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Correlations between Pulp Properties of *Eucalyptus* Clones and Leaf Volatiles Using Automated Solid-Phase Microextraction

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Analysis of biogenic volatile organic compounds (BVOC) of 14 *Eucalyptus* clones has been performed using an automated headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography (GC)/ion trap mass spectrometry (ITMS) method. Correlations between pulp properties of *Eucalyptus* clones and the BVOC of their leaf headspaces were studied. The compounds α-terpineol and the sesquiterpene β-eudesmol were positively correlated with S5, a property related to the hemicelluose content in the pulp. Qualitative results obtained with automated HS-SPME were sufficient to group together the same species and related hybrids through cluster analysis and were confirmed through principal component analysis. A preliminary separation of the essential oils of *Eucalyptus dunnii* through comprehensive two-dimensional gas chromatography (GC × GC) showed ~580 peaks compared to ~60 in a typical GC/ITMS first-dimension chromatogram. The potential of HS-SPME coupled to GC × GC to improve the separation of *Eucalyptus* volatiles and other plant essential oils looks extremely promising for new applications of unsupervised learning methods.

KEYWORDS: Automated solid-phase microextraction (SPME); *Eucalyptus*; biogenic volatile organic compounds (BVOC)' principal component analysis (PCA); cluster analysis (CA); pulp properties

INTRODUCTION

The production of low-cost and high-quality raw material in the pulp and paper industry has been one of the main objectives of current *Eucalyptus* breeding programs in Brazil (*1*). Genetic improvement for this objective usually requires the analysis of pulp properties of selected clones after a 7 year growing period. This type of investigation, even though extremely important, is costly and time-consuming. Some of the pulpwood and pulp properties are inherited in an additive way, such as lignin content, pulp yield, and hemicellulose content, among others (*1*). In general, most of the traits related to wood and pulp quality have high heritability. At the same time, the essential oil traits, such as yield or amount of its components, are also strongly inherited, offering a good chance to improve them by the development of tree-breeding programs (*2*).

The majority of essential oils are complex mixtures of mono- and sesquiterpenoid compounds, also containing minor amounts of other compounds belonging to other classes (*3*).

Chemometric analysis of essential oils has been used for taxonomic studies (*4*), quality control (*3*), analysis of variation in essential oil composition (*5*), investigation of environmental influence in essential oil composition of clones (*6*), and genetic improvement (*6*, *7*).

Distillation is the most common extraction process employed to obtain the essential oil of *Eucalyptus* leaves for such chemometric studies (*8*). Hydrodistillation and or vapor distillation, as well as vacuum distillation, are time-consuming and labor intensive processes (*9*, *10*). Headspace solid-phase microextraction (HS-SPME) has also been applied for chemometric purposes, even though its use was mainly related to the classification of wine (*11*) and other matrices (*12*), and only a few studies on the volatile compounds of different plants have

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Table 1. *Eucalyptus* Species and Hybrids Tested and Some of Their Pulp Properties

tree no.	clone no.	species	S5 (%)	lignin (%)	after screening yield (%)	viscosity (cm^3/g)	whiteness (% ISO)
t1	3330	*E. dunnii*	14.00	18.65	49.89	1226.5	34.60
t2	6874	*E. urophylla* and*globulus maidenii*	11.40	22.95	49.92	1057.0	41.10
t3	1993	*E. saligna*	10.20	23.95	51.99	1140.5	38.35
t4	3341	*E. dunnii*	15.50	22.75	48.41	1249.5	35.95
t5	6873	*E. urophylla* and *globulus maidenii*	12.95	22.95	50.01	1085.0	41.05
t6	952	*E. grandis*	9.80	23.50	50.96	1175.5	38.15
t7	4039	*E. saligna*	9.05	26.60	50.10	1096.5	37.80
t8	6870	*E. saligna* and *globulus maidenii*	13.00	24.00	50.24	1095.0	40.95
t9	4407	*E. grandis* and *urophylla*	10.30	24.00	51.20	1222.0	38.20
t10	4634	*E. grandis* and *urophylla*	8.90	23.95	52.88	1142.0	39.55
t11	2864	*E. saligna*	10.20	26.40	48.54	936.0	41.80
t12	6872	*E. urophylla* and *globulus maidenii*	12.75	22.40	52.53	1073.5	37.60
t13	4587	*E. saligna*	9.05	25.45	50.55	1142.5	37.20
t14	2949	*E. saligna*	9.80	24.50	50.41	1042.0	37.40

