JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of the Genotype from the Content and Composition of the Essential Oil of Lemon Verbena (*Aloysia citriodora* Palau)

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Aloysia citriodora accessions from cultivated material, botanical collections, and wild populations were studied by means of their biomass and essential oil production and composition to assist the selection of the most promising genotype. The study was carried out through both field experiments during two year's time and laboratory processes. Data were evaluated by means of univariate and multivariate techniques. *Aloysia citriodora* intraspecific variation was accounted for by differences in both yield and chemical profiles of the essential oils, but no differences were found in the biomass production. Three chemotypes were identified according to qualitative and quantitative differences of the essential oils. For the higher contents of neral and geranial, Mendoza accession was the most promising to be encouraged for future crops.

KEYWORDS: *Aloysia citriodora*; *Verbenaceae*; lemon verbena; biomass partition; chemotypes

INTRODUCTION

Lemon verbena (*Aloysia citriodora* Palau, *Verbenaceae*) is a perennial, native species of South America (*1*). It shares an important place on the international herbal market due to the sensory and medicinal properties of accumulated essential oil of its leaves (*2–4*). The sensory attributes determine its use as a primary ingredient for infusions and nonalcoholic beverages as well as an aromatic ingredient for the flavor and fragrance industries. The pharmaceutical industry uses lemon verbena for its carminative, antispasmodic, and sedative properties (*3*). The increasing interest in this species has largely contributed to expanding lemon verbena crops in Argentina, Chile, Paraguay, Europe, and Africa Mediterranean regions (*5*).

Differences in the content and composition of the essential oil of lemon verbena have been reported previously (*6*, *7*). Essential oil content ranged between 0.2 and 1% on dry wt. Neral and geranial are the most noticeable compounds present in the essential oil, together with limonene, geranyl acetate, beta-caryophyllene, *ar*-curcumene, and spathulenol. Other compounds were either occasionally quoted for this oil or have been found in specific chemotypes, as carvone, cedrol, 1,8-cineol, thujone isomers, and citronellal (*4*, *8*). However, they are not representative of the typical quality of lemon verbena scent, which is mainly lemon-like, sweet, lightly floral, and herbaceous. Previous studies on lemon verbena have reported that the production and composition of the essential oil are likely to vary according to the part of the plant (*9*), the stage of the plant development (*10*), and the harvesting locations (*7*, *10*, *11*). Considerable evidence has demonstrated that the genotype is a major determinant of how plants acquire and utilize resources that lead to large differences in biomass production (*12*), as well as the synthesis of secondary metabolites (*13*). Furthermore, environment is likely to inflluence the production (*14*, *15*) as well as the quality (*9*, *16*, *17*) of essential oils. Production is also frequently increased by biotic and abiotic stress (*12*, *18*).

Nowadays, the target in lemon verbena modern crop production has focused on the improvement of leaves biomass and essential oil yields, to meet the pattern that industry demands. It is therefore important to characterize and assess the germplasm material available so that the selection of the most promising cultivars is assisted. This research was aimed at identifying and measuring the infraspecific variability in the production of biomass and essential oil, and the essential oil composition in *Aloysia citriodora* accessions from different origins, grown in uniform experimental conditions for two years, so that those materials were identified as commercially valuable on account of their agricultural and industrial utilization.

MATERIALS AND METHODS

Standards. The following reference compounds used for gas chromatography–flame ionization detector–mass spectrometry (GC-FID-

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10.1021/jf0708387 CCC: $37.00
Published on Web 09/20/2007

Table 1. Meteorological Conditions during the Experiments

month	year	rad[a] (MJ m^{-2})	T_{max}[b] (°C)	T_{min}[b] (°C)	T_{me}[b] (°C)
December	2001	470	26.1	18.0	22.0
	2002	466.8	27.3	18.2	22.8
January	2002	427.7	29.6	19.6	24.6
	2003	490.1	30.7	20.2	25.5
February	2002	425.4	28.3	18.3	23.3
	2003	317.2	28.5	18.4	23.5
March	2002	269.6	25.9	18.2	22.1
	2003	258.0	27.0	18.1	22.5
April	2002	288.6	21.6	13.0	17.3
	2003	230.3	22.7	13.3	17.9

[a] Monthly values for solar radiation (rad) are cumulative. [b] Monthly values for maximum (T_{max}), minimum (T_{min}), and mean temperatures (T_{me}) are averages.

