (Giulietti *et al.* 2000) where *P*. subg. *Platycaulon* form a clade, which is sister to *P*. subsect. *Aphorocaulon* and *P*. sect. *Actinocephalus* (both belonging to *P*. subg. *Paepalocephalus*). The results obtained indicate that the distribution of alkanes can be useful as a taxonomic character, although a more detailed inventory of alkanes profiles of species of *Paepalanthus* are needed, based on wide sampling for each species.

Aknowledgments

We thank the financial support of FAPESP, CNPq and FUNDUNESP.

References

Andrade, F.D.P.; Santos, L.C.; Dokkedal, A.L. & Vilegas, W. 1999. Acyl glicosilated flavonoids from *Paepalanthus* species. **Phytochemistry 51**: 411-415**.**

Broschat T.K. & Bogan, M. 1986. Leaf cuticular alkanes of cultivated *Polyscias*. **Biochemical Systematics and Ecology 14**(6): 583-584.

Cowlishaw, M.G.; Bickerstaffe, R. & Young, H. 1983. **Phytochemistry 22**(1): 119-124.

Dokkedal, A.L.; Sano, P.T. & Vilegas, W. 2004. Chemistry in *Paepalanthus* and taxonomic implication. **Biochemical Systematics and Ecology 32**: 503-504.

Eglinton, G.; Gonzalez, A.G.; Hamilton, R.J. & Raphael, R.A. 1962a. Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. **Phytochemistry 1**: 89-102.

Eglinton, G.; Hamilton, R.J. & Martin-smith, M. 1962b. The alkane constituents of some New Zealand plants and their possible taxonomic implications. **Phytochemistry 1**: 137-145.

Eglinton, G. & Hamilton, R.J. 1963. Pp. 543. In: T. Swain (ed.). **Chemical Plant Taxonomy**. London, Academic Press.

Faboya, O.O.P.; Okogun, J.I. & Goddard, D.R. 1980. Leaf wax *n*-alkane constituents of the genus *Khaya*. **Phytochemistry 19**(11): 2462-2463.

Giulietti, A.M. & Hensold, N.C. 1990. Padrões de distribuição geográfica dos gêneros de Eriocaulaceae. **Acta Botanica Brasilica 4**: 133-158.

Giulietti, A.M.; Amaral, M.C. & Bittrich, V. 1995. Phylogenetic analysis of inter- and infrageneric relationships of *Leiothrix* Ruhl. (Eriocaulaceae). **Kew Bulletin 50**: 55-71.

Giulietti, A.M.; Scatena, V.L.; Sano, P.T.; Parra, L.R.; Queiroz, L.P.; Harley, R.M.; Menezes, N.L.; Yseppon, A.M.B.; Salatino, A.; Vilegas, W.; Santos, L.C.; Ricci, C.W.; Bomfim & M.C.P.; Miranda, E.B. 2000. Multidisciplinary studies on neotropical Eriocaulaceae. In: K.L. Wilson & D.A. Morrison (eds.). **Monocots: systematics and evolution**. v.1, Melbourne, CSIRO Publishing.

Gnecco, S.; Bartulin, J.; Becerra, J. & Marticorena, C. 1988. *n*-Alkanes from Chilean Euphorbiaceae and Compositae species. **Phytochemistry 28**(4): 1254-1256.

Herbin, G.A. & Robins P.A. 1968. Studies on plant cuticular waxes - The taxonomy of alkanes and alkenes of the genus *Aloe* (Liliaceae). **Phytochemistry 7**: 239-255.

Manners, G.D. & Davis, D.G. 1984. Epicuticular wax constituents of North American and European *Euphorbia esula* biotypes. **Phytochemistry 23**(5): 1059-1062.

Merino, E.F.; Maestri, D.M. & Planchuelo, A.M. 1999. Chemotaxonomic evaluation of leaf alkanes in species of *Lupinus* (Leguminosae). **Biochemical Systematic and Ecology 27**: 297-301.

Nordby, H.E. & Nagy, S. 1977. Hydrocarbons from epicuticular waxes of *Citrus* peels. **Phytochemistry 16**(9): 1393-1397.

Salatino, M.L.F.; Pereira, H.A.B.; Salatino, A. & Giulietti, A.M. 1988. Alcanos dos capítulos de algumas espécies de Eriocaulaceae. **Boletim de Botânica da Universidade de São Paulo 10**: 55-64.

Salatino, M.L.F.; Salatino, A.; Menezes, N.L. & Mello-Silva, R. 1989. Alkanes of foliar epicuticular waxes of Velloziaceae. **Phytochemistry 28**(4): 1105-1114.

Salatino, A.; Salatino, M.L.F.; Mello-Silva, R. & Duerholt-Oliveira, I. 1991. An appraisal of the plasticity of alkanes profiles of some species of Velloziaceae. **Biochemical Systematic and Ecology 19**(3): 241-248.

Sano, P.T. 2000. *Actinocephalus* (Koern.) sano (*Paepalanthus* sect - *Actinocephalus*), a new genus of Eriocaulaceae, and other taxonomic and nomenclatural changes involving *Paepalanthus* Mart. **Taxon 53**(1): 99-107.

Skorupa, L.A.; Salatino, M.L.F. & Salatino, A. 1998. Hydrocarbons of leaf epicuticular waxes of *Pilocarpus* (Rutaceae): taxonomic meaning. **Biochemical Systematic and Ecology 26**: 655-662.

Smith, R.M. & Martin-Smith, M. 1978. Hydrocarbons in leaf waxes of *Saccharum* and related genera. **Phytochemistry 17**(8): 1293-1296.

Sorensen, P.D.; Totten, C.E. & Piatak, D.M. 1978. **Biochemical Systematic and Ecology 6**(2): 109-111.

Stocker, H. & Wanner, H. 1975. Changes in the composition of coffee leaf wax wiyh development. **Phytochemistry 14**(9): 1919-1920.

Vilegas, W.; Dokkedal, A.L.; Rastrelli, L.; Piacente, S. & Pizza, C. 1999a. New naphtopyranone glycosides from *Paepalanthus vellozioides* and *P. latipes*. **Journal of**

Natural Products 62: 746-749.

Vilegas, W.; Nehme, C.J.; Dokkedal, A.L.; Rastrelli, L.; Piacente, S. & Pizza, C. 1999b. Quercetagetin 7-methyl ether glycosides from *Paepalanthus vellozioides* and *P. latipes.* **Phytochemistry 51**: 403-409.

Wilkinson, R.E. & Kasperbauer, M.J. 1972. Epicuticular alkane content of tobacco as influenced by photoperiod, temperature and leaf age. **Phytochemistry 11**(8): 2439-2442.

Versão eletrônica do artigo em www.scielo.br/abb