been reported (*13*−*15*). However, it has not yet been applied to *Eucalyptus* BVOC. HS-SPME is a very convenient technique for providing the headspace fingerprint of *Eucalyptus* biogenic volatile organic compounds (BVOC) as it is simple, fast, solventless, and promptly automated (*16*).

The fact that pulpwood, pulp properties, and essential oil composition are strongly determined by genetic parameters led to the hypothesis of an eventual genetic precursor among some of these variables. Correlations of the BVOC chemical signature with other physical characteristics of the pulp may be used to identify plants with the desired characteristics without having to perform the time-consuming and expensive direct measurements of those characteristics. Eventual correlations between specific *Eucalyptus* BVOC patterns and pulp properties may be used to advantage in the selection of the best hybrids for genetic improvements, especially if these chemical signatures could be identified at a very early (6 months) age. If promising hybrids could be identified as seedlings rather than waiting for mature wood formation (7 years), significant savings in time and cost could be realized.

In this preliminary work, leaves from mature clones (5−8 years of age) were studied, according to the availability of the pulp and paper industry; however, this first step opens the perspective of using seedlings in the near future.

To verify the hypothesis of a possible genetic linkage between BVOC leaves components and pulp properties, we used an automated extraction (HS-SPME) and analysis [gas chromatography/ion trap mass spectrometry (GC/ITMS)] method of *Eucalyptus* powdered leaves BVOC to investigate potential correlations between these BVOC chromatographic peaks areas and several pulp and paper properties. This method was previously developed and described elsewhere (*17*). The clustering of the *Eucalyptus* clones according to the presence of BVOC components in the headspace of the leaves and to pulp properties was also investigated.

Several recent research works have been published in the past five years describing the application of two-dimensional gas chromatography (GC × GC) for essential oil compound analysis (*18*−*20*), and even less has been seen for coupled SPME-GC × GC (*21*, *22*). In this paper we also present a preliminary work on HS-SPME/GC × GC applied to *Eucalyptus dunnii* essential oil aiming to show the potential of coupling both techniques for pattern recognition of essential oils.

MATERIALS AND METHODS

Plants. Twelve juvenile leaves of 14 *Eucalyptus* clones were sampled on October 16, 2000, and arrived in Canada on October 20, 2000. Leaves were analyzed using HS-SPME from October 21 to November 4, 2000. The *Eucalyptus* species and hybrids under study were selected according to their pulp properties, representing low, average, and high values of relevant pulp properties, as shown in **Table 1**. Each of the clones is labeled t1, t2, ..., t14. The ages of the *Eucalyptus* clones ranged from 5 to 8 years, and hybrids 6870, 6872, 6873, and 6874 were 5 years old. These clones were cultivated in neighboring tree farms in Guaíba city, Rio Grande do Sul state, Brazil, being under similar climate and soil conditions and having been treated with the same levels of nutrients.

Experimental Procedure. For SPME experiments, freshly picked leaves were sampled randomly from the tree canopies of the 14 clones and transported to Canada in Styrofoam boxes containing ice packs. Adult leaves at approximately the same level of development were selected from the tree canopy. Immediately upon arrival, leaves were placed in dry ice [solid CO_2 (−78.5 °C)] until extraction and analysis were performed. Extraction and analysis procedures began the following day. During sample preparation, leaves were first placed in liquid nitrogen. When frozen, the leaves were partially ground in a stainless steel mortar and pestle, after which dry ice was added. Leaves were further ground into a powder, during which time the nitrogen evaporated but the solid CO_2 remained. The mixture of powdered leaves and solid CO_2 was sieved through a 50 mesh (U.S. series) stainless steel sieve. Immediately after the CO_2 had evaporated, powdered leaf (0.05 g) was weighed into 10 mL vials stored on dry ice. Phosphate buffer was added to each vial (2 mL, pH 7.0, 50 mM), whereupon it immediately froze. The temperature was kept below 4 °C to avoid loss of volatile compounds. Once thawed, vials were capped and placed on the temperature-controlled autosampler tray (30 °C), and analysis was initiated. Extraction of headspace BVOC was performed automatically (Combipal autosampler, CTC Analytics, Basel, Switzerland) during 30 min (equilibrium conditions), using a 7 μm PDMS SPME fiber (Supelco, Bellefonte, PA), after at least 24 h at 30 °C. The development of this SPME method is described in detail elsewhere (*23*).