MS) were either obtained or purchased from the following sources, namely, alpha pinene, limonene, 1,8-cineol, citronellal, citral, geranyl acetate, and beta-caryophyllene (VASANA S.A.C.A.I.F. y M.; Villa Martelli, Argentina), sabinene (Dr. Ingrid Loayza, Universidad Mayor de San Simón; Cochabamba, Bolivia), 6-methyl-5-hepten-2-one (Aromatica S.A.; Buenos Aires, Argentina), *cis*-thujone (Sigma-Aldrich de Argentina; Buenos Aires, Argentina), *p*-cymene (Soaljo S.A., Buenos Aires, Argentina), piperitone (R. C. Treatt & Co.; Suffolk, UK), and (−)-caryophyllene oxide (Extrasynthese; Genay, France).

Plant Material. Stem cuttings were collected from plants growing in farms (F) and botanical collections (B), as well as wild plants (W) located in different geographic sites in Argentina (A) and Chile (Ch). The following names were assigned: Botany (B, A), Mendoza (B, A), San Luis (F, A), Salta (W, A), Rancagua (W, Ch), and Talca (W, Ch).

Once collected, the stem cuttings were stored in wet paper and transported at a constant temperature (5 °C), until they were planted in nursery beds located at the Facultad de Agronomía, Universidad de Buenos Aires, Argentina. Voucher specimens were deposited in the Herbarium at Instituto Darwinion (SI 25028 to 25032), San Isidro, Province of Buenos Aires, Argentina.

Site and Growing Conditions. Two pot field experiments were carried out at the Facultad de Agronomía (34° 35′ 5″ lat. S, 58° 29′ long. W and 25 m over sea level), during the 2001–2002 and 2002–2003 spring–summer growing seasons.

Soil samples were taken before the transplanting date to determine initial conditions in both years with the following methods: pH in 1 part soil to 2.5 parts water solution, organic carbon (C) using the Wakely Black method, exchangeable phosphorus (P) using the Bray and Kurtz technique, total nitrogen (N) using the Kjeldahl procedure, and exchangeable potassium (K) level using the 1 N ammonium acetate procedure (*19*). In year 2001, initial soil conditions were pH: 6.31, (C): 1.34, (P): 6.99, (N): 0.15, (K): 1.20, and in year 2002 were pH: 5.68, (C): 1.57, (P): 9.19, (N): 0.14, (K): 1.27.

Meteorological conditions (temperature and solar radiation) were recorded at the Villa Ortúzar meteorological station, Buenos Aires, Argentina, located 200 m away from the experimental site (**Table 1**).

Field Experiment. *Aloysia citriodora* accessions named as Buenos Aires, Mendoza, San Luis, Rancagua, and Talca (assigned names) were studied during the 2001–2002 season (experiment 1) and the accessions from all six origins, including Salta, were studied during the 2002–2003 season (experiment 2). The stem cuttings used in the succeeding years came from the same parental plants. A complete randomized design with five replications was implemented in both experiments.

Previously to the experiments, stem cuttings from each *Aloysia citriodora* accession were placed in nursery beds for rooting. After a 40-day time span (December 1, 2002 and December 5, 2003), one stem cutting from each accession was transplanted to plastic pots (30 L) filled with 25% sand and 75% top soil. Each pot with one plant was considered as the experimental unit for the accession line. Plants were watered as necessary to ensure an optimal water supply. Every 15 days, pots were hand-weeded.

Determination of Biomass and Content and Composition of the Essential Oil. The following procedure was applied to each of the five replications (experimental unit) of each accession: at the beginning of the flowering process, plant shoots were cut at 2 cm over the soil level. At this time point, stems (ST) were separated from whole aerial parts (AE). The content and composition of the essential oil from leaves and inflorescences (LI) as a whole were determined from the fresh material. Immediately after harvesting, a subsample of LI was dried at 70 °C to constant weight to determine the dry weight/fresh weight ratio. Each experimental unit from *Aloysia citriodora* was sliced and hydrodistilled for 1.30 h using a Clevenger apparatus. The essential oil was trapped in 0.1 mL of benzene to improve recovery. Oil was dried over anhydrous sodium sulfate and thereafter stored at −5 °C, until it was analyzed. Content of essential oil was expressed as a percentage of LI dry material (%, v/w) (EOP) and as yield (mL plant^{-1}) (EOY). Finally, five extractions and the analysis of the respective oils were done for each accession.