Hydrodistillation was performed in a modified Clevenger apparatus for 5 h with ∼300 g of fresh leaves and 1 L of deionized water. To avoid the loss of volatile compounds, the refrigeration system was maintained between −4 and 4 °C, by use of a mixture of water and ethylene glycol. The hydrodistilled oil was dried over sodium sulfate and used for the GC × GC experiments and for linear temperature programmed retention indices (LTPRI) calculation.

Pulps of each of the clones were obtained through the same pulping procedure. Analyses of pulp properties were made in duplicate according to standard procedures (ISO 692-1982 for S5; ISO 5351/1-1981 for viscosity; Tappi 222 om-98; for lignin; ISO 2469:1977; ISO 2470:1977; ISO 3688:1977 for ISO brightness), and results are listed in Table 1. Kappa number, the residual lignin content, was constant for all of the pulps (16 ± 0.7).

GC/ITMS. Chromatographic analysis was carried out using a Saturn 4D GC/ITMS system (Varian Associates, Sunnyvale, CA) fitted with a 30 m × 0.25 mm × 0.25 μm HP-5MS column (Agilent, Wilmington, DE) and a septum-equipped programmable injector (SPI). Helium TAG grade (Praxair, Kitchener, ON, Canada) was used as carrier gas. Mass spectra recorded from ITMS were used as total ion chromatogram (TIC).

Table 2. Compounds Detected in the Headspace of Powdered *Eucalyptus* Leaves of 14 Clones