GC-FID-MS analysis was carried out on a Perkin Elmer Clarus 500, with one injector (split ratio: 1:100) connected by a flow splitter to two capillary columns: (a) polyethyleneglycol PM ca. 20 000 and (b) 5% phenyl-95% methyl silicone, both 60 m × 0.25 mm with 25 μ of fixed phase. The whole system operated at a constant flow of 1.87 mL/min. Helium was used as gas carrier. The polar column was connected to a FID, while the nonpolar column was connected to an FID and a quadrupolar mass detector (70 eV) by a vent system (MSVent). Temperature was programmed according to the following gradient: 90–225 °C at 3 °C/min, and then isothermic for 15 min. Injector and both FIDs were set at 255 and 275 °C, respectively. Injection volume was 0.2 μL. Temperatures of the transference line and the ionic source were 180 and 150 °C, respectively; the range of masses (m/z) was 40–300 Da.

Identification of the compounds was performed from the retention indices (relative to C_8–C_{20} *n*-alkanes) in both columns, compared with those of reference compounds, and by comparison of mass spectra using the usual libraries (*20, 21*) and mass spectra obtained from the reference compounds, except *cis*-sabinene hydrate, *ar*-curcumene, epi-cubebol, spathulenol, and tau cadinol, which were tentatively identified considering the MS libraries previously cited and a laboratory-developed mass spectra library built up from components of known oils, and by their corresponding relative indices obtained in both columns. As only comparative data were necessary, a relative percent concentration of the compounds was calculated according to the area of the chromatographic peaks (FID response), assuming all of the response factors were 1. The lowest response obtained from both columns for each component was considered. Five replications of each sample were analyzed, and the data were averaged, expressing the respective standard deviations.

Statistical Analysis. Two plots of Rancagua's accessions from year 2002 were lost, and Salta's accession was only studied in 2002/2003 because it was not possible to collect stem cuttings in time for planting. For these reasons, biomass and essential oil percentage and production were analyzed with analysis of variance (ANOVA) using an incomplete and unbalanced procedure (*22*). The variance homogeneity assumption was not met; therefore, essential oil content values were transformed as $\log(x + 1)$, and ANOVA was carried out on the transformed values. To reveal the relationship among the lemon verbena accessions, the composition data were analyzed by a multivariate statistical procedure. All accessions and essential oil compounds were studied simultaneously using principal component analysis (PCA) (*23–25*). Distinct essential oil components have very different intrinsic variability with variances ranging from 0.03 to 35.72. For this reason, to avoid ordering distortions generated by such differences in variance, PCA was applied on Pearson correlation coefficient (implicit standardization of variables to limit variances). The Pearson correlation coefficient was used to set up the similarity matrix as a way of avoiding ordering distortions generated by marked differences in variances for each compounds. The influence of traits with little variation was in this way standardized to constant variance with those with greater variation (*26*). Data of the neral and geranial contents were analyzed with ANOVA.

RESULTS

Chemical soil properties measured were similar between years ($P > 0.05$). Cumulated radiation was higher in spring–summer

Table 2. Mean Values and ANOVA for the Aerial (AE), Stem (ST), Leaves Plus Inflorescence (LI) Biomass Yields, and Essential Oil Content (EOP and EOY) in Lemon Verbena Accessions

	AE biomass (g)		ST biomass (g)		LI biomass (g)		EOP[a] (mL 100 g^{-1})		EOY[b] (mL plant^{-1})	
accession/year	M[c]	SD[d]	M	SD	M	SD	M	SD	M	SD
					2001/02					
Rancagua	38.4	2.2	21.1	0.8	17.3	2.2	1.6	0.1	0.24	0.03
Talca	40.5	7.6	23.8	5.9	16.8	2.3	0.7	0.3	0.12	0.02
San Luis	46.6	5.4	23.0	5.4	23.7	3.3	1.4	0.2	0.32	0.08
Buenos Aires	40.5	2.6	21.4	2.1	19.1	1.4	1.6	0.6	0.30	0.11
Mendoza	47.6	3.5	23.1	2.5	24.5	2.4	1.7	0.3	0.42	0.06
					2002/03					
Rancagua	21.2	7.4	5.3	2.3	15.8	5.1	1.1	0.2	0.14	0.02
Talca	24.8	4.3	7.9	2.1	16.8	4.2	0.9	0.4	0.14	0.04
San Luis	28.5	6.8	10.8	2.8	17.7	4.7	1.4	0.3	0.22	0.03
Buenos Aires	26.1	6.3	9.1	3.0	17.1	4.6	0.8	0.3	0.12	0.04
Mendoza	25.9	10.5	6.9	3.7	19.0	6.8	1.1	0.4	0.16	0.04
Salta	20.4	2.3	12.5	2.6	7.8	0.4	1.4	0.4	0.10	0.03
$P =$										
year (Y)	<0.0001		<0.0001		0.008		0.01		0.001	
accessions (A)	0.45		0.13		0.07		0.03		0.001	
Y × A	0.74		0.60		0.93		0.08		0.002	