no.	t_R[a] (min)	compound	ip[b]	no.	t_r[a] (min)	compound	ip[b]	no.	t_R[a] (min)	compound	ip[b]
1	3.22	butanoic or propanoic ester	c	**37**	13.06	sesquiterpene	c	**72**	16.59	γ-cadinene	c/b (*29*, *30*)
2	3.34	monoterpene	c	**38**	13.39	sesquiterpene hydrocarbon	c	**73**	17.72	sesquiterpene hydrocarbon	c
3	3.45	α-thujene	a	**39**	13.42	α-terpinenyl acetate	c	**74**	17.81	δ-cadinene	c/b (*25*, *27*, *29*–*31*, *33*)
4	3.56	α-pinene	a	**40**	14.00	α-cubebene	c/b (*30*, *33*)	**75**	17.96	sesquiterpene hydrocarbon	c
5	3.78	camphene	a	**41**	14.07	α-copaene	a	**76**	18.02	sesquiterpene hydrocarbon	c
6	4.30	β-pinene	a	**42**	14.14	sesquiterpene hydrocarbon	c	**77**	18.17	sesquiterpene hydrocarbon	c
7	4.54	β-myrcene	a	**43**	14.45	sesquiterpene hydrocarbon	c	**78**	18.31	calacorene	c
8	4.81	α-phellandrene	a	**44**	14.50	sesquiterpene hydrocarbon	c	**79**	18.38	sesquiterpene	c
9	4.91	ester	c	**45**	14.62	longifolene	c/b (*28*)	**80**	18.50	sesquiterpene	c
10	5.00	ester	c	**46**	14.96	α-gurjunene	a*	**81**	18.53	sesquiterpene	c
11	5.07	α-terpinene	a	**47**	15.08	sesquiterpene hydrocarbon	c	**82**	18.69	sesquiterpene	c
12	5.20	*p*-cymene	a	**48**	15.09	sesquiterpene	c	**83**	18.81	sesquiterpene	c
13/14	5.29	limonene + 1,8-cineole[c]	a	**49**	15.20	β-caryophyllene	a	**84**	18.89	sesquiterpene	c
15	5.47	*cis*-ocimene	a	**50**	15.29	sesquiterpene hydrocarbon	c	**85**	18.97	sesquiterpene	c
16	5.71	*trans*-ocimene	a	**51**	15.42	sesquiterpene hydrocarbon	c	**86**	19.13	spathulenol	c/b (*25*, *28*, *30*, *34*)
17	5.95	γ-terpinene	a	**52**	15.53	β-gurjunene	a*	**87**	19.28	globulol	a
18	6.39	terpene	c	**53**	15.60	sesquiterpene hydrocarbon	c	**88**	19.47	sesquiterpene	c
19	6.64	terpinolene	a	**54**	15.74	aromadendrene	a*	**89**	19.62	sesquiterpene	c
20	6.94	linalool	c/b (*26*, *30*, *31*, *34*)	**55**	15.81	guaiene	c/b (*27*)	**90**	19.62	sesquiterpene	c
21	7.03	ester	c	**56**	16.00	sesquiterpene hydrocarbon	c	**91**	19.73	β-eudesmol	c
22	7.10	ester	c	**57**	16.07	α-humulene	a*	**92**	19.88	sesquiterpene	c
23	7.60	campholenal	c/b (*29*, *31*, *34*)	**58**	16.26	allo-aromadendrene	a*	**93**	20.04	ester	c
24	7.68	monoterpene	c	**59**	16.54	γ-gurjunene	c	**94**	20.20	sesquiterpene	c
25	8.47	ketone	c	**60**		sesquiterpene	c	**95**	20.33	α-eudesmol	c/b (*28*)
26	8.85	borneol	c/b (*25*, *29*, *31*, *34*)	**61**	16.64	γ-muurolene	c/b (*27*)	**96**	20.65	sesquiterpene	c
27	8.89	terpinen-4-ol	a	**62**	16.74	sesquiterpene hydrocarbon	c	**97**	20.73	sesquiterpene	c
28	9.19	α-terpineol	a	**63**	16.90	sesquiterpene hydrocarbon	c	**98**	20.82	sesquiterpene	c
29	9.96	fenchyl acetate	c	**64**	16.95	sesquiterpene hydrocarbon	c	**99**	20.89	sesquiterpene	c
30	10.94	monoterpene	c	**65**	17.12	sesquiterpene hydrocarbon	c	**100**	20.94	sesquiterpene	c
31	11.42	terpene	c	**66**	17.20	sesquiterpene hydrocarbon	c	**101**	21.49	sesquiterpene	c
32	11.74	terpenoid ester	c	**67**	17.14	sesquiterpene hydrocarbon	c	**102**	22.23	sesquiterpene	c
33	12.09	monoterpene	c	**68**	17.25	sesquiterpene hydrocarbon	c	**103**	23.03	sesquiterpene	c
34	12.52	monoterpene	c	**69**	17.42	sesquiterpene hydrocarbon	c	**104**	23.22	sesquiterpene	c
35	12.74	terpenoid ester	c	**70**	17.42	sesquiterpene	c	**105**	23.50	sesquiterpene	c
36	12.79	terpene	c	**71**	17.60	terpenoid ester	c	**106**	23.87	sesquiterpene	c

[a] Retention data obtained with an HP-5MS column. [b] Identification procedure used to assign the family group or identify or tentatively identify the BVOC: a, co-injection with pure compound and comparison with retention data in one to three GC columns; a*, identification made by Dr. W. A. König (Universität Hamburg) using a laboratory-built retention data and mass spectral database of pure compounds; b, tentative identification by comparison with literature LTPRI; c, tentative identification by comparison with mass spectra from NIST 98, Saturn (Varian) libraries and literature retention data. Compounds were considered present in the headspace of a tree if they were detected in at least five leaves from the tree. [c] Not separated.