[a] Expressed as percentage (%, v/w of dry material) in leaves plus flowers (LI). [b] Expressed as yield (mL per plant). [c] Means of five replications (Rancagua in 2002/03, $n = 3$) per clone. [d] Standard deviation (SD ± 1).

Table 3. Major Components (Relative Concentration, Percent) of the Essential Oil from Leaves and Flowers of *Aloysia citriodora* Clone Lines in 2001/2002 and 2002/2003

	Rancagua				Talca				San Luis				Buenos Aires				Mendoza				Salta	
	2001/02		2002/03		2001/02		2002/03		2001/02		2002/03		2001/02		2002/03		2001/02		2002/03		2002/03	
compound/RI[a]/RI[b]	M[c]	SE[d]	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE	M	SE
alpha-pinene/954/1042	0.7	0.1	1.1	0.3	0.8	0.1	0.7	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
sabinene/976/1136	1.6	0.3	2.5	0.5	1.5	0.1	1.9	0.2	0.1	0.0	0.2	0.0	0.1	0.0	0.2	0.0	2.3	0.3	2.7	0.4	5.9	0.3
6-methyl-5-hepten-2-one/986/1344	0.6	0.1	0.8	0.2	0.3	0.1	0.7	0.1	0.8	0.1	0.6	0.0	0.8	0.2	0.7	0.1	0.7	0.1	1.0	0.1	0.4	0.1
para-cymene/1012/1290	0.1	0.0	1.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	1.6	0.0	0.1	0.0	0.0	0.0	0.0	0.0
limonene/1016/1217	12.1	1.7	16.8	3.1	13.9	0.4	13.1	1.0	21.0	0.7	21.9	1.6	20.1	0.7	20.4	0.4	12.7	1.5	12.3	0.8	0.2	0.0
1,8-cineol/1022/1232	5.8	0.3	6.9	1.2	7.6	0.3	5.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1
cis-sabinene hydrate[e]/1071/1463	1.3	0.2	0.7	0.1	0.7	0.1	0.4	0.1	0.8	0.1	0.4	0.1	0.7	0.0	0.3	0.1	0.5	0.1	0.2	0.0	0.3	0.0
cis-thujone/1104/1456	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	1.0	0.0
citronellal/1155/1481	0.6	0.0	0.4	0.0	0.6	0.0	0.0	0.0	0.1	0.0	0.6	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.4	0.3	3.0	0.1
neral/1240/1685	17.7	0.1	17.9	0.6	15.5	0.8	19.5	0.9	21.5	0.4	21.5	0.8	21.6	0.3	21.0	0.2	23.5	0.7	23.9	1.3	22.6	1.1
piperitone/1253/1738	0.6	0.1	0.6	0.1	0.7	0.0	0.5	0.0	0.5	0.1	0.4	0.0	0.5	0.1	0.5	0.1	0.5	0.0	0.5	0.0	0.6	0.1
geranial/1269/1732	21.3	0.4	22.6	1.1	18.3	1.1	25.1	1.3	25.2	0.8	26.8	1.0	25.7	0.6	27.1	0.4	30.8	1.4	32.2	0.8	28.9	1.1
geranyl acetate/1384/1752	1.0	0.1	0.7	0.1	1.0	0.1	1.2	0.2	2.2	0.1	2.1	0.1	2.3	0.1	2.2	0.1	0.5	0.2	0.6	0.2	1.9	1.8
beta-caryophyllene/1422/1617	1.7	0.1	1.4	0.2	1.3	0.1	1.0	0.2	3.0	0.2	1.8	0.2	2.4	0.1	1.5	0.2	1.8	0.2	1.4	0.2	0.2	0.0
ar-curcumene[e]/1483/1678	4.0	0.3	3.3	0.4	3.7	0.1	3.5	0.2	1.9	0.1	2.0	0.1	1.8	0.1	1.9	0.0	2.3	0.1	1.9	0.1	1.6	0.2
epi-cubebol[e]/1493/–	0.0	0.0	0.5	0.0	0.0	0.0	0.1	0.0	0.3	0.0	0.3	0.0	0.1	0.0	0.3	0.1	0.3	0.0	0.7	0.2	0.0	0.0
spathulenol[e]/1578/2118	4.0	0.6	3.0	0.4	4.2	0.3	2.7	0.1	5.1	0.2	5.6	0.4	5.9	0.2	5.7	0.4	3.7	0.3	3.7	0.3	5.2	1.3
caryophyllene oxide/1585/1991	5.4	0.3	4.0	0.7	4.9	0.3	4.3	0.6	3.0	0.1	2.7	0.3	3.4	0.2	3.2	0.3	4.9	0.2	4.1	0.7	3.8	0.7
tau-cadinol[e]/1646/2136	1.4	0.1	0.9	0.3	1.3	0.1	0.9	0.1	1.8	0.1	1.2	0.1	2.0	0.1	1.3	0.0	1.4	0.2	0.7	0.1	0.1	0.0