Operational conditions for the chromatographic separation and detection were as follows: 60 °C, 5 °C/min, 280 °C; temperature of SPI (septum equipped programmable injector), 250 °C; carrier gas, helium at 15 psi (1.5 mL/min); ion trap temperature, 150 °C; transfer line, 240 °C; electron multiplier voltage, 1820−1840 V (optimized daily). Identification of the eluted compounds was performed using the NIST98 MS spectral database. Whenever necessary, these results were also confirmed by comparison with retention data obtained either in an HP-Innowax 30 m × 0.25 mm × 0.50 μm column or in an HP-1MS 60 m × 0.25 mm × 0.25 μm column (Hewlett-Packard). Confirmation was also conducted using a laboratory-built MS spectral database previously collected from chromatographic runs of pure compounds (Sigma Chemical Co., St. Louis, MO; Aldrich Chemical Co., Milwakee, WI; and Fluka Chemie AG, Buchs, Switzerland) performed in the same equipment and conditions. Four compounds present in the headspace of all the leaves were used as chromatographic markers (α-pinene, *p*-cymene, *allo*-aromadendrene, and globulol) in order to monitor retention time variation (*24*). For several components for which pure compounds were not available, comparison with retention data and LTPRI reported in the literature was also used to tentatively identify some of the compounds (*24*−*31*). For determining LTPRI of some of the volatile components of *Eucalyptus* BVOC, a Varian GC 3400C$_x$/FID (Varian Associates) equipped with an HP-5MS column and the same experimental conditions formerly described were used. FID temperature was 250 °C.

Even though this work does not aim to present a quantitative analysis of the BVOC detected, relative amounts of the same compounds are compared in different experiments. For this reason, detector response (GC/ITMS) was investigated in the working range of 5−500 ng and it proved to be linear for the following compounds: 2-carene; 2,5-dimethylstyrene; 1,8-cineole; myrtenal; linalyl acetate; β-caryophyllene; and caryophyllene oxide. The chosen compounds are representative of the following classes of terpenoids: hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes. These classes represent some of the most common compounds found in the BVOC present in *Eucalyptus* oils (*9*). Relative standard deviations of 85−95% of the chromatographic peak areas of BVOC detected in the 14 *Eucalyptus* headspace were <20%.

For GC × GC experiments, an Agilent 6890 gas chromatograph with split/splitless injector and FID, retrofitted with a two-stage thermal modulator (Zoex Corp., Lincoln, NE), was employed. The first dimension column was a DB-1 (4.0 m × 3.5 μm × 100 μm i.d.), and the second dimension column was a DB-1701 (0.5 m × 0.1 μm × 100 μm i.d.). The oven temperature program started with −50 °C/min, initial dwell time of 30 min (to permit focusing on-column during SPME fiber desorption period); initial rate 50 °C to a temperature of 0 °C, followed by a ramp of 3 °C/min to a final temperature of 121 °C. For injection, split mode was used with a split ratio of 0.3, a head pressure of 35.0 psi, constant pressure mode, and an inlet temperature of 250 °C.

The modulation system was a Quad Jet Configuration (PCT Patent Application WO 01/51170PCT/US01/01065 filed January 12, 2001): upstream jet delay, 10 ms; duration, 310 ms; downstream jet delay, 400 ms; duration, 310 ms; jet flow rates (hot and cold). ~15 L/min. The temperature of the detector was 300 °C, H_2 flow was 80.0 mL/min, air flow was 550 mL/min, and there was no makeup gas.

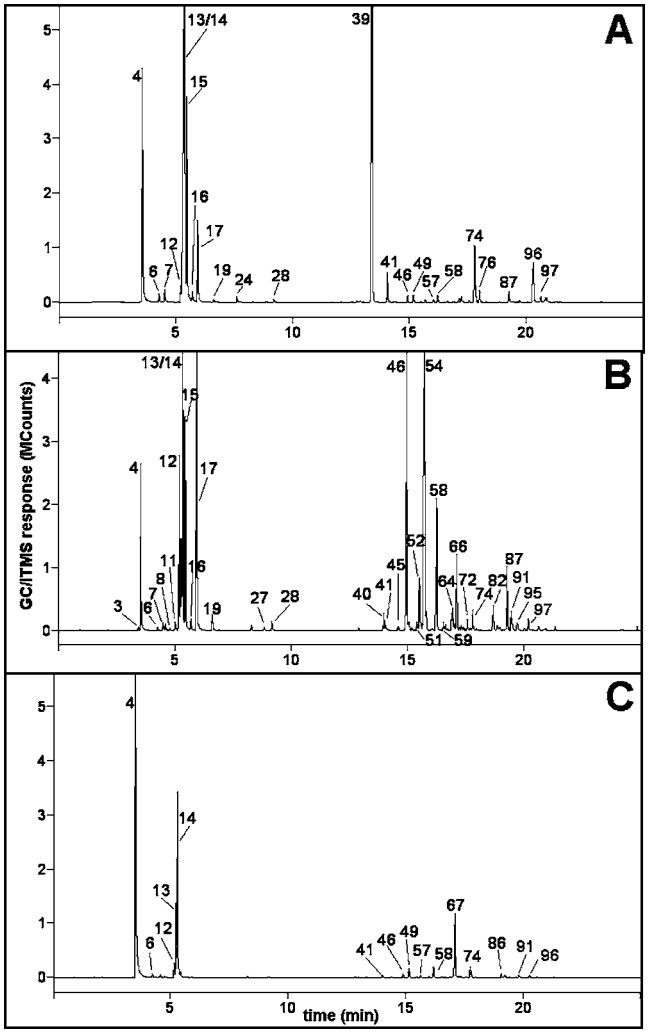

Figure 1. Chromatograms of BVOC of (**A**) hybrid 6874 (*E. urophylla* and *E. globulus maidenii*), (**B**) *E. dunnii*, and (**C**) *E. saligna* powdered leaves.

RESULTS AND DISCUSSION

A hundred and four components were detected in the headspace of 14 *Eucalyptus* clones using SPME, and they are listed in **Table 2** in order of their elution from an HP5-MS column. **Figure 1** shows the chromatographic profile of three different *Eucalyptus* clones. The average area counts (seven replicates, *x* parameter) of the chromatographic peaks of these components and the normalized properties of the pulp (*y* parameter) obtained from each of the 14 *Eucalyptus* clones were plotted in a table (data not shown). Recognition of the same nonidentified compounds from one tree to the other was based on their retention times and similarity of mass spectra. This procedure provided the inclusion of components in the data set or of their peak areas, even when these peak components were not identified.

Possible correlations between areas of the chromatographic peaks of *Eucalyptus* headspace compounds against the properties of the pulp of the same clones were investigated. Panels A and B of **Figure 2** show the positive correlations found between α-terpineol and *β*-eudesmol (tentative identification) and the property S5, respectively. S5 is a property related to the content of hemicelulloses in the pulp; the higher the hemicellulose levels, the better the pulp resistance. A high value of S5 is a desirable pulp characteristic for the paper-making process. Globulol peak area presents a negative correlation with lignin, which is shown in **Figure 2C**. The higher the lignin content in the wood, the more difficult are the cooking and bleaching processes. For this reason, a lower content of lignin is a desirable characteristic in the pulping process. **Table 3** presents the standard residue analysis and other data for the correlations listed above. Even though the number of *Eucalyptus* samples in the test set is small, the results look promising and a greater set of *Eucalyptus* clones with more variety of species and hybrids should be investigated to further confirm the correlations found. The possibility of predicting S5 and/or lignin content in a certain clone without having the cost of a 7 year growing period and subsequent pulping and pulp analysis represents convenient resource savings and makes further research on this matter a scientifically and financially worthy challenge.