[a] Experimental retention index (mean values) on nonpolar column. [b] Experimental retention Index (mean values) on polar column. [c] Means, $n = 5$ (Rancagua in 2002/03, $n = 3$). [d] Standard error. [e] Tentative identification.

2001–2002 (1881 MJ m^{-2}) than in 2002–2003 (1762 MJ m^{-2}). The temperature during growth station in 2001–2002 was lower than in 2002–2003, especially around the flowering stage, previous to harvest (**Table 1**).

Biomass Yield. Significant annual differences were detected in the three biomass measured characteristics (**Table 2**). In 2001/2002 there were higher AE, ST, and LI biomasses. The total AE, ST, and LI biomasses were similarly not affected by genotype ($P = 0.45$, $P = 0.13$, and $P = 0.07$, respectively) (**Table 2**).

Essential Oil Percentage and Yield. The EOP from the accessions ranged from 0.7 to 1.7% (**Table 2**). Quantitative differences among accessions were detected ($P = 0.03$). Rancagua, San Luis, Buenos Aires, and Mendoza had higher EOP than the Talca accession did. The environmental conditions of the second season were more restrictive to the EOP ($P = 0.01$) and EOY ($P = 0.001$). However, the interaction between years and accessions for EOY ($P = 0.002$) showed different responses among accessions when environmental conditions changed (**Table 2**): EOY of Talca did not change between years, while the rest of the accessions always yielded more in 2001–2002 than in 2002–2003 (**Table 2**).

Essential Oil Composition. Qualitative and quantitative analysis of the essential oils showed 25 constituents, 19 of them being the most representative and widespread among the studied accessions (**Table 3**), mainly represented by oxygenated monoterpenes and hydrocarbon and oxygenated sesquiterpenes (71.0–90.5% of the total oils).

The simultaneous evaluation of accessions and composition of the essential oils in both years, with PCA, allowed the detection of two features in *A. citriodora*: the relationships among accessions and the value of compounds for discrimina-

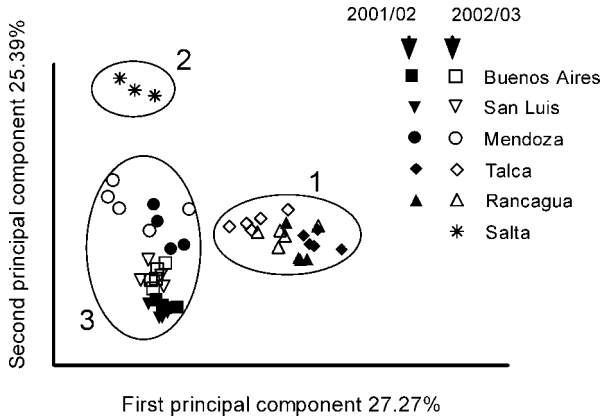

Figure 1. Relationship among *Aloysia citriodora* accessions by means of PCA on the basis of the chemical composition present in LI. Accessions and replicates are represented by full symbols in 2001/02, and empty symbols in 2002/03.