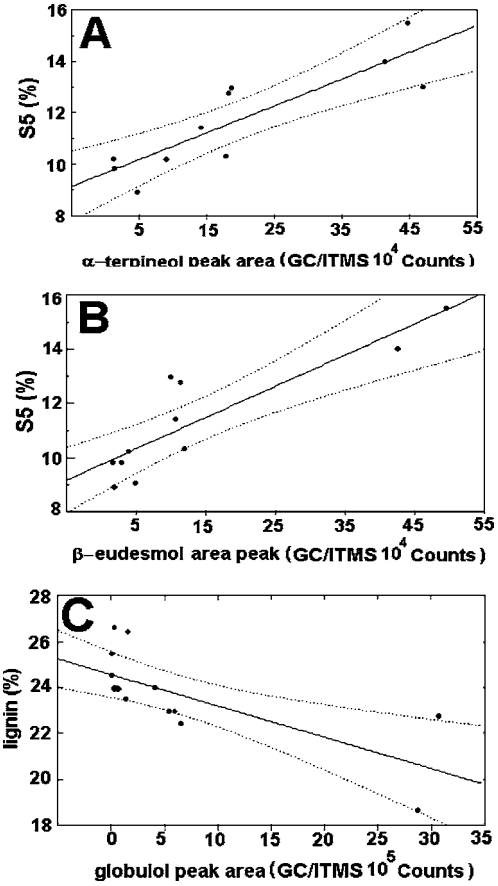

Figure 2. Correlation between S5 and (**A**) α-terpineol and (**B**) eudesmol peak areas and (**C**) correlation between lignin and globulol peak area.

Table 3. Some Correlations among *Eucalyptus* Leaves BVOC and Pulp Properties

headspace compound	pulp property	R^2	standard residues minimum	standard residues maximum	significance
α-terpineol	S5	0.75	−1.56	1.35	0.001
β-eudesmol	S5	0.76	−1.13	1.84	<0.001
globulol	lignin	0.52	−1.42	1.69	0.004

The same set of data was used for exploratory analysis through cluster analysis (CA), and mode Q−principal component analysis (PCA), as shown in **Figures 3** and **4**, respectively. The average area counts of the compounds detected in the headspace of *Eucalyptus* leaf powder were replaced by the number 1, whereas the nondetected components were represented by the number 0 for each *Eucalyptus* clone. A certain compound was considered to be detected if the signal-to-noise ratio of its peak was ≥3 and if it showed up in at least five of the seven replicate extractions and analyses made for each clone.

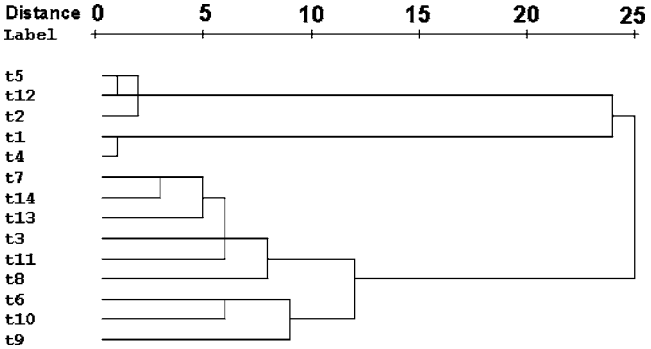

Figure 3. Dendogram of headspace components of 14 *Eucalyptus* clones using the Ward method.

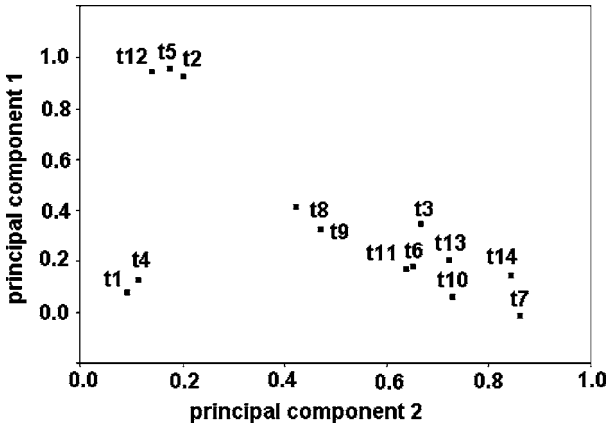

Figure 4. Plot of one and two rotated components resulting from PCA of leaf headspace compounds of 14 *Eucalyptus* clones.

Figure 4 was plotted after varimax rotation of the PCA components and its results confirm the ones obtained by CA, bringing a better visualization of the exploratory analysis results.

CA and PCA plots resulting from the analysis of the headspace components of the *Eucalyptus* clones and the plots obtained after adding the pulp properties to the same data matrix are extremely similar. For this reason, only one of them (the one that takes into consideration only the headspace compounds of *Eucalyptus* leaves) is shown in **Figures 3** and **4**. The exploratory analysis shows that the *Eucalyptus* clones cluster according to their species and also according to their parents' species, as can be seen by three groups in this set of *Eucalyptus* clones. One of them includes t5, t12, and t2, which are all hybrids of *E. urophylla* and *E. globulus maidenii*. A second group encompasses t1 and t4, which are clones of the same *E. dunnii* species. The members of the third group are clones of *E. saligna* (t3, t7, t11, t13, and t14), the hybrid t8, whose parents are *E. saligna* and *E. globulus maidenii*, t6 (*E. grandis*), and two other hybrids (t9 and t10) whose parents are *E. grandis* and *E. urophylla*.

The first three principal components explained 69% of the variance between the clones. Principal component 1 alone explained nearly 42% of the variance among clones and separated *E. dunnii* clones (t1 and t4) and *E. urophylla* and *E. globulus maidenii* hybrids (t2, t5, and t12) from the third group, as shown in **Figure 4**. The substances most closely correlated with component 1 were a sesquiterpene hydrocarbon with a molecular weight of 204 g mol^{-1} and spathulenol.

Components 2 and 3 were of lesser importance, accounting for only 15 and 13% of the variance among the 14 samples, respectively. Component 2 separated the hybrids of *E. urophylla* and *E. globulus maidenii* (t2, t5, and t12) from the other two groups. The substances most closely correlated with these two components were terpenoid esters and sesquiterpene hydrocarbons, respectively.

Preliminary analysis by GC × GC indicated a number of interesting qualitative results. In a typical *Eucalyptus* oil, for example, *E. dunnii*, 580 peaks were readily discernible, as compared to ~60 visible peaks in one-dimensional chromatograms recorded with FID. Coelutions were observed at essentially every conventional one-dimensional (1D) peak. Wherever a 1D chromatogram exhibited an unresolved valley, two or more coeluents were typically observed. Hundreds of GC × GC peaks were observed at trace levels, undetectable in the standard GC chromatograms. One caveat should be mentioned. In the experiments conducted using HS-SPME, ~50% of the trace peaks were due to background, but in this preliminary study, it was not possible to determine the source of the background completely. Even with the background problems observed, however, it is clear that the peak pattern differences between *E. dunii* and *E. saligna* (data not shown) were significant and reproducible. Although these species are also distinguishable on the basis of conventional 1D gas chromatograms, it is clear from the rich peak patterns in the GC × GC images that far more subtle differences could be detected, if present, by GC × GC than by GC alone (*20*−*22*). This raises the possibility of speciating on the basis of GC × GC analysis. More importantly, it suggests that an effort should be made to correlate the detailed chemical compositions revealed by GC × GC with bulk pulp properties.

CONCLUSIONS

Exploratory analysis (CA confirmed by PCA) of the data matrix obtained with an automated HS-SPME method developed for the headspace of *Eucalyptus* leaves powder and pulp properties allowed the classification of a set of 14 *Eucalyptus* clones in three groups. This automated HS-SPME method applied to the headspace of *Eucalyptus* leaf powder proved to be a convenient tool to classify different *Eucalyptus* species and hybrid parent species according to their volatile compounds. This simple and convenient approach can be used to correlate other properties of plants or other living systems to their BVOC emission. SPME has been useful to collect volatile components not only in the laboratory but also directly on-site in vivo with minimum disturbance to the living systems under investigation (*16*, *32*).

Correlations between S5 and α-terpineol and β-eudesmol (tentative identification) peak areas among others were found in this same set of *Eucalyptus* clones. The possibility of replacing time-consuming analysis by easier and faster analysis and also the perspective of establishing chemical markers to precociously access wood and pulp properties are the most attractive aspects of this study. The correlations and the multivariate analysis results are promising and should be confirmed in a further investigation using a larger set of *Eucalyptus* clones and a higher number of identified BVOC. The coupling of SPME with GC × GC analysis appears to be a promising method, which should support multivariate analysis and correlation studies.

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