Table 4. Eigenvalue for Each Principal Component, % of Total Variance Expressed by the % Component and Accumulated Variation

principal component	autovalue	% of variance	% of total accumulated variation
1	5.18	27.27	27.27
2	4.82	25.39	52.66
3	2.44	12.84	65.50
4	1.40	7.35	72.85
5	1.29	6.78	79.63
6	0.97	5.10	84.73
7	0.84	4.44	89.17
8	0.71	3.74	92.91
9	0.46	2.43	95.34
10	0.34	1.79	97.13

Table 5. Trait Contributions to the First Two Principal Components

	correlation r^a	
compound	first	second
alpha-pinene	0.87	0.11
sabinene	0.05	0.92
6-methyl-5-hepten-2-one	−0.34	−0.10
para-cymene	0.00	−0.12
limonene	−0.06	−0.85
1,8-cineol	0.95	0.13
cis-sabinene hydrate	0.43	−0.48
cis-thujone	−0.35	0.79
citronellal	−0.19	0.77
neral	−0.91	0.10
piperitone	0.25	0.21
geranial	−0.85	0.26
geranyl acetate	−0.25	−0.34
beta-caryophyllene	−0.24	−0.81
ar-curcumene	0.86	0.06
epi-cubebol	−0.38	0.01
caryophyllene oxide	0.51	0.25
spathulenol	−0.44	−0.30
tau-cadinol	0.07	−0.85

[a] Coefficient of correlation.

tion among accessions (**Figure 1**). The three first principal components express over 65.50 % of the total variance (**Table 4**). **Figure 1** represents the first and second component combination, which accounts for 52.66% of the total variance (**Table 4**). Both components reflect variability within the accessions, and for each accession, variability between years. A contrast can be observed over the first principal component between Rancagua and Talca and the rest of accessions, while the second principal component presents a contrast between Salta on the one hand, and Mendoza, Buenos Aires, San Luis, Rancagua and Talca on the other hand (**Figure 1**).

Table 6. Comparison of Neral and Geranial Contents in *Aloysia citriodora* Accessions

accessions	neral (%[a])	geranial (%[a])
	2001/2002	
Rancagua	17.7 (0.21)	21.3 (0.8)
Talca	15.5 (1.75)	18.3 (2.39)
San Luis	21.5 (0.93)	25.2 (1.67)
Buenos Aires	21.6 (0.66)	25.7 (1.41)
Mendoza	23.5 (1.36)	30.8 (2.83)
	2002/2003	
Rancagua	17.9 (1.35)	22.6 (2.54)
Talca	19.5 (2.04)	25.1 (2.52)
San Luis	21.5 (1.52)	26.8 (1.89)
Buenos Aires	21.7 (0.46)	27.1 (0.78)
Mendoza	23.9 (2.93)	32.2 (1.86)
Salta	22.6 (1.86)	28.9 (1.81)
$P =$		
year (Y)	0.05	0.0001
accessions (A)	<0.0001	<0.0001
Y × A	0.04	0.01

[a] Figures are means and standard deviations (between brackets).

The correlation between compounds and the first and second principal component (**Table 5**) indicates the discriminating power of compounds: alpha-pinene, 1,8-cineol, neral, geranial, *ar*-curcumene, sabinene, limonene, *cis*-thujone, citronellal, beta-caryophyllene, and tau cadinol. Alpha pinene, 1,8-cineol, and *ar*-curcumene were positively correlated with the first component ($r = 0.87$, 0.95 and 0.86, respectively), whereas neral and geranial were negatively correlated with the same component ($r = -0.91$ and -0.85). Sabinene, *cis*-thujone, and citronellal were positively correlated with the second component ($r = 0.92$, 0.79, and 0.77, respectively), whereas limonene, beta-caryophyllene, and tau cadinol were negatively correlated with the second component ($r = -0.85$, -0.81, and -0.85, respectively).

The PCA analysis allowed identification of three chemotypes among the accessions, as shown in **Figure 1**. Considering the differences in the occurrence and percentage of compounds within the essential oil, they can be characterized as follows:

Chemotype 1: characterized by the occurrence of 1,8-cineol, a lower content of citral (neral and geranial), and a higher percentage of alpha pinene and *ar*-curcumene (accessions from Rancagua and Talca).

Chemotype 2: characterized by a higher percentage of sabinene, *cis*-thujone, and citronellal as well as a lower content of limonene (accession from Salta).

Chemotype 3: characterized by the most representative chemical profile of lemon verbena, with either low content or the absence of 1,8-cineol, citronellal, and *cis*-thujone (accessions from Mendoza, San Luis, and Buenos Aires).

ANOVA test showed some differences in the contents of neral and geranial of lemon verbena LI related to accessions and years (**Table 6**). Even though slight changes in environmental conditions were detected between years, the first year conditions were more restrictive to geranial than to neral. However, the interaction between years and accessions for both compounds ($P = 0.04$ and $P = 0.01$, respectively) showed different responses among accessions.

Discussion. The first year environmental conditions were more favorable to the aerial biomass, EOP and EOY. These responses could have been related to a positive relationship between above-ground biomass and the total intercepted radiation accumulated across the crop growth cycle (*27*), the essential

oil content, and the incident radiation (*28*). Furthermore, according to Croteau et al. (*29*), the synthesis of monoterpenes is closely connected to processes favoring the accumulation of sucrose or photosynthetic analogues, concomitantly with the radiation levels. An increase in photosynthesis enables the formation of terpenoids, while inhibiting their metabolic decomposition. Likewise, it is possible that the EOP increase, together with the EOY and biomass increases (**Table 2**) could show that the essential oil synthesis rate was higher than the biomass accumulation rate in 2001–2002 than in 2002–2003, in accordance to observations made by Koricheva (*30*).

In the present study, the most relevant cause of variability in the essential oil composition was genotype, if compared with the much lower effects of the environment during the tested growing seasons. Our results show that there are important qualitative and quantitative genotypic differences in yield and oil composition among the accessions collected in Argentina and Chile (**Table 2** and **3**, and **Figure 1**), thus stating a clear segregation of three chemotypes. Chemotypes from Rancagua and Talca accessions differed from the other accessions in their preponderance of alpha pinene, 1,8-cineol, and *ar*-curcumene and in the lower contents of neral and geranial, which is a detriment to its aromatic profile. The Salta accession, that is, the second chemotype, had unusually high contents of sabinene, *cis*-thujone, and citronellal, in that all these terpenes are not representative of the typical lemon verbena oil. The third chemotype, embracing the accessions from Mendoza, San Luis, and Buenos Aires, had the best quality of oil for this species in the region, with the typical lemony and fresh herbaceous flavor, therefore being the most promising cluster. The oil composition profile thereof is similar to profiles from other origins (*6*, *11*), with the exception of the steady low yield of 1,8-cineol. In particular, the Mendoza accession, with its higher contents of neral and geranial, is the most promising accession to be encouraged for future crops.

As shown in **Tables 3** and **6**, lemon verbena accessions differed in the stability of the composition of the essential oils when the environmental conditions had changed. The minor content of monoterpenes and the increase of sesquiterpenes during 2001–2002 in relation to 2002–2003 could be explained in part by the different temperatures and volatility of the constituents of the essential oils, as previously suggested by Von Schantz and Ek (*31*). According to these authors, it could be possible that the more volatile monoterpenes (e.g., limonene, neral, geranial) had evaporated more easily than the sesquiterpenes (beta-caryophyllene, *ar*-curcumene, caryophyllene oxide, and spathulenol), thus leading to an altered ratio between them.

Our results show clear infraspecific variability in lemon verbena. The fact that all plants were grown in the same environmental conditions and were analyzed using the same method and under the same conditions allowed a true assessment of the genotypic manifestations, independently from other possible influences.

At the same time, these results provide information on the accessions that currently grow in Argentina, showing not only that the San Luis accessions comply with the aromatic pattern required by the market for this species, but also that Buenos Aires and Mendoza botanical collections should provide a good starting point for the development of lemon verbena cultivars for use by farmers and industry alike, and, particularly, the Mendoza accessions for their high contents of neral and geranial. Our results also show that environmental conditions are important clues to be taken into account not only for changes in biomass and essential oil yields but also for quantitative variations in the essential oil composition. Further studies could be needed to assess how environmental conditions and crop management practices, such as radiation, temperature, plant density, fertilization, and location, can affect those parameters and consequently the yield and quality of the commercialized herb or essential oil.

ACKNOWLEDGMENT

The authors thank the students Claudia Morvillo and Cecilia Conde for their technical assistance, Dr. Carmen Viturro and Amanda Di Fabio for the supply of the Salta and Mendoza accessions, respectively, and Mrs. F. Martins for the English revision.

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Received for review March 21, 2007. Revised manuscript received July 16, 2007. Accepted July 29, 2007. This work is part of the Projects UBACYT BO-19 and PICT R-0284.

JF0708387